INVESTIGATING THE EFFECTS OF AQUEOUS EXTRACT OF PICRALIMA NITIDA FRUIT ON INSULIN HORMONE IN STREPTOZOTOCIN- INDUCED DIABETIC MALE WISTAR RATS

BY

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UNIVERSITY OF BENIN,

BENIN CITY

NOVEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.SC (HONS) MEDICAL BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER, 2025.

CERTIFICATION

We the undersigned hereby certify that the student **NIKPEDE AYIBA-OFEORITSE FAVOUR** carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve same as adequate in scope quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry

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(Head of Department).

_____ _____

EXTERNAL EXAMINER DATE

DEDICATION

This project work is dedicated to God Almighty, for his extravagant love, provision and protection throughout the course of the project.

ACKNOWLEDGEMENT

I would like to appreciate the Almighty God , who provided bountifully for my parents and I making it possible for me to have an education.

I would like to acknowledge my parents, Pastor Austine Nikpede and Mrs. Sonita Nikpede, for their continuous love, support and contribution towards my life and education. I would like to say a big thank you to my siblings, Daniel, Marvelous and Success for all the love and care shown to me.

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by impaired insulin secretion, insulin action, or both, leading to chronic hyperglycaemia and associated complications. The search for plant-based alternatives with antidiabetic potential has gained attention due to the limitations and side effects of conventional therapies. This study investigated the effects of aqueous extract of Picralima nitida fruit on serum insulin levels in streptozotocin-induced diabetic male Wistar rats. Diabetes was induced using streptozotocin, and animals were allocated into five groups: normal control, diabetic control, glibenclamide-treated, low-dose extract, and high-dose extract groups. Serum insulin concentration was quantified using enzyme-linked immunosorbent assay (ELISA).

Results showed that induction of diabetes led to alterations in insulin secretion, with the diabetic control group exhibiting elevated insulin levels compared to the normal control group, suggesting partial β-cell dysfunction with compensatory responses. Glibenclamide treatment produced decreased insulin levels relative to the diabetic control, likely due to the extent of β-cell destruction. The low-dose extract produced insulin levels comparable to glibenclamide, indicating mild insulin-modulating activity. Notably, the high-dose extract produced the highest insulin concentration among all groups, suggesting a dose-dependent stimulatory effect of P. nitida on pancreatic function. Obseved from the results, the extract, particularly at higher doses,may enhance insulin secretion or protect surviving β-cells.

In conclusion, the aqueous fruit extract of Picralima nitida demonstrates potential insulin-modulating activity in streptozotocin-induced diabetic rats. These findings support the possible use of P. nitida as a complementary therapeutic agent for diabetes management. Further studies with larger sample sizes and pancreatic histological evaluations are recommended to better elucidate its mechanism of action.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes mellitus is a major global health challenge characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Changes in carbohydrate, fat and protein metabolism contribute to the development of complications associated with the disease. The hormone Insulin, produced by the β-cells of the pancreatic islets, plays a central role in glucose homeostasis; insufficient insulin secretion or impaired insulin action (insulin resistance) leads to elevated blood glucose levels and various metabolic disturbances.

There is increasing interest in complementary and alternative therapies, including medicinal plants, for the management of diabetes and its related metabolic disorders. In particular, plants with hypoglycaemic, insulin-modulating, antioxidant and anti-inflammatory properties are being explored as adjuncts or alternatives to conventional therapy

The plant species Picralima nitida (family Apocynaceae) is a West African medicinal tree/shrub that has been traditionally used for a variety of ailments — including fever, malaria, pain, gastrointestinal disorders, and more recently for antidiabetic purposes. Studies have documented its phytochemical richness (notably indole alkaloids, saponins, glycosides, tannins) and its pharmacological activities including analgesic, antimalarial, antioxidant and hypoglycaemic effects.

Several experimental studies have indicated that extracts of P. nitida can lower blood glucose levels, improve insulin sensitivity, and reduce associated oxidative stress in rodent models of metabolic disease. For example, one study found that an aqueous seed extract of P. nitida

reduced plasma glucose and insulin levels, and improved insulin resistance in high-fat high-fructose fed rats. Another investigation reported that a methanolic seed extract regulated insulin and blood glucose levels in alloxan-induced diabetic rats. These findings suggest that P. nitida may exert beneficial effects on pancreatic β-cell function and insulin hormone regulation.

Given this background, it becomes scientifically meaningful to study the aqueous extract of P. nitida (rather than merely methanolic or other solvents) and its effects on insulin hormone dynamics in male Wistar rats (a widely accepted experimental model). Such a study can contribute to a deeper understanding of the plant's potential antidiabetic mechanism of action, particularly focusing on insulin hormone levels, pancreatic histology, and downstream metabolic endpoints.

1.2 Statement of the Problem

Despite advances in diabetes management, there remains a considerable burden of metabolic dysfunction, insulin resistance and inadequate glycaemic control worldwide. Conventional antidiabetic drugs often come with side-effects, cost-implications and sometimes limited efficacy in real-world settings. There is therefore a pressing need for novel, safe and affordable plant-based therapeutic options.

While P. nitida has been extensively used in ethnomedicine and some experimental work shows promising hypoglycaemic and insulin-modulating effects, the scientific evidence remains limited and inconsistent. In particular:

1. There is a gap in studies specifically analysing the effects of aqueous extract of P. nitida on insulin hormone levels (as distinct from total glucose lowering).

2. The mechanistic linkage between extract administration, insulin hormone dynamics and pancreatic β-cell/endocrine responses remains only partially elucidated.

3. Many studies focus on mixed metabolic endpoints (e.g., glucose, lipids, oxidative stress) but do not isolate insulin hormone changes and histological pancreatic alterations in male Wistar rats.

4. There is a need for controlled experiments that clearly define dosage, extract preparation, duration and insulin hormone outcomes in a standard experimental model.

Therefore, this research seeks to address these gaps by evaluating the effect of aqueous extract of P. nitida on insulin hormone in male Wistar rats under experimental conditions.

1.3 Aim and Objectives of the Study

General Objective

To investigate the effects of the aqueous extract of Picralima nitida on the insulin hormone in male Wistar rats.

Specific Objectives

1. To prepare and standardise the aqueous extract of P. nitida fruit for administration in male Wistar rats.

2. To determine the effect of different doses of the aqueous extract of P. nitida on serum insulin concentration in male Wistar rats.

3. To assess the effect of the aqueous extract of P. nitida on fasting blood glucose levels in male Wistar rats.

4. To explore the relationship between extract dose and insulin hormone levels.

5. To identify any observable adverse effects or toxicity of the extract in male Wistar rats during the study period.

1.4 Research Hypotheses

Null hypothesis (H_0): The aqueous extract of Picralima nitida has no significant effect on serum insulin concentration in male Wistar rats.

Alternative hypothesis (H_1): The aqueous extract of Picralima nitida significantly increases serum insulin concentration in male Wistar rats.

1.5 Significance of the Study

This study is significant in several respects:

1. It adds to the scientific literature on P. nitida by focusing on insulin hormone dynamics, which is a key mechanistic endpoint in diabetes and metabolic research.

2. The findings may provide evidence for the development of plant-based therapeutic agents or adjuncts targeting insulin regulation, especially in resource-limited settings.

3. For ethnomedicine and pharmacognosy, the study helps validate (or otherwise) traditional claims of P. nitida's antidiabetic uses and may guide further phytochemical or drug-development investigations.

4. Understanding the extract's effect on insulin hormone provides mechanistic insight that might support its clinical translation.

5. The study may inform future toxicological and dose-response research, particularly concerning safety and therapeutic window in animal models.

1.6 Scope and Delimitation of the Study

Scope

1. The study will use male Wistar rats as the experimental model.

2. The plant extract preparation will focus on the aqueous extract of P. nitida fruit

3. Insulin hormone concentration and fasting blood glucose levels will be primary endpoints.

4. A defined duration and set doses will be employed under controlled laboratory conditions.

Delimitations

1. The study does not extend to female rats; thus any sex-specific differences will not be assessed.

2. The extract isn't limited to one plant part (e.g., seeds) rather it comprises of the whole fruit (both the seeds and pulp)

3. The study will not cover long-term chronic toxicity assessments beyond the experimental duration.

4. Other metabolic parameters (e.g., lipid profile, inflammatory markers) may not be comprehensively covered (unless added) as the main focus is insulin hormone.

5. Translation to human application remains speculative and beyond the scope of this thesis.

1.7 Justification of the Study

Despite numerous conventional therapies, the global prevalence of diabetes continues to rise, especially in developing countries where access to synthetic drugs may be limited. Traditional

medicinal plants offer a potentially cost-effective, accessible source of therapeutic agents. Given that P. nitida is already used in African ethnomedicine for diabetes and preliminary studies suggest beneficial effects, further experimental validation is justified.

By investigating the aqueous extract (which is often more accessible and less hazardous than organic solvent extracts) and focusing on insulin hormone responses, the study aligns with a translational paradigm: moving from traditional use through experimental validation toward potential phytotherapeutic development. Moreover, the choice of male Wistar rats ensures replicability and comparability with existing biomedical literature.

1.8 Operational Definition of Terms

Aqueous extract: The preparation of plant material (e.g., seeds of P. nitida) using water as solvent, followed by filtration, concentration and storage for administration.

Insulin hormone (Insulinemia): The concentration of insulin in the blood (typically serum or plasma) measured in μU/mL or equivalent units.

Male Wistar rats: Laboratory rats of the Wistar strain, male sex, commonly used in biological and pharmacological experiments.

Hyperglycaemia: Elevated blood glucose level above the normal fasting range for the species/strain.

Histology: Microscopic examination of tissues (in this study, the pancreas) to assess structural changes, cell morphology, and evidence of damage or regeneration.

Hypoglycaemic effect: A reduction in blood glucose levels resulting from intervention.

Pancreatic β-cells: The insulin-secreting cells located in the islets of Langerhans in the pancreas.

CHAPTER TWO

LITERATURE REVIEW

2.1. INTRODUCTION

Medicinal plants have been used traditionally to manage symptoms of hyperglycaemia and metabolic disease. Ethnopharmacological interest in plant extracts arises from their complex mixtures of bioactive secondary metabolites (flavonoids, alkaloids, saponins, tannins, glycosides) that can act on multiple targets, relative accessibility and cultural acceptability in many regions, and potential to provide lead scaffolds for drug development. Preclinical evidence is commonly sought to validate traditional claims, define mechanisms (e.g., insulin secretion, insulin sensitization, inhibition of carbohydrate-digesting enzymes), and determine safety and dose ranges (reviewed in multiple phytopharmacology surveys).

2.2. OVERVIEW OF PICRALIMA NITIDA

2.2.1. TAXONOMIC CLASSIFICATION

Picralima nitida is a medicinal plant from the genus picralima belonging to the plant family Apocynaceae, a family that comprises many alkaloids producing species with strong pharmacological properties (Teugwa et al., 2013). It is commonly known as Akuamma, and the fruit is often referred to as the Picralima nitida fruit or Akuamma seed. The plant is classified as follows:

Kingdom : Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Picralima

Species: Picralima nitida(Stapf) Th. and H. Durand

It is indigenous to West and Central Africa, particularly in countries such as Nigeria, Ghana, Cameroon, Cote D'Ivoire, and the Democratic Republic of Congo (Inya-Agha, 2006). The plant thrives in tropical rainforests and secondary bushes often growing up to 10 - 12nm in height (Akinloye et al., 2014).

The Apocynaceae family is known for its production of potent indole alkaloids and terpenoids, compounds that contribute to the therapeutic activities of *P. nitida* and other genera in this family(Teugwa et al., 2013 ; Folorunso et al., 2022).

2.2.2. BOTANICAL DESCRIPTION

Picralima nitida is a medium -sized evergreen tree that can reach up to 35 ft in height. The stem bark is greyish brown and exudes a milky latex when cut, a typical characteristic of members of the Apocynaceae family (De Campos et al., 2020). The leaves are simple, opposite and elliptical, with smooth margins and a glossy surface. The flowers are white to cream-coloured, fragrant and occur in small clusters.

The fruit is woody, ellipsoidal capsule usually containing about 6-8 seeds embedded in a fibrous pulp. The seeds are flattened brownish, and covered with a fibrous coat; they are the major part of the plant used for medicinal purposes (Teugwa et al., 2013). The seeds and fruit pulp are

often air-dried, powdered and extracted in water or ethanol for use in traditional medicine and scientific research (Akinloye et al., 2014).

Figure 1: *Picralima nitida* fruit in its natural habitat

Source: Teugwa et al., 2013

2.2.3. PHARMACOLOGICAL EFFECTS

Several in vivo and in vitro studies have supported the pharmacological relevance of picralima nitida.

1. Antidiabetic Effects: Studies have shown that methanolic extracts of picralima nitida reduced fasting blood glucose levels of in alloxan induced diabetic rats, suggesting a hypoglycemic effect (Ezeigbo et al., 2012).

2. Antioxidant activity: The extract has demonstrated dose dependent free radical scavenging ability, which may protect B-cells from oxidative damage.

3. Analgesic and anti-inflammatory effects: These activities, primarily attributed to its alkaloid content, have been well-documented in animal models (Olajide et al., 2021). The alkaloid rich

fraction are thought to inhibit the synthesis of inflammatory mediators such as prostaglandins, thereby reducing pain and inflammation (Okokon and Nwafor 2009).

4. Antimicrobial and Antimalarial Effects: The plant exhibit broad spectrum antimicrobial activity against bacteria and fungi which support its traditional use in treating infections. Additionally, the indole alkaloids have shown anti plasmodial activity for malaria treatment (Tona et al., 2001).

5. Hepato protective and Nephroprotective Effects: Osawe et al., 2015 demonstrated that aqueous extracts of picralima nitida protected liver and kidney tissues from chemically induced damage. Treated animals showed normalized serum liver enzymes - Aspartate Transaminase (AST), Alanine Transaminase (ALT) and kidney function markers (urea, creatinine) , suggesting that the extract can mitigate organ toxicity and oxidative damage.

