INVESTIGATING THE EFFECT OF AQUEOUS PICRALIMA NITIDA FRUIT EXTRACT ON CORTISOL LEVEL IN OXIDATIVE STRESS-AFFLICTED STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR RATS

BY

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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

This is to certify that this project work was carried out by **IGWEKALU HENRY UGOCHUKWU w**ith matriculation number **BMS2101412**, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

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DEDICATION

This research is dedicated to almighty God, for his grace and guidance; to my parents, friends, colleagues and loved ones who have encouraged my endeavours throughout this process, my supervisor who has pushed me to put my best effort into this work and especially to the healthcare professionals and patients who battle conditions involving hyperplasia.

ACKNOWLEDGEMENTS

I express my profound gratitude to God Almighty for His unending love, grace, guidance, and strength throughout the course of this research and my academic pursuit.

I extend my sincere thanks to my project supervisor, Dr. E.S. Oghagbon, for his guidance, constructive criticism, and invaluable support which greatly contributed to the successful completion of this work. My deep appreciation also goes to the Head of Department, Dr. Bobby N. Aguebor-Ogie, for his leadership, encouragement, and academic support. I remain grateful to all my lecturers in the Department of Medical Biochemistry: Prof. (Mrs.) H. A. Oboh, Dr. J. C. Anionye, Dr. Bobby, Mr. Aisosa Eguavoen, Dr. (Mrs.) Eluehike, Dr. (Mrs.) Ikponmwonsa-Eweka, as well as all other lecturers, laboratory technologists, and non-academic staff for their dedication, mentorship, and assistance throughout my academic journey.

My heartfelt appreciation goes to my beloved parents, Mr. And Mrs. Igwekalu, whose prayers, love, and unwavering support have been the backbone of my success. I am equally grateful to my wonderful siblings for their encouragement, care, and continuous belief in me.

My appreciation also goes to my friends for their companionship, motivation, and unwavering support during the course of this work.

To everyone who contributed in one way or another to the success of this research, I express my sincere and heartfelt gratitude.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Diabetes mellitus is a metabolic disorder marked by persistent hyperglycemia, which drives excessive production of reactive oxygen species (ROS) and leads to oxidative stress. A critical yet often overlooked aspect of diabetes pathophysiology is its reciprocal relationship with physiological stress. Oxidative stress can impair the function of the Hypothalamic–Pituitary–Adrenal (HPA) axis, causing an increase in cortisol secretion. Elevated cortisol, in turn, aggravates hyperglycemia and intensifies oxidative damage, creating a vicious cycle that accelerates the onset and progression of diabetic complications. *Picralima nitida*, a medicinal plant traditionally used in diabetes treatment, is known for its antidiabetic and antioxidant properties. However, its potential role in modulating cortisol imbalances associated with diabetes has not been previously examined. This study therefore investigated the protective effects of an aqueous fruit extract of *Picralima nitida* (APN) on hyperglycemia, oxidative stress, and serum cortisol levels in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in adult male Wistar rats using STZ (55 mg/kg), after which the animals received varying doses of APN for a specified treatment period. A group treated with glibenclamide served as the standard drug control. Fasting blood glucose levels were monitored throughout the study, and serum cortisol concentrations were measured using a competitive ELISA technique. The findings revealed that STZ-induced diabetic rats experienced a significant rise in cortisol levels (18.5 ± 0.1 µg/ml) compared with the non-diabetic control group (13.35 ± 2.45 µg/ml), confirming that oxidative stress triggers heightened HPA axis activity. Treatment with APN produced a pronounced reduction in cortisol levels, with the low-dose and high-dose groups showing values of 10.3 ± 1.4 µg/ml and 10.15 ± 1.45 µg/ml respectively. These reductions were statistically significant when compared with both the control and diabetic groups ($P < 0.05$). In addition, APN administration led to marked improvements in fasting blood glucose and oxidative stress markers. In summary, the study demonstrates that *Picralima nitida* effectively lowers blood glucose, mitigates oxidative stress, and corrects cortisol imbalances in diabetic rats. These results underscore its potential therapeutic value in addressing both metabolic disturbances and stress-related abnormalities associated with diabetes mellitus.

CHAPTER ONE

1.1 Background of the study

Diabetes mellitus is a chronic metabolic disorder characterized by chronic hyperglycemia and alterations in carbohydrate, protein, and lipid metabolism (American Diabetes Association, 2013). Its global prevalence is rising at an alarming rate, making it a major public health concern. The streptozotocin (STZ)-induced diabetic model in rats is a well-established method for studying diabetes and its complications. STZ acts as a pancreatic beta-cell toxin, inducing hyperglycemia and triggering a cascade of metabolic disturbances (Gerber and Rutter, 2017).

A key pathophysiological feature of diabetes is oxidative stress, a state defined by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses (Newsholme *et al.,* 2016). Chronic hyperglycemia drives the overproduction of ROS, leading to cellular damage, insulin resistance, and the progression of diabetic complications (Newsholme et al., 2016). Furthermore, a complex bidirectional relationship exists between diabetes and stress. Oxidative stress itself can dysregulate the Hypothalamic-Pituitary-Adrenal (HPA) axis, potentially leading to elevated levels of the stress hormone cortisol. In turn, cortisol can exacerbate hyperglycemia and oxidative stress, creating a vicious cycle that worsens the diabetic condition.

In the search for new therapeutic agents, medicinal plants have gained significant attention. *Picralima nitida* (family Apocynaceae) is a plant used in traditional African medicine for the treatment of diabetes, malaria, and other ailments (Ogbeide, 2025). Scientific studies have begun to validate its ethnomedicinal use. Extracts from different parts of the plant, including the seeds and leaves, have demonstrated antidiabetic and antioxidant properties in animal models (Folorunsho, 2022). For instance, aqueous seed extract of *Picralima nitida* (APN) has been shown to alleviate hyperglycemia, dyslipidemia, and oxidative stress in high-fat high-fructose-fed rats. However, the specific impact of the fruit extract on the cortisol response within the context of STZ-induced diabetes and oxidative stress remains to be elucidated. This research seeks to fill that gap, exploring a potential novel mechanism through which *Picralima nitida* may exert its therapeutic effects.

1.2 Aim of Research

The aim of this research is to investigate the therapeutic potential of the aqueous fruit extract of *Picralima nitida* in ameliorating diabetes and its associated complications, with a specific focus on its effects on cortisol levels and oxidative stress in STZ-induced diabetic male Wistar rats.