However, these studies are largely focused on symptom reduction (e.g blood glucose lowering) and do not provide substantial data on:

I. Changes in pancreatic enzyme activity (amylase, lipase)

II. Histological changes in the islets of Langerhans or acinar cells

III. The effects of aqueous (traditional) extract as most studies use ethanol or methanol based extracts.

2.2.4 PHYTOCHEMICAL COMPOSITION

Several studies have shown that various extracts of this plant are good sources of phytochemicals such as glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins and tannins (Nielsen et al., 2018).

1. Indole Alkaloids: One of the most prominent phytochemical group in picralima nitida are the indole alkaloids, particularly these found in the seeds. They include:

Akuammine

Akuammudine

Picraline

Akuammicine

These alkaloids share structural similarities with opioid and adrenergic compounds and are believed to interact with opioid receptors hence their traditional use for pain relief (Nielsen et al., 2018).

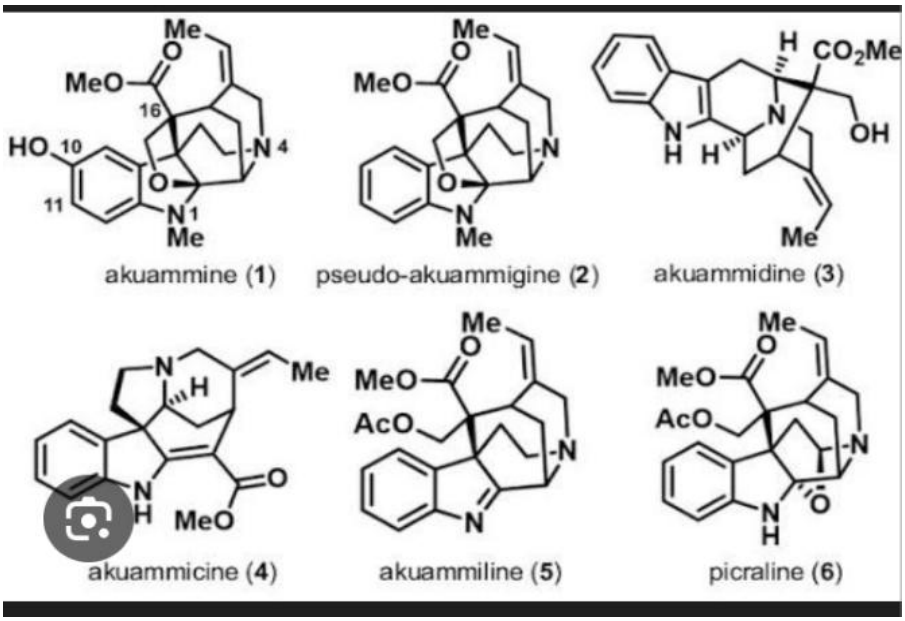

Figure 2: Different alkaloids present in the picralima nitida fruit

Source: Nielsen et al., 2018

2. Flavonoids and Phenolic Acids : Additionally, flavonoids and phenolic acids present in the fruit and leaves have been identified as powerful antioxidants, capable of scavenging free radicals and reducing oxidative stress (Omoregie and Osagie, 2011). These compounds play a significant role in protecting tissues, including the pancreas from oxidative damage - a major contributor to B-cells dysfunction in diabetes mellitus .

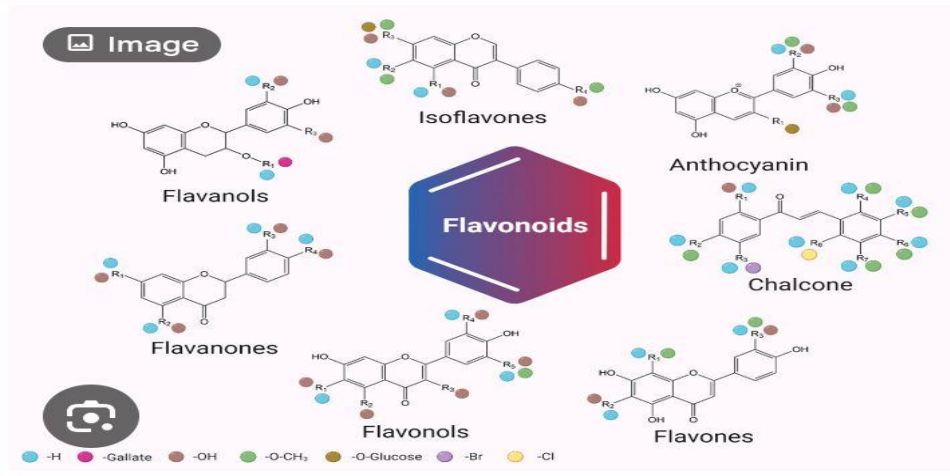

Figure 3: showing Flavonoids as a phytochemical in picralima nitida fruit

Source: Omoregie and Osagie, 2011

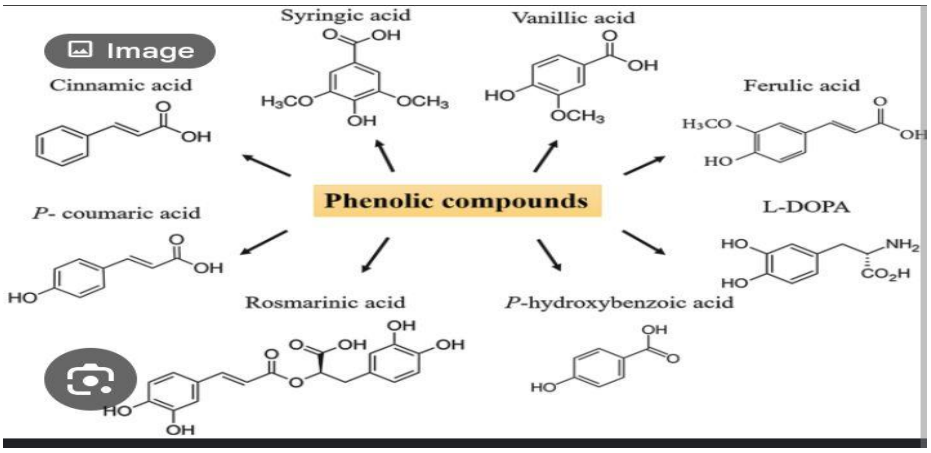

Figure 4: showing different Phenolic compounds

Source: Omoregie and Osagie,2011

3. Saponins and Tannins : Furthermore, saponins and tannins have been reported to exhibit anti-inflammatory and antimicrobial activities, suggesting a potential for modulating immune responses and improving gut health, which indirectly affects metabolic regulation (Ezekwesili et al ., 2014).

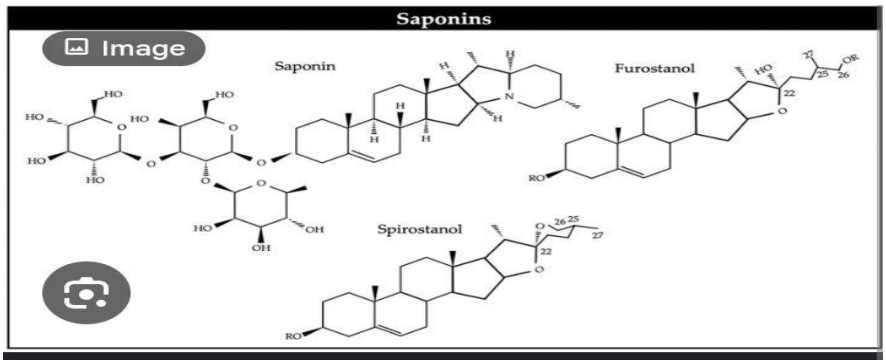

Figure 5: showing Saponins as a phytochemical in picralima nitida fruit

Source: Teugwa et al., 2013

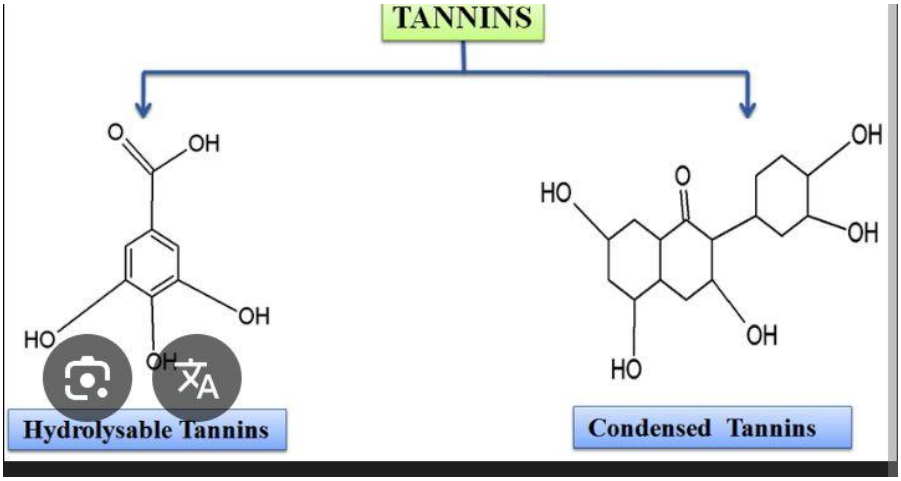

Figure 6: showing different types of Tannins present in picralima nitida fruit

Source: Teugwa et al., 2013

2.3. THE PANCREAS: STRUCTURE AND FUNCTION

2.3.1. ANATOMY OF THE PANCREAS

The pancreas is a vital, elongated, soft, and lobulated retroperitoneal gland located in the upper abdomen. It functions as both an exocrine and endocrine organ and is involved in digestion and metabolic regulation. Structurally and anatomically, it is positioned transversely across the posterior abdominal wall, extending from the concavity of the duodenum (on the right) to the hilum of the spleen (on the left) (Moore et al., 2013).

Shape, Size, and Color

The pancreas is J-shaped or tadpole-shaped in adults, with a length of approximately 12–15 cm, a width of 4–5 cm, and a thickness of 1.5–2 cm. It typically weighs between 70 to 100 grams

(Drake et al., 2015). In fresh specimens, it appears pinkish-tan to yellowish, soft, and lobulated in texture due to the presence of numerous acini and islet clusters.

Borders and Surfaces

The pancreas has three main surfaces; anterior, posterior, and inferior and is described anatomically in four parts:

Head: Lies within the C-shaped curve of the duodenum.

Neck: Narrow portion connecting the head to the body, lying anterior to the superior mesenteric vessels.

Body: Extends leftward across the midline, posterior to the stomach.

Tail: The narrow, leftmost part that approaches the spleen.

The pancreas has superior, inferior, and anterior borders, each associated with major vascular structures like the splenic artery (along the superior border) and the superior mesenteric vessels (posterior to the neck) (Standring, 2016).

Position and Location

Topographically, the pancreas lies at the level of the first and second lumbar vertebrae (L1–L2). It is located in the epigastric and left hypochondriac regions of the abdomen. It is:

Posterior to the stomach

Anterior to the aorta, inferior vena cava, and vertebral column

Medial to the spleen (tail of the pancreas)

In contact with the duodenum (head of the pancreas)

It is also closely related to the bile duct, which passes through or near the head of the pancreas to join the pancreatic duct and open into the duodenum at the ampulla of Vater (Moore et al., 2013).

Pancreatic Duct System

The main duct, the duct of Wirsung, runs through the length of the pancreas and drains into the duodenum at the major duodenal papilla. A smaller accessory duct, the duct of Santorini, may also be present and may drain separately (Drake et al., 2015).

Vascular Supply and Lymphatics

The pancreas receives arterial blood mainly from:

Branches of the celiac trunk (via the splenic artery)

Superior and inferior pancreaticoduodenal arteries

Venous drainage corresponds to the arterial supply and empties into the portal vein. Lymph from the pancreas drains into pancreaticosplenic, pyloric, and superior mesenteric lymph nodes, while innervation is from both sympathetic (via splanchnic nerves) and parasympathetic (via vagus nerve) fibers (Standring, 2016).

Histology of the Pancreas

The pancreas is a heterocrine gland, meaning it performs both exocrine and endocrine functions. Histologically, the pancreas is divided into two main functional components:

Exocrine pancreas (constitutes ~98–99% of pancreatic mass)

Endocrine pancreas (constitutes ~1–2%)

These components differ in structure, function, and cellular composition.

Exocrine Pancreas

The exocrine part is composed of serous acini that produce and secrete digestive enzymes such as amylase, lipase, trypsinogen, chymotrypsinogen, and procarboxypeptidase. These enzymes are delivered to the duodenum via the pancreatic duct system to aid in the digestion of carbohydrates, fats, and proteins (Young et al., 2014).

Each acinus is made up of a cluster of pyramidal epithelial cells with basophilic basal cytoplasm (rich in rough endoplasmic reticulum) and eosinophilic apical zymogen granules, which contain the inactive enzyme precursors. A central lumen collects secretions, which drain into intercalated ducts, and then into intralobular and interlobular ducts, eventually leading to the main pancreatic duct (Ross & Pawlina, 2021).

Supporting tissue includes fibrous stroma, which separates the pancreas into lobules and contains blood vessels, lymphatics, and nerves.

Endocrine Pancreas (Islets of Langerhans)

Scattered throughout the exocrine tissue are clusters of lighter-staining cells called Islets of Langerhans, first described by Paul Langerhans in 1869. These spherical or oval clusters are composed of several types of hormone-secreting cells (Young et al., 2014):

β (Beta) cells: Secrete insulin (about 60–80% of islet cells); centrally located.

α (Alpha) cells: Secrete glucagon (~15–20%); located more peripherally.

δ (Delta) cells: Secrete somatostatin (~5–10%); scattered.

PP (F or gamma) cells: Secrete pancreatic polypeptide.

ε (Epsilon) cells: Secrete ghrelin, primarily in the fetal pancreas.

These cells regulate blood glucose and other metabolic processes. The islets are highly vascularized, allowing hormones to be quickly released into the bloodstream (Ross & Pawlina, 2021).

Microscopic Differences

1). Exocrine tissue stains darker with H&E due to abundant rough endoplasmic reticulum and zymogen granules.

2). Islets appear lighter and more vascular under the microscope.

3). No striated ducts are present in the pancreas (unlike salivary glands), which is a distinguishing histological feature.

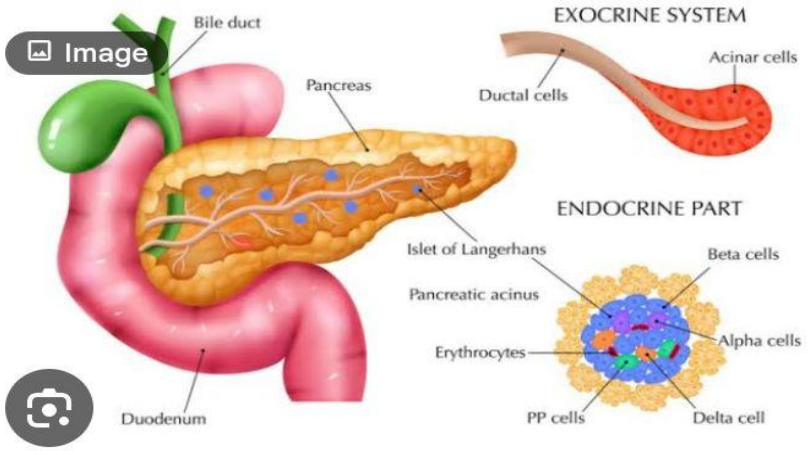

Figure 7: showing the different parts of the Pancreas

Source: Ross and Pawlina, 2021

2.3.2. EXOCRINE FUNCTION OF THE PANCREAS

The exocrine pancreas is responsible for producing and secreting digestive enzymes and bicarbonate-rich fluid into the duodenum, which facilitates the chemical breakdown of food components in the small intestine. This function is essential for proper digestion and nutrient absorption.

Histologically, the exocrine pancreas is made up of pancreatic acini, which are clusters of pyramidal epithelial cells that surround a central lumen. These cells secrete a pancreatic juice that contains enzymes necessary for digesting carbohydrates, proteins, and lipids (Young et al., 2014).

Digestive Enzymes Produced by the Exocrine Pancreas

The exocrine secretion contains three major categories of enzymes:

1. Proteolytic enzymes (protein digestion):

Trypsinogen

Chymotrypsinogen

Procarboxypeptidase These enzymes are secreted in their inactive (zymogen) forms to prevent autodigestion of pancreatic tissue and are activated in the duodenum by enterokinase.

2. Amylolytic enzymes (carbohydrate digestion):

Pancreatic amylase Breaks down complex carbohydrates like starch into simple sugars.

3. Lipolytic enzymes (fat digestion):

Pancreatic lipase

Phospholipase A2

Cholesterol esterase These enzymes hydrolyze fats into free fatty acids and monoglycerides.

In addition to enzymes, the pancreas secretes nucleases such as:

Deoxyribonuclease (DNase)

Ribonuclease (RNase)

These help in the breakdown of nucleic acids (DNA and RNA).

Role of Ductal Cells and Bicarbonate Secretion

The ductal cells of the pancreas play a critical role in producing and secreting bicarbonate ions (HCO_3^-) into the pancreatic fluid. This alkaline secretion:

I).Neutralizes the acidic chyme entering the duodenum from the stomach.

II).Creates an optimal pH (~7.1 to 8.2) for pancreatic enzymes to function effectively (Guyton & Hall, 2021).

The primary stimulus for bicarbonate secretion is secretin, a hormone released from the duodenum in response to acidic gastric contents

Regulation of Exocrine Pancreatic Secretion

Pancreatic exocrine secretion is tightly regulated by both neural and hormonal mechanisms:

Hormonal regulation:

1. Cholecystokinin (CCK): Stimulates acinar cells to secrete enzyme-rich pancreatic juice in response to fats and proteins in the duodenum.

2. Secretin: Stimulates ductal cells to secrete bicarbonate-rich fluid.

Neural regulation:

Vagus nerve (parasympathetic innervation) enhances enzyme secretion via acetylcholine during the cephalic and gastric phases of digestion (Moore et al., 2013).

The coordinated action of these pathways ensures that digestive enzymes and bicarbonate are secreted only when needed, reducing energy waste and preventing self-digestion.

Clinical Relevance of Exocrine Function

In exocrine pancreatic insufficiency (EPI), the pancreas fails to produce enough digestive enzymes, leading to malabsorption, steatorrhea (fatty stools), weight loss, and vitamin deficiencies. Conditions like chronic pancreatitis, cystic fibrosis, and pancreatic cancer can impair exocrine function (Stevens et al., 2020).

Measurement of enzymes like serum amylase and lipase are commonly used biomarkers for pancreatic inflammation and pancreatitis in both clinical and experimental research settings.

2.3.3. ENDOCRINE FUNCTION OF THE PANCREAS

The endocrine pancreas plays a central role in glucose homeostasis and metabolic regulation through the secretion of hormones directly into the bloodstream. These hormones are essential for regulating carbohydrate, lipid, and protein metabolism. The endocrine tissue is composed of

specialized cell clusters known as the Islets of Langerhans, first described by Paul Langerhans in 1869 (Young et al., 2014).

Although the endocrine portion comprises only 1–2% of the total pancreatic mass, its function is critical for maintaining normal physiological balance.

Islets of Langerhans: Structure and Cell Types

The Islets of Langerhans are well-vascularized, lightly stained clusters of endocrine cells dispersed throughout the exocrine tissue of the pancreas. These islets are composed of five main types of hormone-secreting cells, each with specific metabolic roles (Ross & Pawlina, 2021):

Cell Type	Percentage	Location in Islet	Hormone Secreted	Function
β (Beta) cells	60 - 80 %	Central	Insulin	Lowers blood glucose by promoting uptake and storage
α (Alpha) cells	15 - 20 %	Peripheral	Glucagon	Increases blood glucose via glycogenolysis and gluconeogenesis
δ (Delta) cells	5 - 10 %	Scattered	Somastostatin	Inhibit insulin, glucagon and GI hormone secretion
PP (F) cells	< 5 %	Rare	Pancreatic polypeptide	Regulates exocrine secretion and GI motility
ε (Epsilon) cells	< 1 %	Sparse	Ghrelin	Stimulates hunger and growth hormone release

These hormones are secreted in response to blood nutrient levels, autonomic nervous signals, and hormonal feedback from the gut.

Major Endocrine Hormones and Their Functions

1. Insulin

It is secreted by β-cells in response to elevated blood glucose. It facilitates glucose uptake by cells, promotes glycogenesis, lipogenesis, and protein synthesis. Insulin also inhibits gluconeogenesis and lipolysis.A deficiency or resistance to insulin results in diabetes mellitus type 1 or 2 (Guyton & Hall, 2021).

2. Glucagon

It is secreted by α-cells when blood glucose is low. Glucagon stimulates glycogenolysis, gluconeogenesis, and lipolysis to increase blood glucose levels. It acts primarily on the liver to maintain glucose availability during fasting.

3. Somatostatin

It is secreted by δ-cells; acts as a paracrine inhibitor. It suppresses the release of insulin, glucagon, and other gastrointestinal hormones like gastrin and secretin. It helps regulate the timing and balance of endocrine signaling.

4. Pancreatic Polypeptide

It is secreted by PP cells. It modulates both exocrine pancreatic secretions and appetite regulation and also influences hepatic glycogen levels and gastrointestinal motility.

5. Ghrelin

It is produced by ε-cells, especially during the fetal period. It plays a role in stimulating appetite and regulating energy balance (Moore et al., 2013).

2.4 OVERVIEW OF INSULIN HORMONE

Insulin is a vital peptide hormone responsible for maintaining glucose homeostasis in the body. It is secreted by the β-cells of the pancreatic islets of Langerhans and plays a central role in regulating carbohydrate, lipid, and protein metabolism. The hormone facilitates glucose uptake by tissues, promotes glycogenesis, and inhibits gluconeogenesis and lipolysis. Dysregulation in insulin synthesis, secretion, or action results in metabolic disorders such as diabetes mellitus, which is characterised by chronic hyperglycaemia and oxidative stress (Folorunso et al., 2022).

The action of insulin is crucial in maintaining the balance between glucose production and utilization, ensuring a stable energy supply to cells. Its deficiency or impaired function, as observed in streptozotocin (STZ)-induced diabetic rats, leads to cellular energy deprivation, increased free fatty acid levels, and oxidative stress. These are conditions that Picralima nitida extracts have been reported to ameliorate through antioxidant and insulin-modulating mechanisms (De Campos et al., 2020; Teugwa et al., 2013).

2.4.1 STRUCTURE OF INSULIN

Insulin is a small polypeptide hormone consisting of 51 amino acids arranged in two chains: an A-chain with 21 amino acids and a B-chain with 30 amino acids. These two chains are linked by two inter-chain disulfide bonds and one intra-chain disulfide bond within the A-chain, which stabilizes its tertiary structure (Folorunso et al., 2022). The molecular weight of insulin is approximately 5.8 kDa.

The primary structure of insulin is highly conserved among mammals, highlighting its physiological importance. Structurally, insulin is synthesized initially as preproinsulin, which contains a signal peptide sequence that directs the nascent peptide to the rough endoplasmic reticulum. Upon cleavage of this signal peptide, proinsulin is formed. This intermediate contains the A and B chains connected by a connecting peptide (C-peptide) (Teugwa et al., 2013). In the Golgi apparatus, specific endopeptidases cleave the C-peptide, producing the active insulin molecule that is stored in secretory granules within β-cells.

The structural integrity of insulin is critical for its binding to insulin receptors on target cells, initiating intracellular signaling cascades that enhance glucose transport. Structural modification or oxidative damage to insulin molecules can impair receptor binding, leading to insulin resistance, a condition often linked with diabetes and oxidative stress (De Campos et al., 2020).

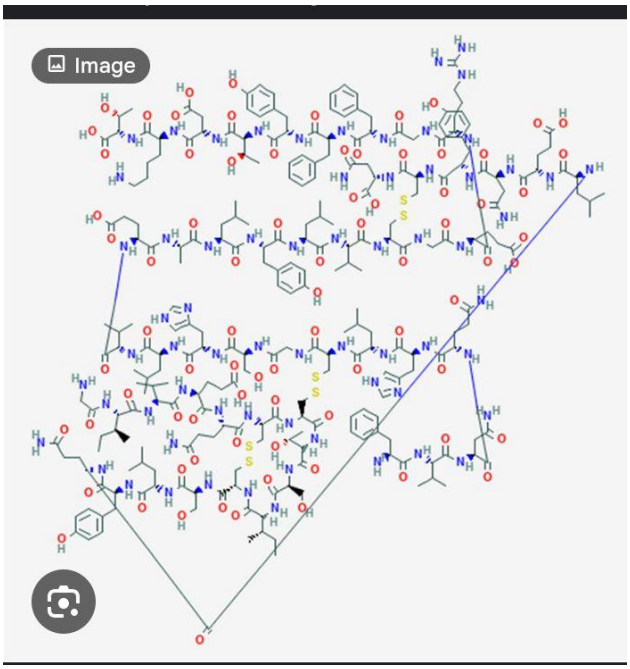

Figure 8: showing the 2 dimensional structure of the Insulin hormone

Source: Teugwa et al., 2013

2.4.2 BIOSYNTHESIS AND SECRETION OF INSULIN

The biosynthesis of insulin occurs exclusively in the β-cells of the pancreas, primarily located in the islets of Langerhans. The process begins with transcription of the insulin gene, producing mRNA, which is translated into the preproinsulin polypeptide on ribosomes bound to the rough endoplasmic reticulum (Akinloye et al., 2014). The preproinsulin undergoes enzymatic cleavage to form proinsulin, which is transported to the Golgi apparatus where it is packaged into secretory vesicles. Within these vesicles, proinsulin is cleaved into insulin and C-peptide by prohormone convertases (PC1 and PC2).

Insulin secretion is tightly regulated by blood glucose concentration. When plasma glucose levels rise, such as after a meal, glucose enters β-cells through the GLUT2 transporter and undergoes metabolism to generate ATP. The rise in the ATP/ADP ratio leads to closure of ATP-sensitive potassium (KATP) channels, causing membrane depolarization. This depolarization triggers voltage-dependent calcium channels (VDCCs) to open, allowing calcium influx, which in turn stimulates exocytosis of insulin-containing granules (Folorunso et al., 2022).

In diabetic conditions, such as those induced by STZ in experimental models, the β-cells undergo oxidative and nitrosative stress, leading to cellular damage and impaired insulin synthesis and release (De Campos et al., 2020). Streptozotocin selectively destroys pancreatic β-cells by entering via the GLUT2 transporter, leading to DNA fragmentation and depletion of NAD^+ and ATP. This results in hypoinsulinaemia and persistent hyperglycaemia (Inya-Agha, 2006).

Interestingly, extracts of Picralima nitida have shown potential to enhance insulin secretion and protect β-cells from oxidative damage. Teugwa et al. (2013) and Folorunso et al. (2022) both reported that aqueous and ethanolic extracts of P. nitida improved insulin levels and restored

pancreatic function in diabetic rats, suggesting a possible β-cell regeneration or antioxidant protective mechanism.