1.3 Scope of the Study

This study will be an experimental laboratory-based investigation. The scope is limited to:

1. Subject: The use of adult male Wistar rats.
2. Disease Model: Induction of diabetes using a single intraperitoneal injection of Streptozotocin (STZ).
3. Intervention: Administration of aqueous fruit extract of *Picralima nitida* at varying doses.
4. Parameters: The analysis will be confined to assessing blood glucose, serum cortisol levels.

1.4 Objective of the Study

The specific objectives of this study are:

1. To induce a stable and validated diabetic state in male Wistar rats using Streptozotocin.
2. To prepare the aqueous extract from the fruits of *Picralima nitida*.
3. To evaluate the antidiabetic effect of the extract by measuring its ability to lower fasting blood glucose levels in the diabetic rats.
4. To determine the effect of the aqueous fruit extract of *Picralima nitida* on serum cortisol levels in the STZ-induced diabetic rats.
5. To correlate the changes in cortisol levels with the improvements in hyperglycemia and oxidative stress.

1.5 Significance of the Study

This research is significant for several reasons:

- Scientific Validation: It will provide scientific data to validate or refute the traditional use of *Picralima nitida* fruit for managing diabetes, contributing to the standardization of herbal medicine.
- Novel Insight: It explores a less-studied mechanism of action, modulation of the stress hormone cortisol, which could provide a novel explanation for the plant's antidiabetic and antioxidant properties.
- Potential for Drug Discovery: The findings could identify the aqueous fruit extract of *Picralima nitida* as a promising source for developing new, multi-target therapeutic agents for diabetes that address both hyperglycemia and the associated oxidative stress and hormonal dysregulation.
- Public Health Contribution: Given the rising global burden of diabetes and the need for affordable and accessible treatments, this study contributes to the exploration of locally available natural resources as potential adjuncts or alternatives to conventional therapies.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

In many African countries, up to 80% of the population relies on herbal medicines for primary healthcare, particularly for women's health issues (e.g., infertility, pregnancy) and common ailments like diarrhoea. Medicinal plants are deeply embedded in cultural practices and provide livelihoods for local communities in developing countries through harvesting and selling and are often perceived as safer and more natural alternatives to synthetic drugs, driving their demand. However, only a fraction of medicinal plants have been rigorously tested for efficacy and safety. For example, many herbs used in traditional medicine lack clinical trial data leading to a lack of scientific standardisation. *Picralima nitida* is one of the many herbs garnering attention for it's proven antioxidant and antimicrobial properties (Adeola *et al.,* 2024). This chapter reviews the effects of *Picralima nitida* on cortisol level in a biological system.

2.2 PICRALIMA NITIDA: BOTANICAL AND PHYTOCHEMICAL OVERVIEW

Botanical Description

Picralima nitida is a medicinal plant from the Apocynaceae (Dogbane) family growing as a medium-sized to large tree with a relatively smooth pale grey bark that exudes a milky latex. It's leaves are large oblong shaped, it's fruit large and globose with it's skin green which turns an orange-brown upon maturity. Each fruit contains numerous seeds and are extremely bitter. Their native range include the Tropical West and Central Africa and can be commonly found in Nigeria, Ghana and Cameroon (Akabassi *et al.,* 2023). They're commonly known in English as the African Peach, in Yoruba as "Erin" and Igbo as "Osu".

Fig 1.0: Unripe picralima nitida fruit

Source: Akabassi et al., 2023

2.2.1 Pharmacological Effects

Modern scientific studies have confirmed multiple pharmacological properties, primarily attributed to it's indole alkaloids (e.g., akuammine, akuammicine, pseudo-akuammigine) (Creed *et al.,* 2020). Some of the effects include:

i. **Analgesic and Opiodergic Activity:** Alkaloids like akuammine and pseudo-akuammigine act as μ-opioid receptor agonists while akuammicine is a potential κ-opioid receptor agonist (Creed *et al.,* 2020).

ii. **Anti-inflammatory and Antioxidant Effects:** Ethanolic seed extracts inhibited NF-κB and MAPK signaling in human neuronal cells, reducing inflammation (Olajide *et al.,* 2014) while the aqueous seed extract reduced lipid peroxidation (malondialdehyde levels) and enhanced antioxidant capacity in high-fat diet-induced oxidative stress in rats (Folorunsho *et al.,* 2022).

iii. **Antidiabetic and Metabolic Regulation:** Akuammicine stimulates glucose uptake in adipocytes. Aqueous seed extract reduced hyperglycemia, insulin resistance and dyslipidemia in high-fat high-fructose fed rats by upregulating glucokinase and CPT-1 expression. Aqueous seed extract also increased high density lipoprotein (HDL) cholesterol and decreased triglycerides, low density lipoprotein (LDL) and atherogenic indices (De Campos *et al.,* 2020).

iv. **Antimicrobial Activity:** Picralima nitida ethanolic seed extract inhibited the growth of Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi and Streptococcus pneumoniae (Dapaah *et al.,* 2017).

v. **Antitussive and Expectorant Effects:** Picralima nitida ethanolic extract reduced tracheal phenol red secretion (muco-supressant) and stabilized mast cells, explaining its traditional use for cough (Dapaah *et al.,* 2017).

vi. **Antidepressant and Anxiolytic Effects:** Total crude alkaloid fraction reduced immobility time in forced swim and tail suspension tests in mice indicating antidepressant-like effects and also increased open-arm entries in elevated plus maze tests, suggesting anxiolytic activity mediated via noradrenergic pathways *(Okyere et al., 2025).*

vii. **Haematological Effects:** Stem bark extract increased red blood cell count, hemoglobin and hematocrit, suggesting potential for treating anemia. It also elevated white blood cell counts, indicating immune-stimulatory effects (Ogbeide, 2025).

Phytochemicals Composition

Picralima nitida is characterised by a rich and diverse profile of bioactive compounds, predominantly indole alkaloids such as Akuammine, Akuammicine, Akuammidine and other phytochemical classes present in the plant including Saponins, Flavonoids, Tannins, Terpenoids (Creed *et al.,* 2020). The synergistic action of these compounds underpins the plant's wide range of traditional uses.