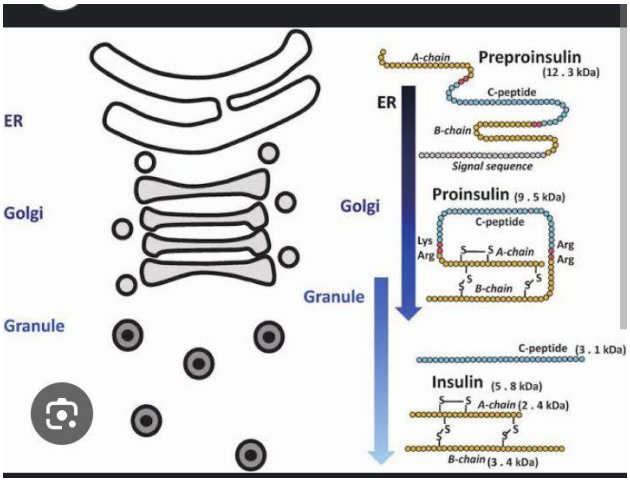

Figure 9: showing the synthesis of insulin

Source: Folorunsho et al., 2022

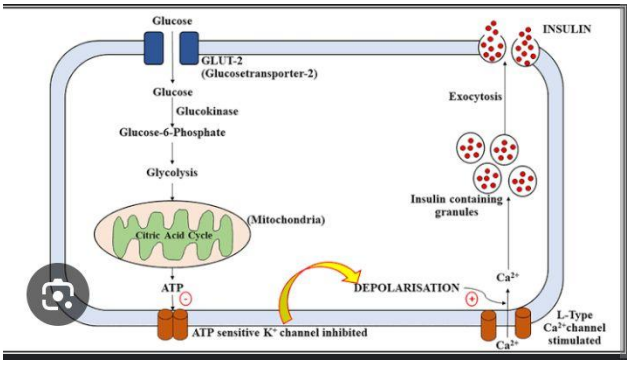

Figure 9: showing the process of insulin secretion

Source: Folorunsho et al., 2022

2.4.3 PHYSIOLOGICAL ROLE OF INSULIN IN GLUCOSE HOMEOSTASIS

Insulin plays a central role in maintaining glucose homeostasis by promoting the uptake, utilization, and storage of glucose, while inhibiting its production and release. After a meal, when blood glucose levels rise, insulin facilitates glucose uptake into muscle and adipose tissues via activation of GLUT4 transporters, thereby lowering plasma glucose levels (Teugwa et al., 2013).

In the liver, insulin promotes glycogenesis (the conversion of glucose to glycogen for storage) and inhibits gluconeogenesis and glycogenolysis. It also suppresses lipolysis in adipose tissue, preventing excessive release of free fatty acids into circulation (De Campos et al., 2020). In the skeletal muscles, insulin enhances both glucose uptake and amino acid transport, thereby promoting protein synthesis and inhibiting proteolysis (Akinloye et al., 2014).

The insulin signaling pathway involves binding of insulin to its receptor, a tyrosine kinase receptor, leading to autophosphorylation and activation of downstream signaling proteins such as insulin receptor substrate (IRS), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (Akt). These signals regulate glucose metabolism and gene expression associated with cellular growth and differentiation (Folorunso et al., 2022).

When insulin secretion is insufficient or when tissues become resistant to its action, glucose accumulates in the bloodstream, resulting in hyperglycaemia and metabolic imbalance. Chronic hyperglycaemia contributes to oxidative stress and inflammation, damaging pancreatic cells and other tissues (De Campos et al., 2020).

Experimental studies have shown that treatment with aqueous extract of Picralima nitida can significantly lower blood glucose levels and increase serum insulin concentration in diabetic rats.

These effects are attributed to the antioxidant and insulin-sensitizing properties of the plant's bioactive compounds, such as indole alkaloids and flavonoids (Teugwa et al., 2013; Folorunso et al., 2022). Thus, P. nitida may help restore glucose homeostasis by enhancing insulin secretion, improving receptor sensitivity, and protecting β-cells against oxidative injury.

2.4.4 INHIBITION OF INSULIN

The regulation of insulin involves both stimulatory and inhibitory pathways that maintain physiological glucose balance. The inhibition of insulin secretion or action is mediated by several hormones, enzymes, and inflammatory molecules, which operate through various biochemical pathways. This section reviews the molecules responsible for insulin inhibition and their mechanisms of action.

1. Somatostatin

Somatostatin is a peptide hormone secreted by the delta (δ) cells of the pancreas and hypothalamus. It acts as a potent inhibitor of insulin secretion by binding to G-protein-coupled somatostatin receptors (SSTR2 and SSTR5) on pancreatic β-cells. This interaction results in the inhibition of adenylyl cyclase activity, leading to a reduction in cyclic AMP (cAMP) levels, suppression of calcium influx, and inhibition of insulin granule exocytosis(Guyton and Hall, 2021).

2. Catecholamines (Epinephrine and Norepinephrine)

Catecholamines, including epinephrine and norepinephrine, are released in response to stress and exercise. They inhibit insulin secretion via activation of α2-adrenergic receptors on β-cells. This receptor engagement triggers Gαi-mediated suppression of adenylate cyclase activity, thereby

lowering intracellular cAMP and inhibiting insulin release (Guyton and Hall, 2021). Additionally, catecholamines promote hepatic glucose production, further counteracting insulin activity.

3. Glucagon

Although primarily involved in increasing blood glucose levels, glucagon also inhibits insulin secretion indirectly. Elevated glucagon stimulates somatostatin release and attenuates β-cell responsiveness to glucose through paracrine interactions within the islet microenvironment (Edgerton et al., 2009).

4. Growth Hormone (GH)

Growth hormone decreases insulin sensitivity, particularly in adipose tissue, by promoting lipolysis and increasing circulating free fatty acids. These fatty acids impair glucose uptake and utilization, leading to insulin resistance. GH also modulates insulin receptor substrate (IRS) signaling, contributing to decreased insulin effectiveness (Guyton and Hall, 2021).

5. Cortisol

Cortisol, a glucocorticoid hormone, is known for its inhibitory effects on insulin action. It enhances hepatic gluconeogenesis, reduces peripheral glucose uptake, and increases lipolysis, resulting in elevated blood glucose levels. Chronic cortisol elevation, as seen in prolonged stress or Cushing's syndrome, impairs insulin sensitivity and interferes with GLUT-4-mediated glucose transport (Guyton and Hall, 2021).

6. Tumor Necrosis Factor-alpha (TNF-α)

TNF-α is an inflammatory cytokine implicated in obesity-induced insulin resistance. It inhibits insulin signaling by promoting serine phosphorylation of insulin receptor substrate-1 (IRS-1),

thereby impairing downstream pathways necessary for glucose uptake into cells (Guyton and Hall, 2021). Elevated TNF-α levels are commonly observed in metabolic syndrome.

7. Free Fatty Acids (FFAs)

Free fatty acids, particularly in the context of obesity, contribute to insulin inhibition through lipotoxic effects. They induce oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress within β-cells and insulin-responsive tissues. This results in impaired insulin secretion and worsened insulin sensitivity (Guyton and Hall, 2021).

2.5 DIABETES MELLITUS

2.5.1 DEFINITION AND GENERAL OVERVIEW

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (Teugwa et al., 2013). It is one of the most common endocrine diseases worldwide, leading to disturbances in carbohydrate, lipid, and protein metabolism. The chronic elevation of blood glucose levels eventually results in long-term damage, dysfunction, and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels (De Campos et al., 2020).

Under normal physiological conditions, insulin regulates glucose homeostasis by facilitating glucose uptake into tissues and suppressing hepatic glucose production. In diabetic conditions, however, either the pancreas produces insufficient insulin (as in Type 1 diabetes) or the body's cells become resistant to insulin's effects (as in Type 2 diabetes) (Folorunso et al., 2022). The resultant metabolic imbalance leads to impaired energy utilization, oxidative stress, and inflammatory responses.

According to Inya-Agha (2006), diabetes mellitus is not only a biochemical disorder but also a multifactorial disease, influenced by genetic, environmental, and lifestyle factors. The condition has become a major global health concern due to its increasing prevalence and association with obesity, sedentary lifestyle, and aging populations.

2.5.2 ETIOLOGY AND PATHOPHYSIOLOGY

The etiology of diabetes mellitus is complex and involves the interplay of genetic predisposition and environmental influences. The two main pathological mechanisms underlying the disease are insulin deficiency and insulin resistance (Akinloye et al., 2014).

In Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), autoimmune destruction of pancreatic β-cells leads to absolute insulin deficiency. This type is commonly seen in younger individuals and is characterized by rapid onset and dependency on exogenous insulin for survival.

In Type 2 diabetes mellitus (T2DM), the predominant mechanism is insulin resistance, a condition in which target tissues such as skeletal muscles, adipose tissue, and the liver fail to respond appropriately to insulin (De Campos et al., 2020). Initially, the pancreas compensates by secreting more insulin, but over time, β-cell exhaustion occurs, resulting in relative insulin deficiency and persistent hyperglycaemia.

The pathophysiological features of diabetes also include increased oxidative stress and the generation of reactive oxygen species (ROS), which damage cellular macromolecules and contribute to β-cell dysfunction (Folorunso et al., 2022). In STZ-induced diabetes, streptozotocin causes selective necrosis of β-cells by entering through the GLUT2 glucose transporter, resulting in DNA fragmentation, depletion of NAD^+, and impaired ATP synthesis. This process mimics

the pathophysiological mechanism of human diabetes, making it a reliable experimental model (Inya-Agha, 2006).

Plants such as Picralima nitida have been shown to attenuate the pathophysiological effects of diabetes through antioxidant and insulin-sensitizing mechanisms (Teugwa et al., 2013). Their phytochemicals may scavenge free radicals, protect β-cells, and enhance insulin secretion, thereby reversing hyperglycaemia (De Campos et al., 2020).

2.5.3 CLASSIFICATION OF DIABETES MELLITUS

Diabetes Mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is classified into four major types based on etiological factors and clinical presentation, according to the World Health Organization (WHO, 2020) and the American Diabetes Association (ADA, 2022).

1. Type 1 Diabetes Mellitus (T1DM)

Type 1 Diabetes Mellitus is an autoimmune condition that leads to the destruction of insulin-producing β-cells in the pancreas. This results in an absolute deficiency of insulin. The disease typically presents in childhood or adolescence but can develop at any age. Both genetic predisposition and environmental triggers, such as viral infections, are implicated in its onset (Atkinson, Eisenbarth and Michels, 2014). Lifelong insulin replacement is required to manage this form of diabetes, and failure to do so may result in diabetic ketoacidosis.

2. Type 2 Diabetes Mellitus (T2DM)

Type 2 Diabetes Mellitus is the most prevalent form, accounting for approximately 90–95% of diabetes cases globally. It is characterized by insulin resistance and a progressive decline in

insulin secretion. Key risk factors include obesity, sedentary lifestyle, poor dietary habits, aging, and genetic susceptibility (DeFronzo et al., 2015). Unlike Type 1, Type 2 diabetes has a gradual onset and may remain undetected for several years. Early diagnosis and management through lifestyle modification and pharmacological interventions are crucial to reducing complications.

3 Gestational Diabetes Mellitus (GDM)

Gestational Diabetes Mellitus refers to glucose intolerance first recognized during pregnancy. It is associated with increased risks of adverse maternal and fetal outcomes, such as preeclampsia, fetal macrosomia, and the need for cesarean delivery. Women diagnosed with GDM also have a higher risk of developing Type 2 diabetes in the future (American College of Obstetricians and Gynecologists, 2021). Proper screening and management are essential to ensure favorable outcomes.

4. Other Specific Types of Diabetes

This category comprises less common forms of diabetes resulting from specific genetic defects, diseases of the exocrine pancreas, endocrine disorders, infections, autoimmunity, or drug-induced causes (WHO, 2020). Examples include:

Maturity-Onset Diabetes of the Young (MODY): A monogenic form caused by mutations affecting insulin production.

Pancreatogenic (Type 3c) Diabetes: Secondary to disorders like pancreatitis or pancreatic neoplasms.

Drug or Chemical-Induced Diabetes: Associated with chronic use of glucocorticoids, thiazides, or certain antipsychotics.

These forms require tailored diagnostic and therapeutic approaches based on the underlying cause (ADA, 2022).

2.5.4. BIOCHEMICAL ALTERATIONS IN DIABETES

Diabetes mellitus leads to widespread biochemical alterations due to insulin deficiency and hyperglycaemia. The major biochemical changes include disturbances in carbohydrate, lipid, and protein metabolism, as well as oxidative imbalance and inflammatory responses (Folorunso et al., 2022).

1. Carbohydrate Metabolism:

Lack of insulin impairs glucose uptake by tissues and enhances hepatic gluconeogenesis, leading to elevated blood glucose levels. Persistent hyperglycaemia results in glycosylation of proteins and formation of advanced glycation end-products (AGEs), which contribute to vascular and tissue damage (De Campos et al., 2020).

2. Lipid Metabolism:

Insulin normally inhibits lipolysis; thus, insulin deficiency leads to increased breakdown of triglycerides in adipose tissue, releasing free fatty acids into circulation. These are converted to ketone bodies in the liver, leading to diabetic ketoacidosis in severe cases (Teugwa et al., 2013).

3. Protein Metabolism:

In diabetes, the body enters a catabolic state with increased proteolysis and reduced protein synthesis. Muscle wasting and loss of body weight are common consequences of prolonged insulin deficiency (Akinloye et al., 2014).

4. Oxidative Stress and Antioxidant Imbalance:

Chronic hyperglycaemia induces oxidative stress by generating reactive oxygen species (ROS) and reducing antioxidant enzyme activity. This imbalance damages β-cells and worsens insulin resistance (Folorunso et al., 2022). Antioxidant-rich plants like Picralima nitida have been shown to counteract oxidative stress, restore antioxidant enzyme activities, and improve glycaemic control in diabetic rats (De Campos et al., 2020; Teugwa et al., 2013).

2.5.5 EXPERIMENTAL INDUCTION OF DIABETES USING STREPTOZOTOCIN (STZ)

Overview of Streptozotocin

Streptozotocin (STZ) is one of the most commonly used agents for the experimental induction of diabetes mellitus in laboratory animals, particularly rodents. It was first isolated in 1956 from the soil bacterium Streptomyces achromogenes and initially identified for its antibiotic properties against certain bacterial infections (Teugwa et al., 2013). However, later studies revealed that STZ possessed selective toxicity to the insulin-producing β-cells of the pancreatic islets of Langerhans, which led to its application in experimental diabetes research (Folorunso et al., 2022).

Chemically, STZ is a naturally occurring nitrosourea derivative of glucose, with the molecular formula $C_8H_{15}N_3O_7$ (Lenzen, 2008). It appears as a pale yellow crystalline powder, highly soluble in water but unstable in neutral or alkaline solutions, hence it is usually prepared freshly in a cold citrate buffer (pH 4.5) before administration to maintain potency and reduce degradation (Rees and Alcolado, 2005). The compound's glucose moiety enables it to selectively

enter β-cells through the low-affinity glucose transporter 2 (GLUT2), which is abundantly expressed on pancreatic β-cells (Ghasemi et al., 2023).

The mechanism of action of STZ primarily involves the induction of DNA alkylation and fragmentation within β-cells. Once inside the cell, STZ causes DNA damage by transferring its methyl group to DNA bases, which activates poly (ADP-ribose) polymerase (PARP), an enzyme that attempts to repair DNA breaks but depletes the cell's NAD^+ and ATP reserves in the process. The resulting oxidative stress, energy failure, and nitric oxide (NO) generation ultimately lead to β-cell necrosis (Lenzen, 2008). This destruction of β-cells mimics the insulin-deficient state characteristic of type 1 diabetes mellitus (T1DM).

STZ is generally administered via intraperitoneal (i.p.) injection or intravenous (i.v.) injection, depending on the experimental design (Deeds et al., 2011). A single high dose (typically 50–70 mg/kg body weight) induces severe hyperglycaemia within 48–72 hours, while multiple low doses can be used to mimic gradual autoimmune destruction of β-cells. The choice of dosage and route affects the onset and severity of diabetes, with intraperitoneal injection being most common due to ease of administration and consistent results (Deeds, 2011).

The results of STZ administration include a marked rise in fasting blood glucose levels, polyuria, polydipsia, and significant reduction in serum insulin concentration (Teugwa et al., 2013). Histopathological examination of the pancreas typically reveals degeneration and necrosis of islet β-cells, confirming diabetes induction (Lenzen, 2008).

STZ is preferred over alloxan as a diabetogenic agent due to its higher reproducibility, greater β-cell selectivity, and less susceptibility to spontaneous remission of hyperglycaemia (Ghasemi et al., 2023). Alloxan's mechanism of β-cell damage depends largely on reactive oxygen species

generation, which may vary between individual animals and species, leading to inconsistent results (Lenzen, 2008). Moreover, STZ has a well-characterized dose-response relationship, allowing better control of diabetes severity and mortality rate in experimental models (Ghasemi et al., 2023).

Therefore, streptozotocin remains the gold standard for chemically inducing diabetes in laboratory animals, providing a reliable and reproducible model for investigating antidiabetic agents such as the aqueous extract of Picralima nitida fruit.

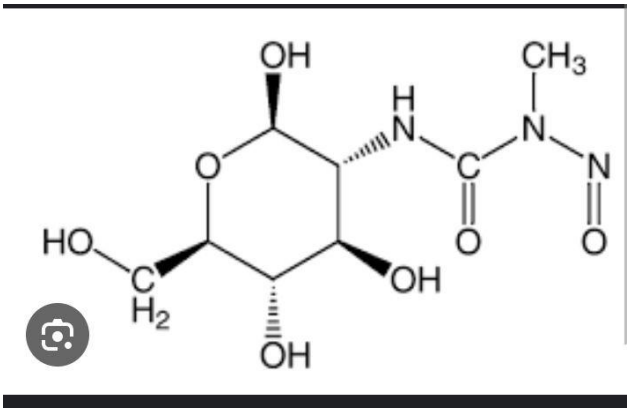

Figure 10: showing the chemical structure of Streptozotocin

Source Deeds et al., 2011

Induction of Diabetes Using Streptozotocin

Streptozotocin (STZ) is a naturally occurring nitrosourea compound that is widely used for the experimental induction of diabetes mellitus in laboratory animals, particularly rats and mice. It selectively destroys pancreatic β-cells due to its affinity for the GLUT2 transporter, which facilitates its entry into β-cells (Inya-Agha, 2006).

Once inside the cells, STZ causes DNA alkylation, fragmentation, and activation of poly-ADP-ribose polymerase (PARP), leading to depletion of cellular NAD^+ and ATP. The resulting oxidative stress and necrosis of β-cells lead to insulin deficiency and hyperglycaemia, mimicking human Type 1 diabetes (De Campos et al., 2020).

The induction of diabetes using STZ is dose-dependent. Typically, a single intraperitoneal injection of STZ at doses ranging from 40–60 mg/kg body weight is sufficient to induce stable hyperglycaemia in rats (Teugwa et al., 2013). After induction, blood glucose levels are monitored to confirm diabetic status before commencing treatment with plant extracts or standard drugs like Daonil (glibenclamide).

Studies by Teugwa et al. (2013) and Folorunso et al. (2022) demonstrated that administration of aqueous or ethanolic extracts of Picralima nitida significantly reduced blood glucose levels and increased serum insulin in STZ-induced diabetic rats, suggesting a β-cell protective and insulin-stimulating effect. Similarly, De Campos et al. (2020) reported that P. nitida seed extract prevented hyperglycaemia and oxidative stress in high-fat/high-fructose-fed rats, confirming its potential as a natural antidiabetic agent.

2.6 ANTIDIABETIC AGENTS AND STANDARD TREATMENT

2.6.1 OVERVIEW OF ANTIDIABETIC AGENTS

Antidiabetic therapy refers to all forms of treatment aimed at maintaining normal blood glucose levels and preventing the long-term complications of diabetes mellitus. These include pharmacological agents, dietary management, exercise, and use of medicinal plants with hypoglycaemic activity (Akinloye et al., 2014).

Pharmacological treatment involves the use of oral hypoglycaemic agents (OHAs) and insulin therapy, depending on the type and severity of diabetes. Oral agents are commonly used in Type 2 diabetes mellitus (T2DM) to stimulate insulin secretion, enhance insulin sensitivity, or inhibit glucose absorption, while insulin therapy remains essential for Type 1 diabetes (Teugwa et al., 2013).

In developing countries, high cost, limited availability, and side effects of synthetic drugs have encouraged the use of medicinal plants such as Picralima nitida as alternative or complementary therapies for diabetes (Inya-Agha, 2006). These plants often contain bioactive compounds with antioxidant, insulinotropic, and β-cell protective properties that can help restore glucose homeostasis.

2.6.2 CLASSIFICATION OF ANTIDIABETIC AGENTS

Antidiabetic (or antihyperglycemic) agents can broadly be classified based on how they lower blood glucose, their site of action, and/or their therapeutic role. According to pharmacology and clinical-diabetes review sources, common classes include: insulin secretagogues, insulin sensitizers, alpha-glucosidase inhibitors, sodium-glucose cotransporter- 2(SGLT2) and dipeptidyl peptidase-4 (DPP-4) (De Campos et al., 2022, Folorunso et al., 2022)

1. Insulin Secretagogues

Insulin secretagogues are a class of oral antidiabetic drugs that stimulate the pancreas to release more insulin. They act by binding to sulfonylurea receptors (SUR) on pancreatic β-cells, leading to the closure of ATP-sensitive potassium channels. This causes depolarization of the cell membrane, allowing calcium influx, which triggers insulin secretion (De Campos et al., 2022)

Examples:

Sulfonylureas: glibenclamide, glipizide

Meglitinides: repaglinide, nateglinide

These agents are effective in type 2 diabetes patients with residual pancreatic function but have a risk of hypoglycemia and weight gain (Folorunsho et al., 2022).

2. Insulin Sensitizers

Insulin sensitizers improve the body's response to insulin without increasing insulin secretion. They primarily act on peripheral tissues to enhance glucose uptake or decrease hepatic glucose production. The two main subclasses are biguanides and thiazolidinediones.

i. Biguanides (e.g., metformin) reduce hepatic gluconeogenesis and increase insulin sensitivity in muscle cells.

ii. Thiazolidinediones (e.g., pioglitazone) act on peroxisome proliferator-activated receptor gamma (PPAR-γ) to regulate gene transcription involved in glucose and lipid metabolism (De Campos et al., 2022).

These agents have minimal risk of hypoglycemia and are foundational in type 2 diabetes management (Folorunsho et al., 2022).

3. Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors delay carbohydrate digestion and absorption in the small intestine. They competitively inhibit the alpha-glucosidase enzyme in the brush border of the intestine, reducing postprandial blood glucose spikes (De Campos et al., 2022).

Examples:

Acarbose

Miglitol

Since their action is localized in the gut, their systemic effects are minimal, but they may cause gastrointestinal side effects such as flatulence and diarrhea (Folorunsho et al., 2022).

4. Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors

SGLT2 inhibitors are a newer class of oral antidiabetic agents that lower blood glucose by preventing its reabsorption in the kidney. They act by inhibiting the SGLT2 protein in the proximal renal tubules, promoting glucosuria and reducing plasma glucose levels (De Campos et al., 2022).

Examples:

Canagliflozin

Empagliflozin

Dapagliflozin

Beyond glucose control, this class offers cardiovascular and renal benefits, making it useful in patients with comorbidities (Folorunsho et al., 2022).

5. Dipeptidyl Peptidase-4 (DPP-4) Inhibitors

DPP-4 inhibitors enhance the incretin system by blocking the enzyme dipeptidyl peptidase-4, which degrades incretin hormones like GLP-1 (glucagon-like peptide-1). This increases the

levels of active incretins, stimulating glucose-dependent insulin release and suppressing glucagon secretion (De Campos et al., 2022).

Examples:

Sitagliptin

Vildagliptin

Linagliptin

These drugs are weight neutral and carry a lower risk of hypoglycemia, making them well-tolerated in many patients (Folorunsho et al., 2022).

Each drug class targets specific pathways involved in glucose regulation. However, sulfonylureas such as Daonil (glibenclamide) remain the most widely used oral agents due to their effectiveness in lowering blood glucose and improving insulin release (Akinloye et al., 2014).

2.6.3 GLIBENCLAMIDE - A STANDARD ANTIDIABETIC TREATMENT

Discovery and Development of Glibenclamide

Glibenclamide, a second-generation sulfonylurea, was introduced in the early 1970s as an oral hypoglycaemic agent to treat non-insulin-dependent diabetes mellitus (type 2 diabetes) (Ghasemi et al., 2023). Over the decades, its use in both clinical and experimental settings has become well-established, and it now serves as a benchmark drug in many rodent studies of antidiabetic extracts (Silva Frederico et al., 2015).

Chemical Nature and Form of Glibenclamide

Chemically, glibenclamide is a sulfonylurea derivative characterised by the molecular formula $C_{23}H_{28}ClN_3O_5S$. It is delivered in crystalline powder form which is formulated into oral tablets—commonly 5 mg tablets under the brand name "Glanil" or generic equivalents (Teugwa et al., 2013). In research literature, the drug is administered orally in rodent studies, matching its human formulation.

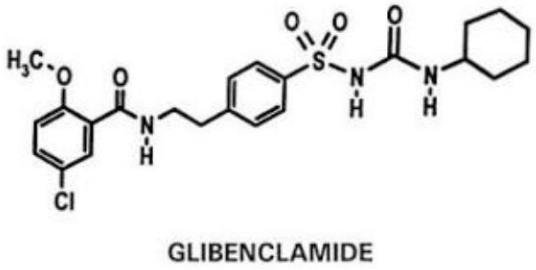

Figure 11: showing the chemical structure of Glibenclamide

Source: Akinloye et al., 2014

Mechanism of Action of Glibenclamide

The hypoglycaemic effect of glibenclamide is primarily mediated through pancreatic β-cells: it binds to the sulfonylurea receptor 1 (SUR1) component of ATP-sensitive potassium (K_ATP) channels on the β-cell membrane, causing closure of these channels, membrane depolarisation, opening of voltage-gated calcium channels, calcium influx, and resultant exocytosis of insulin-containing granules (Silva Frederico et al., 2015). Additionally, extra-pancreatic effects have

been reported, including improved peripheral glucose uptake and insulin sensitivity in muscle and liver cell (Silva Frederico et al., 2015).

Results of Action

Clinically and experimentally, glibenclamide induces a significant increase in plasma insulin concentration and a lowering of blood glucose levels in diabetic models that retain residual β-cell function (Silva Frederico et al., 2015). In experimental studies, glibenclamide is widely used as the standard drug against which plant extracts (e.g., extracts of Picralima nitida) are compared to assess relative hypoglycaemic and insulin-modulating efficacy (Silva Frederico et al., 2015). The reproducibility of its action and well-understood mechanism make it a reliable comparator in animal diabetes research.

Glanil As A Standard Drug

Glanil, the trade name for glibenclamide, is a second-generation sulfonylurea that has been extensively used as a standard antidiabetic agent in both clinical and experimental settings. It serves as the reference drug in studies evaluating the antidiabetic potential of plant extracts such as Picralima nitida (Teugwa et al., 2013; Inya-Agha, 2006).

Glibenclamide exerts its hypoglycaemic effect by stimulating insulin secretion from pancreatic β-cells. It binds to sulfonylurea receptors (SUR1) located on the ATP-sensitive potassium (KATP) channels of β-cell membranes. This binding closes the KATP channels, causing membrane depolarization, opening of voltage-gated calcium channels, and subsequent influx of

calcium ions. The increased intracellular calcium triggers exocytosis of insulin-containing granules, resulting in elevated plasma insulin levels (Akinloye et al., 2014).

Apart from enhancing insulin release, glibenclamide also increases peripheral glucose utilization and improves hepatic glycogen synthesis (Folorunso et al., 2022). Its glucose-lowering effect, however, depends on the presence of functional β-cells, and prolonged use can cause β-cell exhaustion or hypoglycaemia.