2.2.2 Alkaloids

The major bioactive compound present in *picralima nitida* are the alkaloids. They're responsible for most of the plant's medicinal properties. They include:

i. **Akuammine**: Acts as a μ-opioid receptor agonist with analgesic properties. Also exhibits antimalarial activity (Creed *et al.,* 2020).
ii. **Akuammidine**: Also a μ-opioid receptor agonist with demonstrated antinociceptive effects in animal models. Also shows antiplasmodial properties (Creed *et al.,* 2020).
iii. **Pseudo-akuammigine:** Binds to μ-opioid receptors and has shown anti-inflammatory and analgesic action in rat models (Creed *et al.,* 2020).
iv. **Akuammicine**: A potential κ-opioid receptor agonist. Also stimulates glucose uptake in adipocytes, indicating potential antidiabetic effects (Hennessy *et al.,* 2024).
v. **Akuammiline**: Limited direct opioid efficacy reported but often isolated alongside other bioactive Alkaloids. Structural studies suggest potential role in overall extract activity (Creed *et al.,* 2020).
vi. **Picraline**: Evaluated for opioid receptor activity but shows low efficacy in bioassays leaving its pharmacological profile less defined (Creed *et al.,* 2020).
vii. **2-Hydroxyakuammiline**: A newly identified alkaloid with ongoing research. Preliminary studies focus on isolation and structure characterization rather than confirmed efficacy (Laryea *et al.,* 2024).
viii. **Alstonine**: Shows significant antiplasmodial effects indicating potential for malaria treatment (Laryea *et al.,* 2024).

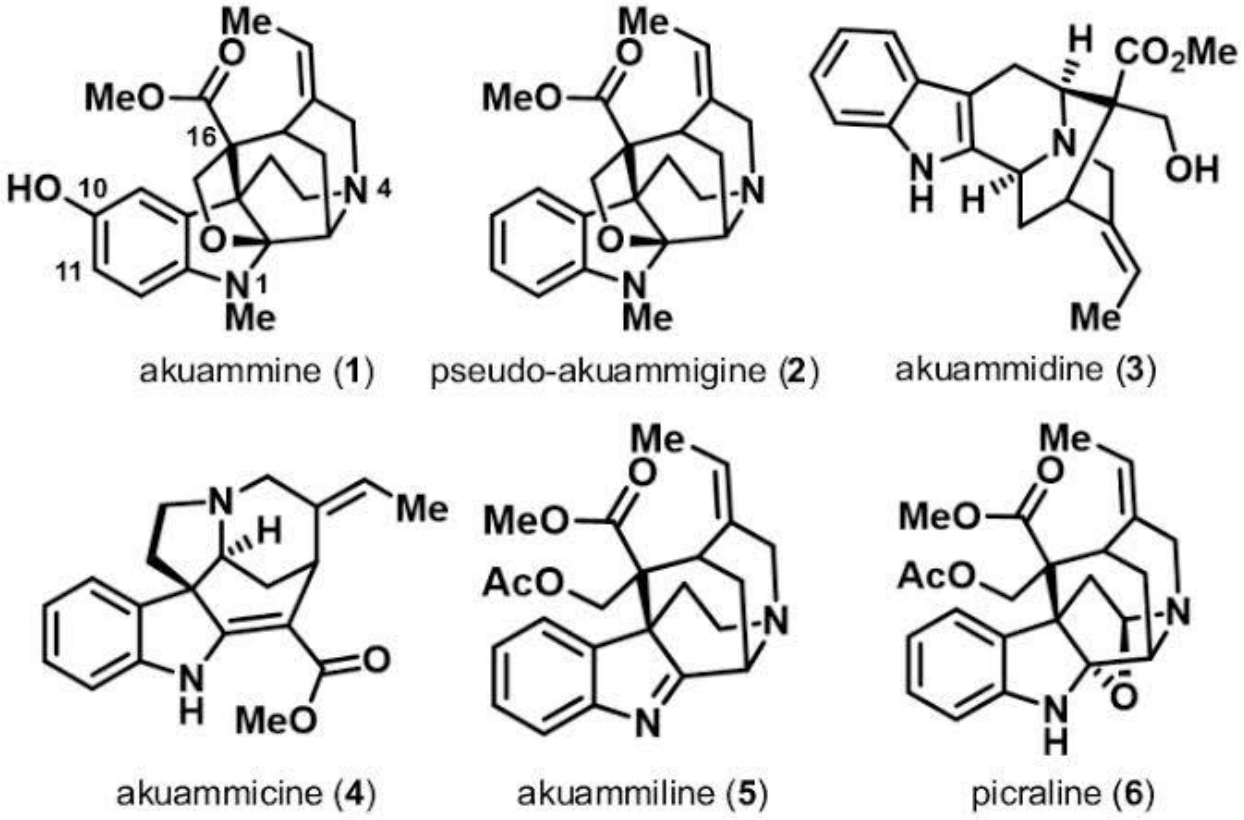


Fig 1.1: Major alkaloids present in Akuamma

Source: Creed *et al.,* 2020

Other phytochemical classes contribute to the pharmacological properties of *Picralima nitida* including Flavonoids which exhibit antioxidant activity, phenolic compounds which enhance antioxidant and anti-inflammatory effects, Tannins which contribute to the plants antimicrobial properties, carotenoids which provide antioxidant support, and anthocyanins which contribute to antioxidant and anti-inflammatory activities (Olumese *et al.,* 2023).

2.3 What is Cortisol?

Cortisol is a steroid hormone produced primarily by the zona fasciculata of the adrenal cortex. It is often called the body's primary "stress hormone" due to its critical role in the stress response, but it also regulates numerous other physiological processes, including metabolism, immune function, and cardiovascular homeostasis. Its secretion is governed by the hypothalamic-pituitary-adrenal (HPA) axis and follows a circadian rhythm, peaking in the early morning and reaching its lowest levels at night (Keller-Wood, 2015).

2.3.1 Synthesis Pathway of Cortisol

Cortisol is synthesized in the zona fasciculata of the adrenal cortex via a multi-step enzymatic process known as steroidogenesis. The starting material, cholesterol, is majorly derived from low-density lipoprotein (LDL) in the bloodstream, taken up by adrenal cells via receptor-mediated endocytosis (Smith, 2014). The initial and rate-limiting step is the transport of cholesterol into the mitochondria, mediated by the Steroidogenic Acute Regulatory (StAR) protein. Within the mitochondria, cholesterol is converted to pregnenolone by the enzyme Cholesterol Side-Chain Cleavage Enzyme (CYP11A1) (Papadopoulos and Miller, 2012). Pregnenolone is subsequently converted to progesterone in the endoplasmic reticulum by 3β-hydroxysteroid dehydrogenase (3β-HSD). The pathway then proceeds through a series of hydroxylation reactions: Progesterone is converted to 17α-hydroxyprogesterone by 17α-Hydroxylase (CYP17), which is then converted to 11-deoxycortisol by 21-Hydroxylase (CYP21A2). The final step, catalyzed by the mitochondrial enzyme 11β-Hydroxylase (CYP11B1), converts 11-deoxycortisol into active cortisol (Azhar *et al.,* 2020). It is critical to note that deficiencies in these enzymes, most commonly CYP21A2, lead to Congenital Adrenal Hyperplasia, a disorder characterized by impaired cortisol synthesis and compensatory androgen excess (Carvalho *et al.,* 2021).