Experimental Relevance:

In research involving STZ-induced diabetic rats, Glanil is used as the standard control drug to evaluate the efficacy of natural extracts (Teugwa et al., 2013). Since STZ partially destroys β-cells, glibenclamide's response in these models indicates the level of surviving β-cell function. Comparison with Picralima nitida extract helps determine whether the extract exerts a β-cell protective or regenerative effect similar to or greater than that of glibenclamide (De Campos et al., 2020).

2.6 4 LIMITATIONS AND SIDE EFFECTS OF CONVENTIONAL ANTIDIABETIC AGENTS

Despite their efficacy, synthetic antidiabetic agents such as sulfonylureas and biguanides are associated with several adverse effects and limitations. Glibenclamide, for example, can induce severe hypoglycaemia, weight gain, gastrointestinal disturbances, and, with chronic use, pancreatic β-cell depletion (Akinloye et al., 2014).

Additionally, long-term therapy often results in drug resistance, requiring higher doses or drug combinations to maintain glycaemic control. Metformin, though safer, may cause lactic acidosis

in patients with renal impairment, while thiazolidinediones can lead to fluid retention and liver toxicity (De Campos et al., 2020).

In contrast, medicinal plants are considered safer and more holistic in managing diabetes, often acting through multiple biochemical pathways. Extracts from Picralima nitida, for instance, exhibit potent antioxidant, hypoglycaemic, and insulinotropic effects with minimal toxicity (Teugwa et al., 2013; Folorunso et al., 2022). These plant-based treatments have gained attention as adjuncts or alternatives to conventional therapy, especially in regions with limited access to synthetic drugs.

2.6.5 COMPARATIVE MECHANISM: GLIBENCLAMIDE VS PICRALIMA NITIDA EXTRACTS

While both Glibenclamide and Picralima nitida extract lower blood glucose, their mechanisms of action differ in scope and biochemical targets.

Glibenclamide primarily acts on pancreatic β-cells to stimulate insulin secretion via KATP channel modulation (Akinloye et al., 2014).

Picralima nitida Extract, on the other hand, exerts a multifaceted mechanism, including:

a). β-cell protection and regeneration, thereby preserving endogenous insulin synthesis.

b). Antioxidant activity, which neutralizes reactive oxygen species (ROS) produced during hyperglycaemia (Teugwa et al., 2013).

c). Enhancement of insulin sensitivity in peripheral tissues, improving glucose uptake and utilization (Folorunso et al., 2022).

d). Inhibition of carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, thereby reducing postprandial hyperglycaemia (De Campos et al., 2020).

According to Inya-Agha (2006), aqueous extracts of P. nitida fruit contain alkaloids, flavonoids, and saponins that act synergistically to restore metabolic balance and normalize insulin function. Compared to glibenclamide the extract provides additional antioxidant and anti-inflammatory benefits, making it a potentially safer and more sustainable therapeutic option for diabetes management.

2.6.6 RATIONALE FOR USING GLIBENCLAMIDE AS STANDARD DRUGS

Glibenclamide is widely accepted as a standard reference drug in experimental studies on diabetes due to its reliable hypoglycaemic action, well-understood mechanism, and comparability with plant-based extracts (Teugwa et al., 2013). Its inclusion allows researchers to evaluate the relative efficacy and safety of natural compounds such as Picralima nitida under controlled conditions.

Moreover, Glanil's ability to stimulate insulin secretion provides a clear benchmark for assessing whether the test extract enhances insulin release, protects β-cells, or improves glucose metabolism via non-pancreatic mechanisms (De Campos et al., 2020). This comparison helps in identifying potential therapeutic pathways and justifying the integration of traditional remedies into modern antidiabetic therapy.

2.7 EXPERIMENTAL ANIMAL MODEL

Rodent models are widely used to evaluate antidiabetic agents and to probe insulin physiology. Male Wistar rats are a common choice owing to their stable baseline metabolic profiles, well-characterized physiology, and availability. Chemical induction of diabetes in rodents is usually

performed with alloxan or streptozotocin (STZ) to create hypoinsulinaemic states resembling Type 1 diabetes, or with lower-dose STZ combined with dietary manipulation (high-fat/high-fructose) or nicotinamide to model insulin resistance and partial β-cell dysfunction resembling Type 2 diabetes (Ghasemi et al., 2023; Qamar et al., 2023).

Each model has strengths and limitations: STZ and alloxan produce reproducible β-cell injury but differ mechanistically (alkylation vs. redox stress), while diet-induced and genetic models better capture insulin resistance but are slower and more resource-intensive (Singh et al., 2024). Selection of an appropriate model should match the experimental question, example, whether the extract's effect on insulin secretion (β-cell function) or on insulin sensitivity (peripheral action) is the principal concern.

2.7.1 ETHICAL CONSIDERATIONS IN ANIMAL EXPERIMENTATION

Animal experimentation is an essential component of biomedical research, particularly in studying physiological and pathological mechanisms relevant to human health. However, the use of animals in research raises significant ethical concerns, necessitating the application of strict ethical guidelines to ensure humane treatment. When using male Wistar rats in scientific studies, as in this project, adherence to ethical principles is critical to maintain scientific integrity and public trust.

1. The 3Rs Principle

One of the foundational frameworks guiding ethical animal use is the 3Rs principle—Replacement, Reduction, and Refinement. This concept was first articulated by Russell and Burch in 1959 and remains a cornerstone of animal research ethics (Russell and Burch, 1959).

Replacement involves using alternative methods (e.g., in vitro studies, computational models) to avoid or replace the use of animals where possible.

Reduction seeks to minimize the number of animals used without compromising statistical validity. This includes appropriate experimental design and statistical planning.

Refinement refers to modifying procedures to minimize pain, suffering, and distress, and to enhance animal welfare.

In the context of Wistar rat experiments, these principles guide researchers to use the smallest effective number of animals, employ humane endpoints, and ensure continuous monitoring (Festing and Wilkinson, 2007).

2. Legal and Institutional Oversight

Most countries have stringent regulations governing the use of animals in research. In Nigeria and many other countries, ethical approval from an Institutional Animal Care and Use Committee (IACUC) or a similar ethics committee is mandatory prior to the commencement of experiments (Lawrence, 2010). These bodies ensure studies comply with local and international guidelines, including the EU Directive 2010/63/EU and the U.S. Public Health Service Policy.

3. Humane Housing and Care

Animal welfare extends beyond the conduct of experiments to include proper housing, feeding, environmental enrichment, and veterinary care. The Guide for the Care and Use of Laboratory Animals emphasizes the need for species-specific housing conditions that promote physical and psychological well-being (National Research Council, 2011). For male Wistar rats, this includes

adequate space, social grouping when appropriate, and appropriate temperature and lighting cycles.

4. Minimization of Pain and Distress

An essential ethical consideration is the prevention and alleviation of pain and distress. This requires the use of appropriate anesthetic, analgesic, and euthanasia methods. Pain must be recognized and managed according to standardized protocols (Hawkins et al., 2011). Additionally, humane endpoints should be established—criteria by which animals are removed from a study if they experience undue suffering.

5 Scientific Justification

Ethical research mandates that the use of animals must be scientifically justified. Researchers must demonstrate that animal models, such as Wistar rats, are necessary for the intended research and that there are no viable alternatives. The research must have the potential to advance knowledge or lead to meaningful health benefits (Garner, 2014).

6. Reporting and Transparency

Finally, ethical reporting involves transparent documentation of methods, animal handling, and outcomes. Compliance with reporting guidelines such as ARRIVE (Animal Research: Reporting of In Vivo Experiments) helps ensure reproducibility and ethical accountability (Kilkenny et al., 2010).

2.8 SUMMARY OF LITERATURE AND RESEARCH GAPS

Numerous studies have explored the biological and pharmacological activities of Picralima nitida, a plant traditionally used in African herbal medicine for treating diabetes, malaria, fever, and gastrointestinal disorders (Teugwa et al., 2013; Akinloye et al., 2014). Phytochemical analyses have confirmed the presence of alkaloids such as akuammicine, akuammine, and picraline, which contribute to its antioxidant, antimicrobial, and hypoglycaemic effects (Folorunso et al., 2022). These bioactive compounds have been reported to modulate oxidative stress and possibly enhance pancreatic β-cell function, supporting its traditional use in diabetes management (De Campos et al., 2020).

Experimental evidence indicates that Picralima nitida extracts exhibit antidiabetic potential through mechanisms involving reduction of blood glucose levels and protection of pancreatic tissues. Teugwa et al. (2013) demonstrated that methanolic and aqueous extracts of P. nitida significantly reduced blood glucose and improved serum insulin in streptozotocin-induced diabetic rats. Similarly, Folorunso et al. (2022) reported that ethanolic extract of P. nitida fruit enhanced antioxidant enzyme activity and preserved pancreatic histo-architecture, suggesting a protective effect against oxidative β-cell damage. These studies imply that the plant may influence insulin secretion or insulin sensitivity.

On the other hand, the mechanisms by which P. nitida exerts its insulin-modulating effects remain incompletely understood. Most earlier studies have primarily focused on blood glucose and lipid profiles, with limited attention to direct quantification of insulin hormone and pancreatic histology (Akinloye et al., 2014). Moreover, variations in extraction solvent, dosage, and duration of treatment across studies have produced inconsistent results, making it difficult to draw definitive conclusions on its efficacy and mechanism of action (De Campos et al., 2020).

Animal models of diabetes, particularly those induced by streptozotocin (STZ) provide a reproducible platform for evaluating such plant extracts. STZ selectively destroys pancreatic β-cells, causing insulin deficiency and hyperglycaemia (Lenzen, 2008). However, only few published reports have investigated P. nitida extract specifically in STZ-induced diabetic male Wistar rats and evaluated its direct impact on serum insulin concentration using enzyme-linked immunosorbent assay (ELISA) methods.

Furthermore, while glibenclamide (Daonil) remains a standard reference drug for evaluating oral antidiabetic agents (Silva Frederico et al., 2015), comparative studies between Daonil and P. nitida fruit extract on insulin levels and pancreatic function are scarce. The majority of available literature assesses glucose reduction rather than insulin regulation or pancreatic repair, leaving an important research gap in understanding whether P. nitida enhances insulin synthesis or simply improves peripheral glucose uptake.

Therefore, a critical need exists for detailed studies investigating the effect of aqueous extract of Picralima nitida fruit on insulin hormone concentration and pancreatic histology in experimentally diabetic male Wistar rats, using Daonil as standard treatment. Such studies will not only validate the ethnopharmacological claims about P. nitida but also clarify its mechanism of action whether through β-cell regeneration, insulin secretion stimulation, or antioxidative protection. This research aims to fill that gap by providing biochemical and histological evidence for the plant's potential therapeutic effect on insulin regulation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment and Apparatus

The following materials and equipment were used during the study:

Glassware and Consumables:

Cotton wool

Chloroform

Dissecting set

Methylated Spirit

Distilled water

Plain, EDTA and lithium heparin sample containers

Syringes (5 mL and 10 mL)

Insulin syringe (1mL)

16–18 gauge stainless-steel gavage needle

Nose mask

Lancets

Hand gloves

3.1.2 Machines and Instruments:

1. Glucometer (NEWSPRING Glucose Meter, China; Model KF-B12)
2. 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 pH meter, China; Model PHS-25)

3.2. RESEARCH DESIGN

The purpose of this biomedical experimental investigation was to examine the effects of Picralima nitida fruit aqueous extract on insulin hormone levels in streptozotocin- induced diabetic male Wistar rats. The study used twenty-four(24) male Wistar rats that were physiologically normal weighing between 107.6g and 169.3g that were obtained from the Department of Anatomy, University of Benin, Nigeria The animals were kept in regular laboratory conditions at the Department of Anatomy, University of Benin, Nigeria, in clean, well-ventilated plastic cages with soft wood shavings(saw-dust) for bedding. Before the experiment started, the rats were acclimated to a controlled environment for four(4) weeks, which included a temperature of 22 ± 2°C, a relative humidity of 50–60%, and a 12-hour light/dark cycle.

The remaining rats were provided water ad libitum (freely) and conventional rat feed(Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude

protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus, during this acclimation period. The Departmental Animal Ethics Committee granted ethical approval for the study, and all methods complied with worldwide guidelines for the humane treatment of laboratory animals. After acclimation, the twenty four (24) rats were divided into five (5) groups of five (5) rats each except for Group 0 that has only four (4) rats based on their initial body weight ranges: 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 5 (148.3g – 154.5g). To facilitate easy identification, the rats were individually labelled using coloured markers (Genital Violet) on specific body parts, including the hand, leg, back, head, and tail while some were plain (unmarked). Each rat was assigned a unique identification number based on the part of the body where the colour was applied.

During the experiment, the rats were closely monitored for any sign of distress, behavioural changes, or alterations in their physical conditions. Their weights were recorded weekly to observe any significant changes that might indicate the effects of experimental procedures. Also, their fasting blood glucose was recorded weekly (between the hours of 7:30am and 8;30am). The rats were kept under optimal care and all handling procedures were designed to minimize stress and maintain their well being.

At the end of the experimental period (10 days), the rats were sacrificed humanely using chloroform anaesthesia, following ethical guidelines for animal research. Blood samples were collected via cardiac puncture where necessary in labeled sample containers (lithium heparin, plain) and kept in a refrigerator to preserve their integrity until analysis.

This study was conducted following the ethical guidelines outlined by the relevant ethics board of University of Benin. Throughout the study, efforts were made to ensure the humane treatment of the animals and to adhere strictly to ethical practices in biomedical research.

3.3 PLANT MATERIALS

3.3.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Fresh fruits of Picralima nitida (about 300 in numbers, weighing between 0.2kg-0.3kg of which many were unripe and few ripe ones) were purchased from Oliha market, along Siloko Road, Benin City, Edo state, Nigeria. The plant material was authenticated and identified by a botanist in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, where a voucher specimen was deposited for reference. The Voucher Number given was UBH-P424.

3.3.2 PREPARATION OF PICRALIMA NITIDA EXTRACT

The fruits were initially sorted out in order to remove soil, stones, insects, rotten or mouldy ones and only fresh, healthy, clean, and mature fruits were used. The fresh fruits were washed thoroughly with clean water to remove dirt and debris. The rind (outer part) was manually peeled off, and the seeds and the pulp were cut into smaller sizes and divided into six(6) batches for easy drying Thereafter, they were air-dried under shade for 7–10 days to prevent photo-degradation of bioactive compounds. The dried material was further oven-dried at 45°C for about four(4) weeks to ensure complete moisture removal. The oven-dried material was weighed to be 2,202.19g and then ground into fine powder using an electric grinder (Dry Herb Industrial Grinder, Model SYB-18B). The grinding process was thorough to ensure that a smooth fine powder was obtained, which would facilitate efficient extraction of the bioactive compounds.

The powdered material was weighed, and 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 with intermittent stirring to facilitate extraction of the bioactive compounds. The mixture was filtered using a muslin cloth, separating the filtrate (solvent + bioactive compounds) away from the moist grounded matter. The filtrate was freeze-dried using a Freeze Dryer (BIOBASE Freeze Dryer, China; Model BK- FD10S) to obtain a solid residue. This process involves freezing and sublimation of the water content under low temperature and pressure resulting in a stable dried extract. The resulting dried extract was weighed to determine the percentage yield and stored in an airtight container at 40°C until use. At the end of the freeze drying process, a total yield of 1,271.98g (57.78%) of the extract was obtained.

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.

The preparation method ensured that the extract retained its bioactive properties while providing a safe and effective preparation for experimental use.

3.4 EXPERIMENTAL ANIMAL DESIGN

The study used twenty four (24) male Wistar rats that were physiologically normal weighing between 107.6g and 169.3g that were obtained from the Department of Anatomy, University of Benin, Nigeria The animals were kept in regular laboratory conditions at the Department of Anatomy, University of Benin, Nigeria, in clean, well-ventilated plastic cages with soft wood

shavings(saw-dust) for bedding. Before the experiment started, the rats were acclimated to a controlled environment for four(4) weeks, which included a temperature of 22 ± 2°C, a relative humidity of 50–60%, and a 12-hour light/dark cycle. One out of the thirty-five rats died during acclimatisation process possibly because of stress from transportation to new environment or inability to adapt to new environment, hence, the number used.

The remaining rats were provided water ad libitum (freely) and conventional rat feed(Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus, during this acclimation period. The Departmental Animal Ethics Committee granted ethical approval for the study, and all methods complied with worldwide guidelines for the humane treatment of laboratory animals.

3.4.1 EXPERIMENTAL GROUPING

Twenty four (24) rats were divided into five (5) groups of five (5) rats each except for Group 0 that has only four (4) rats, shown as follows:

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

Group 4: Diabetic rats treated with Picralima nitida extract (500 mg/kg).

The grouping was based on the mean body weight of the animals, which ranged between 107.69 g and 183.59 g. To facilitate easy identification, the rats were individually labelled using coloured markers (Genital Violet) on specific body parts, including the hand, leg, back, head,

and tail while some were plain (unmarked). Each rat was assigned a unique identification number based on the part of the body where the colour was applied.

After diabetes was confirmed, the rats were regrouped as stated above but only two(2) rats in each group except for the positive control having four(4) rats.

3.5 INDUCTION OF DIABETES USING STREPTOZOTOCIN (STZ)

i) Preparation of Streptozotocin Solution

Streptozotocin (STZ) was used to induce diabetes mellitus in experimental rats. The streptozotocin was freshly 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. The preparation was done under low light conditions to prevent degradation, and the resulting solution was stored in a refrigerator until use.

ii)Dosage Calculation for 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 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 mg ×154 g/1000 g

= 9.24 mg

Hence, 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

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

iii) Diabetes was induced in overnight-fasted rats using a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body weight, freshly dissolved in 0.1 M citrate buffer (pH 4.5). 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 post-induction using a glucometer. Rats with fasting blood glucose levels above 200mg/dL were considered diabetic. However, after six(6) days awaiting the others to be induced in order to have a larger number of rats induced with diabetes, all their fasting blood glucose dropped possibly due to reduced food intake due to sickness/anorexia resulting in hypoglycemia. The rats that were remaining were re-induced as four died after the first inducing due to hypoglycemia.

iii)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 to 0.1L(100mL).

pH and Weight Calculations

The pH was calculated using the Handerson–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

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. The prepared buffer was stored under refrigeration to prevent degradation over time.

iv)Re-inducing of Diabetes using Streptozotocin (STZ)

The streptozotocin was freshly prepared by dissolving 0.20g of streptozotocin 0.05M citrate buffer (pH 4.5). The buffer solution was mixed thoroughly until the streptozotocin completely dissolved. The preparation was done under low light conditions to prevent degradation, and the resulting solution was stored in a refrigerator until use.

v)Dosage Calculation for Streptozotocin

The dosage of Streptozotocin used for re-induction was 40 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

Hence, 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

Each rat's dose and volume was determined individually using the same formula to ensure accuracy. The calculated volume of streptozotocin solution was then administered

intraperitoneally to each rat. Diabetes was induced in overnight-fasted rats using a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 40 mg/kg body weight, freshly dissolved in 0.05M citrate buffer (pH 4.5). 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 post-induction using a glucometer. Rats with fasting blood glucose levels above 110 mg/dL were considered diabetic.

vi)Preparation of Citrate Buffer

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).

pH and Weight Calculations

The pH was calculated using the Handerson–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

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 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. The prepared buffer was stored under refrigeration to prevent degradation over time.

vii)Post-Induction Care

After the administration of Streptozotocin, the rats were given 0.4% glucose solution orally for 24 hours to prevent hypoglycemic shock. Subsequently, the rats were maintained on a standard diet and water ad libitum.

viii)Confirmation of Diabetes

Seventy-two (72) hours after STZ injection, fasting blood glucose levels were determined using a glucometer. Rats showing fasting blood glucose levels above 110mg/dL were considered diabetic and selected for further studies.

3.6 ADMINISTRATION OF PICRALIMA NITIDA

The aqueous extract of Picralima nitida fruit (pulp and seeds) as previously described was administered to the experimental groups (G4-G5) . Group 0 (G0) served as the control (negative control) which received only water and feed alongside citrate buffer based on the volume given to rats in other groups with similar weights, providing a baseline for comparison to determine the effects of the extract and Group 2 (G2) which served as positive control received glibenclamide, a standard antidiabetic medicine in order to compare the extract's activity with the standard antidiabetic medicine.

Weights were used to allocate the rats into five (5)groups of five(5) rats each. The oral gavage technique (Turner et al., 2011; Diehl et al. 2001) was used to introduce the aqueous extract of Picralima nitida into the rats.. Rats in the various Groups that were confirmed diabetes were isolated from those that were non- diabetic and regrouped randomly into five (5) experimental groups of two(2) rats each, except the positive control group, having four(4) rats. Diabetic rats in G4 and G5 received the extract via oral gavage in addition to feed and water in doses of 200mg/kg and 500mg/kg respectively. Throughout the treatment period no signs of poisoning and animal deaths were observed.

3.6.1 DOSAGE CALCULATION OF PICRALIMA NITIDA

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

For a standard dose of 200mg/kg;

200mg of extract is required for 1kg body weight of rat

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

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

$$1000 \text{ g}$$

$$= 27.32 \text{ mg}$$

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.

This method was used for all the rats in G4 and G5 that was confirmed diabetes, ensuring that each rat received the appropriate extract dosage according to their body weight. 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.

3.7 ADMINISTRATION OF GLIBENCLAMIDE (GLANIL)

Glibenclamide (glanil) was used as the standard oral antidiabetic drug for comparison with rat groups given the aqueous Picralima nitida extract. The drug was obtained from RX Pharmacy, Ekosodin, Benin City, Edo state Nigeria.

The drug was administered to only Group 2 (G2) as per the experimental design. The dose was determined according to the animal body weight by U. S. Food and Drug Administration (FDA, 2005). Based on this conversion, glibenclamide was administered at a dose of 5mg/kg body weight (Diehl et al., 2001; Turner et al., 2011). The required dose was prepared freshly by dissolving the appropriate amount of the drug tablet (5mg) powder in distilled water to ensure uniform dispersion. The solution was gently stirred to obtain homogeneity before administration.

Administration was performed by oral gavage using a calibrated syringe and stainless-steel gavage needle. The drug was given once daily at the same time each morning, to minimize diurnal variation in glucose metabolism. The total volume administered did not exceed 5mg/kg body weight to prevent gastric discomfort.

Throughout the treatment period, animals had free access to standard laboratory chow and water. The negative control and positive control (Diabetic Group) were monitored daily for changes , food and water intake activity level and clinical signs of stress or intolerance.

3.7.1 MONITORING OF BLOOD GLUCOSE AND GLIBENCLAMIDE RESPONSE

Blood glucose levels were measured weekly from tail-vein samples using a glucometer to monitor the response to glibenclamide treatment. The glibenclamide-treated diabetic group

served as the positive control, providing a reference for comparing the hypoglycaemic and biochemical effects of Picralima nitida extracts.

All procedures were conducted according to institutional ethical guidelines for animal care and the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 2011).

3.7.2 DOSAGE CALCULATION FOR GLIBENCLAMIDE

The dosage of glibenclamide for the rats in Group 2 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.

The required amount of glibenclamide was dissolved or suspended in a small amount of distilled water to achieve a uniform dose before administration.

Similarly, the dosage calculation was done for all the rats in Group 2 (G2).

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. Blood glucose measurements were taken after an overnight fast using a glucometer (Newspring Glucose Meter, Model KF-B12, China). Blood samples were obtained from the tail tip using a sterile lancet, and the corresponding glucose values were recorded.

Body weights were measured using a high-precision weighing scale (NEWSPRING Digital Scale, China; Model NS- 790).

Note: The results presented below include the baseline weight and weight before induction of each rat, as well as the baseline fasting blood glucose levels and the fasting blood glucose before and after induction.

Table 3.1 showing Rat Groups, Weights and Fasting Blood Glucose Levels

Group	Label	Treatment (Dose)	Rat ID	Baseline FBG (mg/dl)	Baseline weight (g)	FBG before induction	Weight before induction	FBG 4 days after induction

G0	Normal control	Distilled H2O	C1 C2 C3 C4	81 79 68 77	169.3 163.5 155.0 167.7	83 81 79 90	197.5 176.0 189.4 192.0	70 67 72 59
G1	Diabetic control	Streptozotocin (60mg/kg)	S1 S2 S3 S4 S5	52 68 56 88 70	143.2 145.6 144.0 148.2 144.9	76 83 86 94 95	169.1 160.8 182.1 178.2 154.7	72 77 47 125 34
G2	Glibenclamide	Glibenclamide (5mg/kg)	M1 M2 M3 M4 M5	76 76 90 76 68	123.5 124.1 126.6 127.5 128.7	104 81 88 99 83	136.1 158.8 139.4 145.6 157.3	232 144 88 133 83
G3	P. Nitida low	200 mg/kg	D1 D2 D3 D4 D5	72 70 58 85 72	138.7 140.5 140.7 141.8 142.2	74 104 72 77 79	142.6 152.6 156.2 161.0 161.1	52 135 43 279 130
G4	P. Nitida high	500mg/kg	H1 H2 H3 H4 H5	77 72 65 50 85	151.8 154.3 152.1 154.5 148.3	90 97 76 67 94	175.6 166.0 166.2 182.4 149.4	194 250 263 90 238

3. 9 SAMPLE COLLECTION

Good hygiene was maintained in the animal house through regular cleaning of cages and replacement of sawdust bedding. The health and general well-being of the rats were closely monitored, and food and water were provided daily.

On the tenth (10th) day, the rats were fasted overnight, re-weighed, and subsequently sacrificed. Anesthesia was induced using chloroform to minimize pain and distress. The animals were euthanized in a chloroform chamber, after which a lateral incision was made in the abdominal cavity. Blood samples were then collected by cardiac puncture and dispensed into plain sample bottles.

The blood samples were stored at 4°C until analysis. Prior to biochemical evaluation, the samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The resulting serum was used to determine levels of insulin hormone using standard biochemical procedures.

3.10. BIOCHEMICAL ASSAY

The estimation of insulin in streptozotocin (STZ)-induced diabetic male Wistar rats primarily uses the Enzyme-Linked Immunosorbent Assay (ELISA) kit, which operates on the principle of competitive binding or sandwich assay. The procedure involves inducing diabetes, collecting blood samples, preparing serum, running the assay, and calculating concentrations using a standard curve.

Principle

The principle of the ELISA for insulin quantification is typically based on a competitive binding method.

Wells in a microplate are pre-coated with secondary antibodies (e.g., anti-insulin antibodies).

During the assay, a known amount of enzyme-labeled insulin (conjugate) and the unknown concentration of insulin in the rat serum samples (or standards) compete for binding sites on the antibodies.

The more insulin present in the sample, the less labeled conjugate will bind to the well.

After washing away unbound components, a substrate is added, producing a color change. The intensity of the color produced is inversely proportional to the concentration of insulin in the original sample.

An alternative is the "sandwich" ELISA, where the color is directly proportional to the insulin concentration. The specific kit's instructions must be followed.

Method and Procedure

The general procedure involves the following steps:

1. Diabetes Induction: Male Wistar rats receive a single high dose of STZ (e.g., 50-60 mg/kg body weight) dissolved in cold 0.1 M citrate buffer (pH 4.5) via intraperitoneal (i.p.) or intravenous injection.

2. Confirmation of Diabetes: After 48-72 hours, measure fasting blood glucose levels using a glucometer from a tail vein blood sample. Rats with levels exceeding 200 mg/dL (or 11 mmol/L) are considered diabetic and selected for the study.