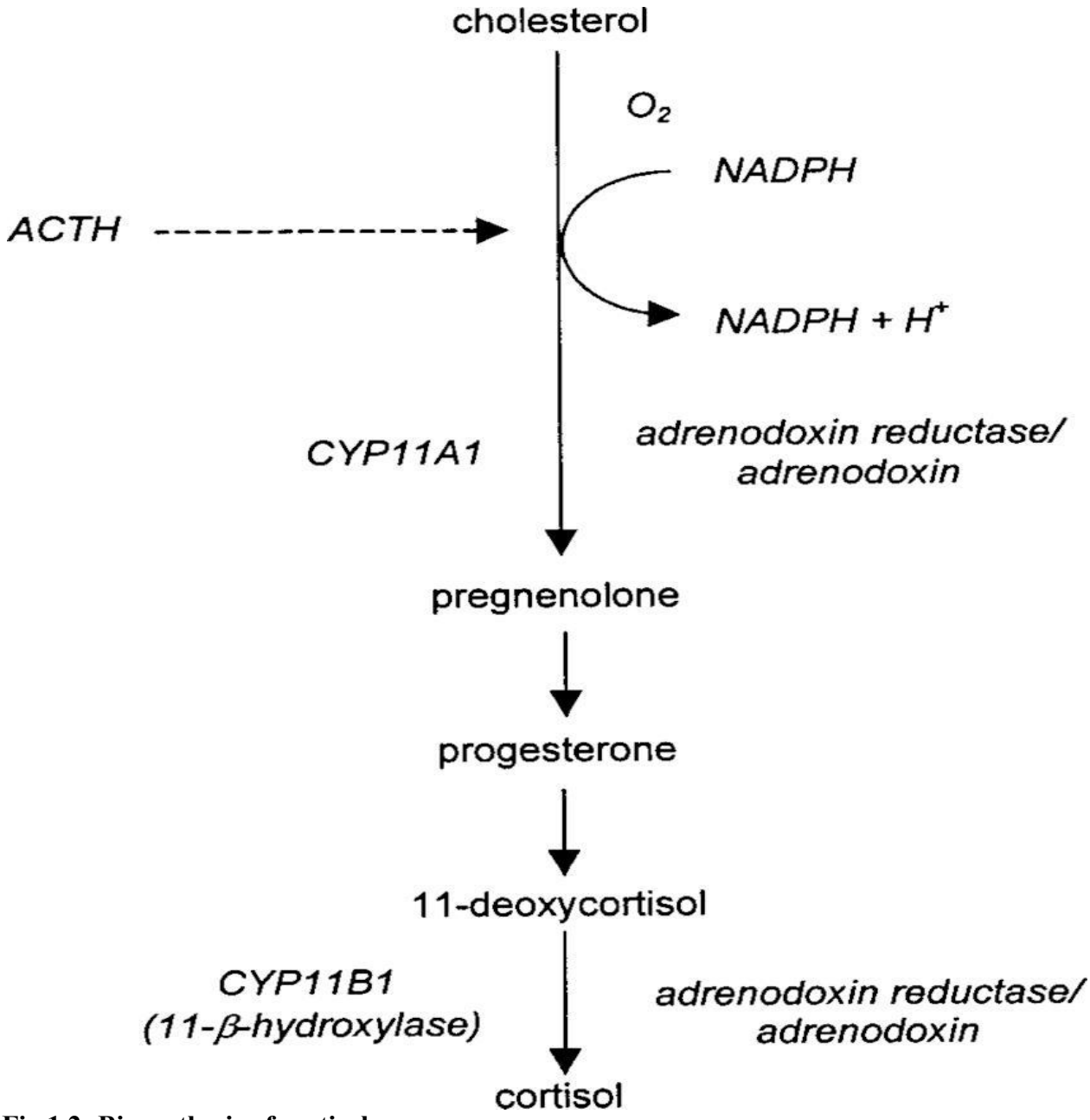


Fig 1.2: Biosynthesis of cortisol

Source: Papadopoulos and Miller, 2012

2.3.2 Secretion and Transport of Cortisol

Secretion is directly coupled to synthesis and is exclusively controlled by the HPA axis. In response to various stimuli (circadian rhythm, stress, etc.), at the hypothalamus, neurosecretory neurons in the paraventricular nucleus (PVN) of the hypothalamus synthesize and release Corticotropin-Releasing Hormone (CRH) into the hypophyseal portal blood system (Sukhareva, 2021). CRH binds to receptors on corticotroph cells in the anterior pituitary, stimulating the synthesis and secretion of Adrenocorticotropic Hormone (ACTH) into the systemic circulation. ACTH binds to high-affinity melanocortin-2 receptors (MC2R) on the cells of the zona fasciculata in the adrenal cortex (Ruggiero and Lalli, 2016). This binding triggers a cAMP-mediated signaling cascade that has two critical effects:

- Acute (Minutes): It stimulates the transport of cholesterol into the mitochondria by increasing the synthesis and phosphorylation of the StAR protein (Steroidogenic Acute Regulatory Protein), which is the true acute regulator of steroidogenesis. This leads to a rapid burst of cortisol synthesis and secretion.
- Chronic (Hours/Days): It promotes the transcription of genes encoding the steroidogenic enzymes (CYP11A1, CYP17, etc.) and the MC2R itself, thereby increasing the long-term capacity of the adrenal gland to produce cortisol.

Once synthesized, cortisol is lipid-soluble and diffuses freely out of the adrenal cell and into the bloodstream. In the blood, approximately 90% of cortisol is bound to proteins, primarily corticosteroid-binding globulin (CBG or transcorrin), and to a lesser extent, albumin. Only the free, unbound fraction is biologically active (Meyer *et al.,* 2016).

2.3.3 Regulation of Cortisol Secretion

A. The Core HPA Axis Pathway

The cascade begins with the release of Corticotropin-Releasing Hormone (CRH) from neurosecretory neurons in the hypothalamus. CRH, transported via the hypophyseal portal system, stimulates corticotroph cells in the anterior pituitary to secrete Adrenocorticotropic Hormone (ACTH) into systemic circulation (Papadimitriou and Priftis, 2009). ACTH then binds to melanocortin-2 receptors on adrenal cells, triggering a cAMP-mediated signaling cascade that rapidly increases StAR protein activity and chronically upregulates the expression of steroidogenic enzymes, resulting in cortisol production and secretion (Ruggiero and Lalli, 2016).