3. Blood Sample Collection: At the end of the study period, after an overnight fast (typically 12 hours with free access to water), rats are humanely euthanized (e.g., under anesthesia followed by cervical dislocation). Blood is collected via cardiac puncture or retro-orbital venous plexus.

4. Serum/Plasma Separation:

Allow the blood to clot for 30 minutes at room temperature (if using serum tubes).

Centrifuge the blood samples at approximately 1500–3000 rpm for 10–15 minutes.

Collect the supernatant (serum or plasma) into labeled Eppendorf tubes.

Store the samples at -20°C (or -80°C for long-term storage) until analysis

5. ELISA Assay Performance:

Bring all reagents and samples to room temperature before use.

Add standards of known insulin concentration and the collected serum/plasma samples to the appropriate wells of the ELISA plate, following the manufacturer's specific instructions (e.g., from Crystal Chem or Mercodia).

Add necessary antibodies, conjugates, and wash the plate multiple times between incubation steps.

Add substrate solutions and then a stop solution to halt the reaction.

Measure the optical density (OD) or absorbance at the recommended wavelength (typically 450 nm) using a microplate reader within 10 minutes of adding the stop solution.

Calculation

Obtain Mean Absorbance: Calculate the average absorbance values for each standard and sample.

Generate Standard Curve: Plot the mean absorbance values (Y-axis) against the corresponding known insulin concentrations of the standards (X-axis).

This can be done manually on semi-log graph paper or using specialized software.

A four-parameter logistic (4PL) or a log-logit curve fit is typically recommended for accurate results.

Determine Sample Concentration: Use the standard curve to interpolate the concentration of insulin in each unknown sample based on its measured absorbance value. If the samples were diluted before the assay, multiply the obtained concentration by the dilution factor to get the final concentration in the original serum/plasma.

CHAPTER FOUR

RESULTS

4.1 Effects of Aqueous Extract of Picralima nitida Fruit on Serum Insulin Levels in Streptozotocin-Induced Diabetic Male Wistar Rats

Serum insulin concentrations (uU/mL) were determined using ELISA to assess the effect of the aqueous fruit extract of Picralima nitida on pancreatic β-cell function in STZ-induced diabetic male Wistar rats.

Group	Rat ID	Insulin Levels uU/ml
Normal Control	**C3**	**12**
	C4	**17.8**
Diabetic control	**A2**	**28.8**
	S4	**15.9**
Glibenclamide treated	**D1**	**8.7**
	D2	**11.9**
Low Dose	**M1**	**10.5**
	M4	**12.7**
High Dose	**A1**	**19.6**
	A4	**28.9**

4.2 Descriptive Statistics

Table 4.1 below shows the mean serum insulin values and standard error of mean (SEM) for each experimental group.

Table 4.1: Serum Insulin Levels (Mean ± SEM) N=2

Group	Mean	SEM
Normal control	**15.25**	**15.25 ± 2.55**
Diabetic control	**22.25**	**22.25 6± .35#**
Glibenclamide treated	**10.30**	**10.30 ± 1.60**
Low Dose	**11.60**	**11.60 ± 1.10**
High Dose	**24.25**	**24.25 ± 4.65**

4.3 Comparative Analysis Between Experimental Groups

The normal control rats showed a basal insulin concentration of 15.25 ± 2.55 uU/mL.

Induction of diabetes with streptozotocin resulted in an elevation of serum insulin (22.25 ± 6.35 uU/mL), reflecting β-cell dysfunction and an initial compensatory response.

Administration of glibenclamide produced a reduction in insulin levels (10.30 ± 1.60 uU/mL) compared to both the normal and diabetic control groups.

Low-dose extract treatment showed moderate insulin levels (11.60 ± 1.10 uU/mL), similar to the glibenclamide group.

High-dose extract produced the highest insulin concentration among all groups (24.25 ± 4.65 uU/mL), indicating a potential stimulatory effect of Picralima nitida extract on insulin secretion.

4.4 Graphical Representation

The mean serum insulin levels are graphically presented in Figure 4.1.

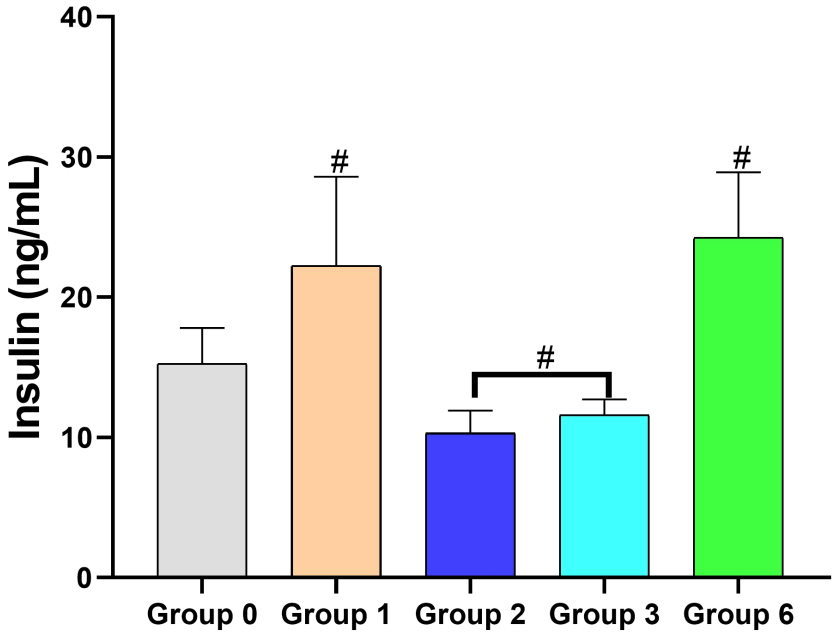

Figure 4.1: Effect of *Picralima nitida* on insulin concentrations of Sprague Dawley rats. Values are expressed as mean ± SEM. # represent statistical significant at P < 0.05 when compared to group 0.

The result shows that group 1 and 6 significantly increased insulin levels when compared to group 0. However, there was a significant reduction in insulin levels in group 2 and 3 when compared to group 0.

4.5 Biological Activity

Insulin levels increased after STZ administration, confirming successful induction of diabetes.

Glibenclamide and low-dose extract groups showed reduced insulin levels relative to diabetic control.

The high-dose extract produced the highest insulin concentration among all experimental groups.

Although differences among groups were not statistically significant ($p > 0.05$), the biological trend suggests a dose-dependent effect of Picralima nitida extract on insulin secretion.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigated the effect of aqueous fruit extract of Picralima nitida on serum insulin concentration in streptozotocin-induced diabetic male Wistar rats. Streptozotocin (STZ) is a well-known diabetogenic agent that selectively destroys pancreatic β-cells, leading to impaired insulin synthesis and secretion. In this study, ELISA analysis revealed varying responses in insulin levels following extract administration, and these findings provide insight into the potential insulin-modulating properties of P. nitida.

The normal control group exhibited a baseline serum insulin concentration of 15.25 ± 2.55 uU/mL, representing typical physiological insulin levels in healthy rats. In contrast, the diabetic control group recorded a higher insulin concentration (22.25 ± 6.35 uU/mL). Although STZ generally causes β-cell destruction leading to decreased insulin, some animals experience an early compensatory hyperinsulinemia due to partial β-cell injury or insulin resistance in peripheral tissues. The observed rise in insulin levels in the diabetic control rats may therefore indicate the presence of residual β-cell function responding to STZ-induced metabolic stress.

Treatment with glibenclamide resulted in a decreased insulin concentration (10.30 ± 1.60 uU/mL) relative to the diabetic control group. Glibenclamide is a sulfonylurea drug known to enhance insulin secretion by closing ATP-sensitive potassium channels in β-cells. The lower insulin values observed in this study could be attributed to the extent of β-cell destruction by STZ, which may have limited the capacity for glibenclamide-induced insulin release. This finding

suggests that at the administered dose, glibenclamide may not fully restore β-cell stimulation in severely diabetic conditions.

Administration of the low-dose extract of P. nitida produced an insulin concentration of 11.60 ± 1.10 uU/mL, which was comparable to that of the glibenclamide group. This suggests that lower concentrations of the extract may exert mild stimulatory or protective effects on β-cells, although not strong enough to significantly elevate circulating insulin levels.

Interestingly, the high-dose extract group exhibited the highest insulin concentration (24.25 ± 4.65 uU/mL), surpassing even the diabetic control group. This observation suggests a possible dose-dependent effect, where higher concentrations of P. nitida fruit extract may stimulate surviving β-cells or enhance insulin secretion through antioxidant, anti-inflammatory, or membrane-stabilizing mechanisms. The phytochemical constituents of P. nitida including alkaloids, flavonoids, tannins, and saponins are known to possess hypoglycemic and cytoprotective properties, which may contribute to enhanced β-cell function or reduced oxidative stress.

Overall, the results indicate a promising dose-dependent improvement in insulin secretion with high-dose Picralima nitida extract, suggesting that the fruit may have beneficial effects on β-cell activity in diabetes.

5.2 Conclusion

This study investigated the effects of aqueous fruit extract of Picralima nitida on serum insulin levels in streptozotocin-induced diabetic male Wistar rats. The findings demonstrated that:

STZ induction disrupted insulin homeostasis, as reflected by elevated insulin levels in diabetic control rats.

Glibenclamide and low-dose extract showed modest effects on insulin levels, likely due to partial β-cell destruction.

High-dose P. nitida extract produced the greatest increase in serum insulin concentration, suggesting a dose-dependent stimulatory or protective effect on pancreatic β-cells.

In conclusion, aqueous extract of Picralima nitida fruit may possess insulin-modulating activity, especially at higher doses. Further studies involving larger sample sizes, histological assessment of pancreatic tissue, and evaluation of blood glucose levels are recommended to fully establish its antidiabetic potential and mechanism of action.

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APPENDICES

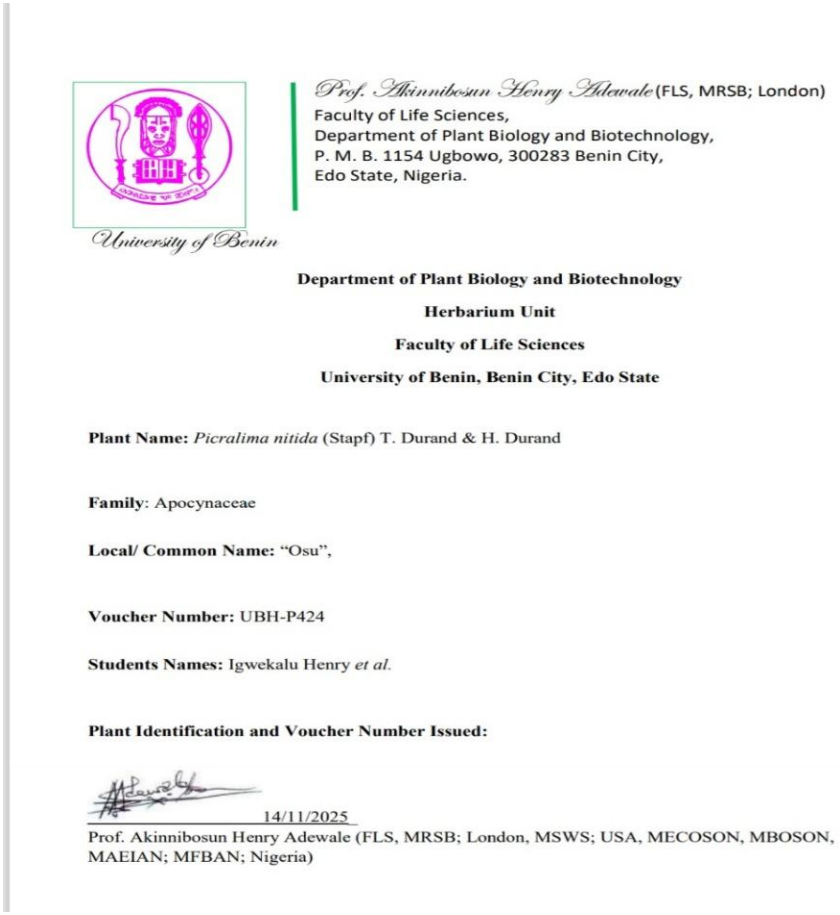
Appendix I: Grouping and Identification of Rats

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
G2	5	143.2 – 148.2	M1- Plain
			M2- Tail
			M3- Two Legs
			M4- Head
			M5- Leg
G3	5	129.0 – 136.9	L1- Two legs
			L2- Tail & Back
			L3- Head & Tail
			L4- Two legs & Back
			L5- Back
G4	5	138.7 – 142.2	D1- Legs & Back
			D2- Tail
			D3- Leg
			D4- Hand

			D5- Plain
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 and Biotechnology



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P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *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)

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 fruit.

Chopped Picralima nitida nitida

Droed Picralima fruits

Grounded Picralima nitida

Extraction of Picralima nitida

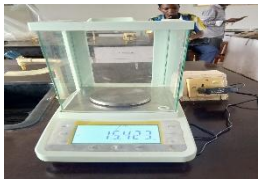
Extract of Picralima nitida

Freeze-dried Picralima.

Freeze-drying machine

Oven

nitida



Weighing Scale

Figure IV b: Acclimation of rats

Figure IV c: **Table A IV c Showing Effect of *Picralima nitida* on insulin concentrations in STZ induced diabetic Male Wistar rats.**

Parameters (ng/mL)	Group 0	Group 1	Group 2	Group 3	Group 6
Insulin	15.25 ± 2.55	22.25 ± 6.35#	10.3 ± 1.6#	11.6 ± 1.1#	24.25 ± 4.65#

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. **Centrifuged Blood samples**

Blood samples

Figure IV f: Streptozotocin obtained from Bridge Biotech Limited

Place bought **Producer.** **Streptozotocin powder**