B. Negative Feedback Loops (The Primary Regulator)

Cortisol itself is the primary regulator of its own levels through negative feedback. Elevated cortisol levels inhibit the secretion of both CRH from the hypothalamus and ACTH from the pituitary (Papadimitriou and Priftis, 2009). This feedback occurs on two timescales: a fast feedback (within minutes) that inhibits release, and a delayed feedback (over hours) that suppresses the synthesis of CRH and ACTH. This mechanism is the basis for the adrenal suppression observed with prolonged therapeutic glucocorticoid use (Keller-Wood, 2015).

C. Diurnal Rhythm and Stress Modulation

The HPA axis exhibits a pronounced circadian rhythm, independent of sleep, driven by the suprachiasmatic nucleus. Secretion peaks in the early morning (around 6-8 a.m.) and reaches its nadir around midnight (Udi, 2025).

This baseline rhythm can be overridden by stress. Physical, psychological, or inflammatory stressors activate higher brain centers, which signal the hypothalamus to amplify CRH release, leading to a significant surge in cortisol to mobilize energy and modulate immunity (Sitorus and Silitonga, 2025).

D. Other Factors

- **ACTH Pulsatility:** ACTH is released in pulses, which results in a corresponding pulsatile secretion of cortisol. The amplitude of these pulses is highest in the morning, contributing to the diurnal peak (Lightman and Conway-Campbell, 2024).
- **Immune System:** Inflammatory cytokines, particularly Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α), can directly stimulate the HPA axis, explaining the elevated cortisol levels during illness and inflammation (Nava *et al.,* 2025).

2.3.4 Physiological Roles and Functions of Cortisol

Cortisol exerts its effects on nearly every organ and tissue in the body. Its primary roles can be categorized as follows:

A. Metabolism Regulation (Glucocorticoid Action)

Cortisol is a key metabolic hormone that influences the metabolism of carbohydrates, proteins, and fats.

- **Glucose Homeostasis**: Cortisol increases blood glucose levels by stimulating gluconeogenesis (synthesis of glucose from non-carbohydrate sources) in the liver and reducing glucose uptake in muscle and adipose tissue. It also promotes glycogenolysis (breakdown of glycogen into glucose) in the liver (Qaid and Abdelrahman, 2016).
- **Anti-Insulin Effect:** It reduces the uptake and utilization of glucose by peripheral tissues (like muscle and fat), making more glucose available for the brain and other vital organs.
- **Protein and Lipid Metabolism:** Cortisol promotes proteolysis (breakdown of proteins) to release amino acids for gluconeogenesis. It also stimulates lipolysis (breakdown of fats) to release free fatty acids for energy production (Qaid and Abdelrahman, 2016).

B. Stress Response

- Cortisol is a key mediator of the body's stress response. It works synergistically with catecholamines (e.g., adrenaline) to maintain alertness and provide energy during acute stress. In chronic stress, cortisol helps sustain energy mobilization but can lead to detrimental effects if dysregulated (Sitorus and Silitonga, 2025).
- The HPA axis is activated in response to stress, leading to cortisol release. This involves the hypothalamus secreting corticotropin-releasing hormone (CRH), which stimulates the pituitary to release adrenocorticotropic hormone (ACTH), ultimately prompting cortisol production (Papadimitriou and Priftis, 2009).

C. Anti-inflammatory and Immunosuppressive Actions

- Cortisol has anti-inflammatory and immunosuppressive effects. It inhibits the production of pro-inflammatory cytokines (e.g., IL-12, IFN-gamma, TNF-alpha) and reduces the migration of immune cells (e.g., neutrophils) to inflammation sites (Strehl *et al.,* 2019).
- It promotes a shift from Th1-mediated (cellular) immunity to Th2-mediated (humoral) immunity, which can alter the body's ability to combat certain infections.
- Chronic stress and elevated cortisol can lead to glucocorticoid resistance, resulting in non-resolving inflammation and increased susceptibility to illnesses (Knezevic *et al.,* 2023).

D. Cardiovascular Function

- Cortisol helps maintain blood pressure by enhancing the sensitivity of blood vessels to vasoconstrictors like catecholamines and angiotensin II. It also regulates electrolyte balance by promoting sodium retention in the kidneys (Nadagouda, 2025).
- Dysregulated cortisol levels can contribute to hypertension (high blood pressure) or hypotension (low blood pressure) (Sitorus and Silitonga, 2025).

E. Nervous System and Cognitive Effects

- Cortisol influences mood, motivation, and fear processing through its actions on the amygdala and hippocampus. It also modulates memory formation, particularly for emotionally charged events.
- Chronic elevation of cortisol is linked to neurodegenerative diseases (e.g., Alzheimer's and Parkinson's) and major depressive disorder due to HPA axis dysregulation and neuroinflammation (Knezevic *et al.,* 2023).

F. Other Functions

- Cortisol regulates the sleep-wake cycle by supporting wakefulness in the morning and promoting relaxation at night (Elder *et al.,* 2014).
- It plays a role in bone metabolism by inhibiting bone formation and promoting resorption, which can lead to osteoporosis in excess states (Hardy *et al.,* 2018).

2.4 Deficiencies Associated with Cortisol

Cortisol is a very important hormone in the body. Pathologic conditions arising from problems in cortisol level possess significant clinical implications. Some of those conditions include:

- **Adrenal Insufficiency (Hypocortisolism)**

  Adrenal insufficiency is characterized by inadequate cortisol production and can be primary (Addison's disease), secondary (pituitary dysfunction), or tertiary (hypothalamic dysfunction or exogenous glucocorticoid withdrawal) (Charmandri *et al.,* 2014).

  i. Primary Adrenal Insufficiency (Addison's disease)

  Causes: Autoimmune adrenalitis is the most common cause. Other causes include infections, adrenal hemorrhage, and genetic disorders like familial glucocorticoid deficiency (FGD) (Charmandri *et al.,* 2014).

Symptoms:

- Fatigue, weight loss, and hypotension.
- Hyperpigmentation due to elevated ACTH levels.
- Salt craving and electrolyte imbalances (hyponatremia, hyperkalemia) due to concomitant mineralocorticoid deficiency.

Diagnosis:

Low morning cortisol levels with elevated ACTH. Confirmation via ACTH stimulation test (Ospina *et al.,* 2016).

ii. Secondary and Tertiary Adrenal Insufficiency

Causes: Pituitary tumors, surgery, or chronic exogenous glucocorticoid use (most common cause of tertiary insufficiency) (Charmandri *et al.,* 2014).

Symptoms: Similar to primary insufficiency but without hyperpigmentation or electrolyte imbalances (mineralocorticoid function is preserved) .

Diagnosis: Low ACTH and cortisol levels. Insulin tolerance test or ACTH stimulation test may be used for confirmation (Ospina *et al.,* 2016).

- **Familial Glucocorticoid Deficiency (FGD)**

Genetic Causes: Mutations in genes like MC2R (ACTH receptor), MRAP (ACTH receptor accessory protein), and NNT (nicotinamide nucleotide transhydrogenase) impair cortisol synthesis (Meimaridou *et al.,* 2013).

Symptoms: Presents in infancy or childhood with hypoglycemia, failure to thrive, hyperpigmentation, and recurrent infections,
Treatment: Glucocorticoid replacement therapy.

- **Neonatal Cholestasis and Hypoglycemia**

- Cortisol deficiency in infants can present with neonatal cholestasis (liver dysfunction) and hypoglycemia, often resolving with hydrocortisone replacement .
- Mechanisms may involve altered bile acid transport and hepatic function due to cortisol deficiency (Al-Hussaini *et al.,* 2012).

- **Adrenal Crisis**

  - A life-threatening emergency characterized by hypotension, hypoglycemia, hyponatremia, and shock. It can be triggered by stress, infection, or abrupt withdrawal of glucocorticoid therapy .

  - Treatment: Immediate parenteral glucocorticoid administration and fluid resuscitation.

2.5 Diagnostic Testing

- **Serum Cortisol Levels:** Measured at 8 AM and 4 PM to assess diurnal variation. Loss of rhythm suggests dysregulation .
- **ACTH Stimulation Test:** Used to diagnose adrenal insufficiency. Blunted cortisol response indicates insufficiency .
- **Dexamethasone Suppression Test:** Used to diagnose hypercortisolism (Cushing's syndrome) .
- **Salivary Cortisol:** Useful for assessing free cortisol levels and circadian rhythm.

2.6 Diabetes Mellitus

Diabetes Mellitus is a chronic, metabolic disorder characterized by hyperglycemia (elevated blood glucose levels) resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2013). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Balaji *et al.,* 2019). Its global prevalence has reached pandemic proportions, with an estimated 537 million adults living with diabetes in 2021, a number projected to rise to 643 million by 2030. This surge is largely driven by the increased prevalence of Type 2 Diabetes, associated with urbanization, sedentary lifestyles, and obesity (Hossain *et al.,* 2024).

2.6.1 Classification and Types of Diabetes Mellitus

The current classification system, endorsed by the World Health Organization (WHO) and the American Diabetes Association (ADA), includes several distinct types, with Type 1 and Type 2 being the most prevalent.

A. Type 1 Diabetes Mellitus (T1DM): T1DM is an autoimmune disorder characterized by the selective destruction of insulin-producing β-cells in the pancreatic islets of Langerhans. This

leads to an absolute deficiency of insulin (Mauvais and van Endert, 2025). This form of diabetes is initiated in genetically susceptible individuals (e.g., those with HLA-DR3 or HLA-DR4 alleles) triggered by environmental factors such as an enteroviral infection (Alves *et al.,* 2024). After initiation, the process begins when the immune system, specifically T-lymphocytes, mistakenly identifies pancreatic beta-cell antigens as foreign. This leads to the production of autoantibodies (GADA, IA-2A, ZnT8A) which serve as markers of the disease, often appearing years before symptoms (Mauvais and van Endert, 2025). It's progression is a slow, insidious destruction of beta-cells occurs over months or years. Symptoms only manifest when 80-95% of beta-cell function is lost, leading to an absolute insulin deficiency.

B. Type 2 Diabetes Mellitus (T2DM): T2DM is characterized by a combination of insulin resistance in peripheral tissues (muscle, liver, and adipose) and a relative insulin secretory defect by pancreatic β-cells. The body produces insulin, but not enough to overcome the resistance (Czech, 2017). In other words, T2DM is defined by a cycle of insulin resistance and beta-cell dysfunction.

- **Insulin resistance:** The liver, muscle, and adipose tissue become less responsive to insulin. In the liver, this leads to uncontrolled hepatic gluconeogenesis even in the fed state. In muscle, it impairs glucose uptake. Adipose tissue releases increased free fatty acids (FFAs), which further worsen insulin resistance and contribute to lipid accumulation in the liver and muscle (lipotoxicity) (da Silva Rosa *et al.,* 2020).
- **Beta-Cell Dysfunction:** Initially, beta-cells compensate for insulin resistance by secreting more insulin (hyperinsulinemia). Over time, due to genetic factors and glucotoxicity (the toxic effect of chronic high glucose on beta-cells), they fail to compensate. The progressive decline in beta-cell function is the hallmark of T2DM progression (Saisho, 2025).

T2DM has a strong genetic predisposition and is highly associated with modifiable risk factors, including obesity (particularly abdominal), physical inactivity, and an unhealthy diet (Chandrasekaran and Weiskirchen, 2024). It's onset is usually insidious and can be asymptomatic for years. Symptoms, when they appear, are similar to T1DM but may also include fatigue, blurred vision, and slow healing of cuts or sores. It is more common in adults, but incidence is rising in younger populations due to obesity.

C. Gestational Diabetes Mellitus (GDM): GDM is defined as glucose intolerance with onset or first recognition during pregnancy. It results from the insulin-antagonizing effects of hormones produced by the placenta (e.g., human placental lactogen, progesterone), which can lead to insulin resistance in a genetically susceptible woman (Lois *et al.,* 2010). Pregnancy induces a state of natural insulin resistance to divert glucose to the growing fetus. In women who cannot increase their insulin secretion sufficiently to overcome this resistance, GDM develops (Mora-Ortiz and Rivas-Garcia, 2024). GDM poses risks to both the mother (increased risk of pre-eclampsia and future T2DM) and the fetus (macrosomia, neonatal hypoglycemia) (Mora-Ortiz and Rivas-Garcia, 2024).

Other specific types of diabetes include:

- Genetic defects of β-cell function such as Maturity-Onset Diabetes of the Young (MODY) caused by mutations in genes like HNF1A, GCK, HNF4A.
- Genetic defects in insulin action such as syndromes of severe insulin resistance.

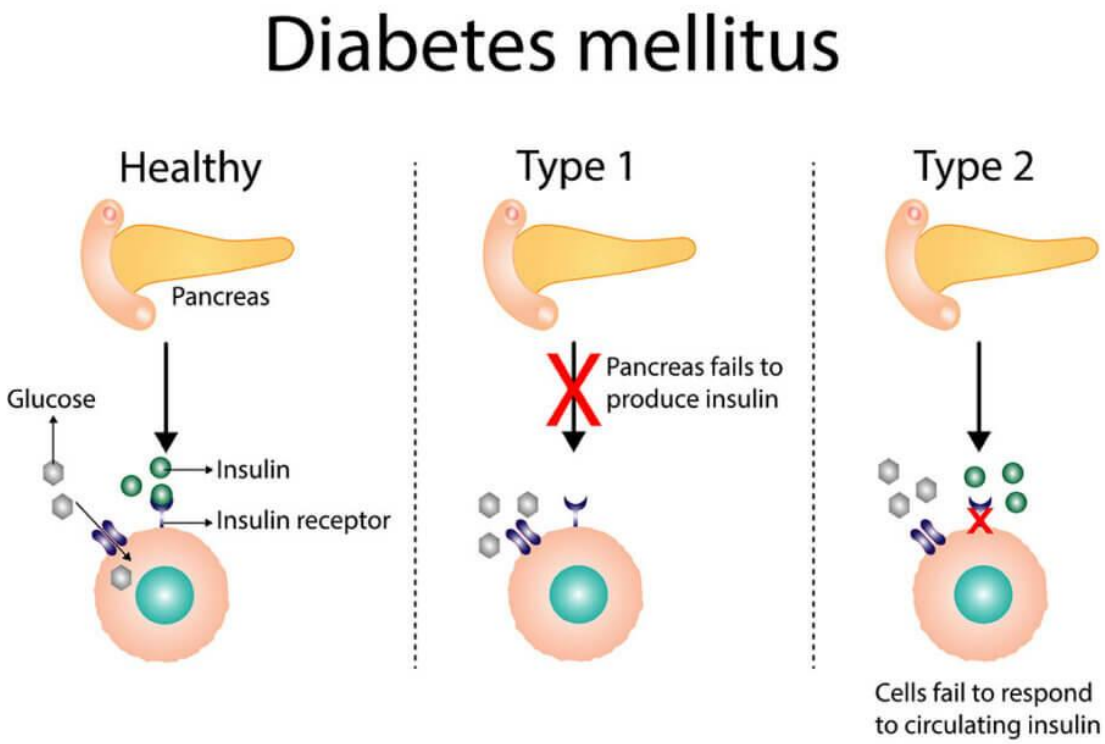


Fig 1.3: Diabetes Mellitus and its types

Source: American Diabetes Association, 2013

- Diseases of the exocrine pancreas including pancreatitis, cystic fibrosis and hemochromatosis which can destroy beta cells.
- Drug or chemical induced diabetes e.g., due to glucocorticoids, antipsychotics or in the treatment of HIV/AIDS.

2.7 The Interplay Between Cortisol and Oxidative Stress in Diabetes Mellitus

In diabetes mellitus, a destructive bidirectional relationship exists between cortisol and oxidative stress. This relationship forms a vicious cycle: hyperglycemia-driven oxidative stress can dysregulate the Hypothalamic-Pituitary-Adrenal (HPA) axis, leading to elevated cortisol levels, which in turn exacerbates oxidative stress and further worsens glycemic control. This cycle contributes significantly to the progression and complications of both Type 1 and Type 2 diabetes (Subba *et al.,* 2021).

2.7.1 How Cortisol Exacerbates Oxidative Stress in Diabetes

A. Aggravation of Hyperglycemia: Cortisol stimulates gluconeogenesis in the liver, providing more substrate (glucose) for hyperglycemia-induced oxidative stress. Cortisol also aggravates hyperglycemia by reducing glucose uptake in muscle and adipose tissue because cortisol is a counter-regulatory hormone that opposes insulin action, contributing to sustained high blood glucose levels (Dey and Lakshmanan, 2013). Persistent hyperglycemia is a major driver of oxidative stress through four well-established pathways:

- Increased Polyol Pathway Flux: Consumes NADPH, depleting the glutathione (GSH) regeneration system, a key cellular antioxidant.
- Advanced Glycation End-product (AGE) Formation: AGEs and their receptors (RAGE) generate reactive oxygen species (ROS).
- Activation of Protein Kinase C (PKC): PKC activation can stimulate NADPH oxidase, a major ROS-producing enzyme.
- Hexosamine Pathway Flux: Alters gene expression in ways that can promote oxidative stress.

B. Promotion of Lipolysis

Cortisol stimulates the breakdown of triglycerides in adipose tissue, releasing free fatty acids (FFAs) into the circulation. Elevated FFAs increase the rate of fatty acid beta-oxidation in

mitochondria, which inherently increases the production of ROS as a byproduct (Chandrasekaran and Weiskirchen, 2024).

Ectopic fat deposition in the liver and muscle further promotes insulin resistance and ROS generation via mitochondrial dysfunction and endoplasmic reticulum stress.

C. Direct Impairment of Antioxidant Defences

Cortisol can directly suppress the body's endogenous antioxidant systems. Studies have shown that glucocorticoids can decrease the levels of glutathione, one of the most critical intracellular antioxidants (Patani *et al.,* 2023). Cortisol may also suppress the activity or expression of key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

2.7.2 How Oxidative in Diabetes Promotes Cortisol Secretion

The influence is not unidirectional; oxidative stress itself can stimulate the HPA axis. Oxidative stress acts as a potent physiological stressor. Inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α), whose production is often increased by ROS, are powerful stimulators of CRH and ACTH release (Nava *et al.,* 2025).

Chronic stress and inflammation, hallmarks of diabetes, can lead to glucocorticoid resistance at the level of the pituitary and hypothalamus. This impairs the normal negative feedback loop, resulting in sustained hypersecretion of cortisol despite already high circulating levels (Sharma and Singh, 2020).

2.8 Streptozotocin (STZ) and it's Mechanism of Action

STZ is a glucosamine-nitrosourea compound produced by the bacterium *Streptomyces achromogenes*. Its diabetogenic effect is a result of its selective cytotoxicity towards pancreatic beta cells, which occurs through several interconnected processes (Zhu, 2022).

STZ's structural similarity to glucose allows it to be preferentially taken up by pancreatic beta cells through the GLUT2 glucose transporter. This is the primary reason for its selective toxicity to these cells (Zhu, 2022). Once inside the cell, STZ breaks down, releasing its methylnitrosourea moiety, a potent DNA alkylating agent. This DNA damage triggers the activation of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP). Excessive PARP

activation leads to a profound depletion of cellular NAD+ and ATP, effectively shutting down cellular energy metabolism and leading to necrosis (Fahrer and Christmann, 2023). STZ metabolism also contributes to the generation of reactive oxygen species (ROS), including superoxide radicals and nitric oxide. Beta cells are particularly vulnerable to oxidative stress due to their relatively low levels of antioxidant enzymes. The resulting oxidative damage to proteins, lipids, and DNA further accelerates cell death (Gerber and Rutter, 2017). Beyond causing cell death, STZ can also directly inhibit glucose-induced insulin secretion, partly by damaging mitochondrial DNA and impairing the critical signaling function of mitochondrial metabolism in beta cells (Gerber and Rutter, 2017).

2.9 Streptozotocin (STZ)-induced Diabetes Mellitus Models

Table 1: Preparation of Diabetic Experimental Models Using STZ

Model Type	Typical STZ Protocol	Key Characteristics	Primary Application
Type 1 Diabetes Model	Single high-dose (e.g., 50-65 mg/kg in rats; 100-200 mg/kg in mice via IP or IV injection)	Induces massive β-cell necrosis via DNA alkylation, leading to severe insulin deficiency and rapid-onset hyperglycemia	Studying diabetic complications (nephropathy, retinopathy), pathophysiology of insulin deficiency, and screening insulin-sensitizing drugs
Type 2 Diabetes Model	Low-dose single injection or multiple injections (e.g., 35 mg/kg), often combined with High-	Causes partial β-cell impairment and insulin resistance. HFD induces obesity and insulin resistance prior	Studying the interplay between insulin resistance and β-cell

	Fat Diet (HFD) or nicotinamide pre-treatment	to STZ, which then causes moderate β-cell loss	dysfunction, and metabolic syndrome

2.9.1 Advantages of the Streptozotocin (STZ)-Induced Diabetes Model

- **Clinical Relevance:** The structural, functional, and biochemical alterations observed in STZ-induced diabetes closely resemble those found in human diabetes, making it a valuable model for studying the pathogenesis of the disease and its associated complications (Zhu, 2022).
- **Cost-Effectiveness and Simplicity:** Genetically engineered spontaneous diabetes models are often expensive and complex to maintain. In contrast, inducing diabetes with STZ is a relatively straightforward and inexpensive procedure (Qamar *et al.,* 2023).
- **Reproducibility:** With careful standardization of protocols (dose, route, animal strain), the model can produce a consistent and reproducible diabetic phenotype (Ghasemi and Jeddi, 2023).
- **Flexibility:** By varying the dose and administration regimen, researchers can create models for both Type 1 and Type 2 diabetes, allowing for the investigation of different aspects of the disease (Ghasemi and Jeddi, 2023).

2.9.2 Disadvantages of the Streptozotocin (STZ)-Induced Diabetes Model

- **Model Fidelity:** The chemically-induced, rapid destruction of beta cells is mechanistically different from the slow, autoimmune-mediated destruction characteristic of human Type 1 diabetes. For Type 2 diabetes, STZ models may not fully replicate the complex pathophysiology of the human disease (Bauer *et al.,* 2023).
- **Variability and Technical Challenges:** The model's reproducibility can be affected by numerous factors, including the animal's species, strain, age, gender, and nutritional status. STZ is also unstable and must be prepared carefully in a citrate buffer, and the optimal dosing can vary significantly between animal models (Ghasemi and Jeddi, 2023).
- **Mortality and Non-Specific Toxicity:** High doses of STZ can cause acute mortality, often due to severe hypoglycemia from the sudden release of insulin from dying beta cells or later from ketoacidosis. STZ can also have toxic effects on other organs, notably

the kidneys, which can confound studies on diabetic complications (Al-Mahmood *et al.,* 2016).

- **Ethical Considerations:** The induction of diabetes causes significant animal suffering, including weight loss, polyuria, and polydipsia. Stringent ethical oversight and measures to minimize suffering (e.g., insulin therapy, pain management) are required (Qamar et al., 2023).

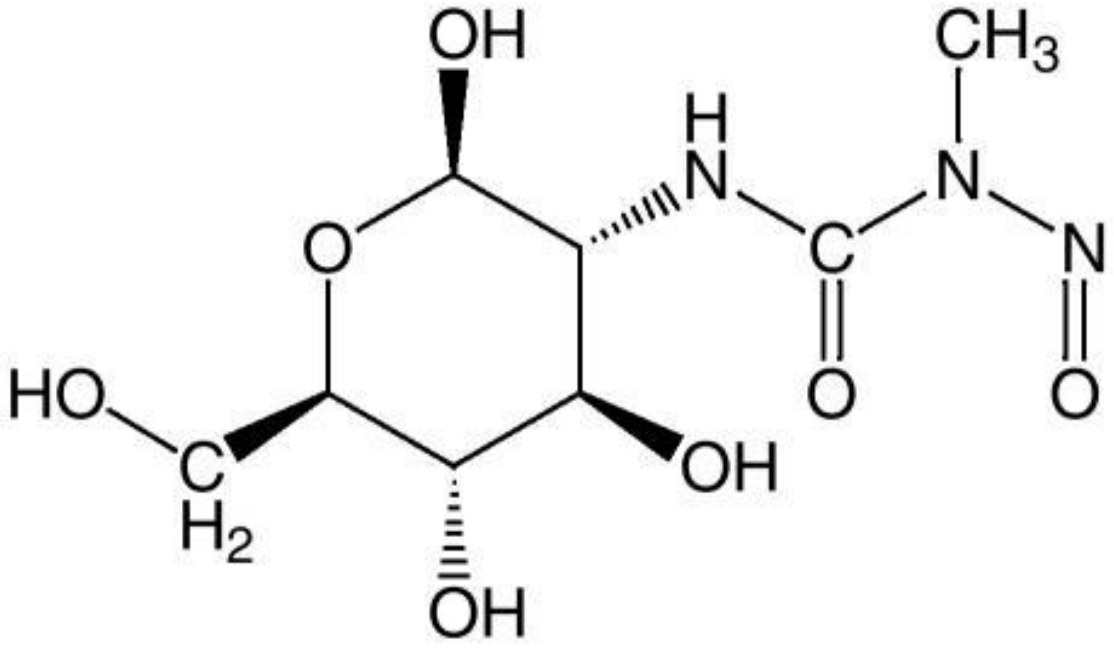


Fig 1.4: Structure of Streptozotocin

Source: Zhu, 2022

CHAPTER THREE

MATERIALS AND METHODS

3.1 Equipment and Apparatus

The following materials and equipment were used during the study:

A. 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

B. Machines and Instruments:

- Glucometer (NEWSPRING Glucose Meter, China; Model KF-B12)
- Weighing scale (NEWSPRING Digital Scale, China; Model NS- 790)
- Storage system (HAIER THERMOCOOL, China; Model HRF- 3500X)
- Grinder (Dry Herb Industrial Grinder, Model SYB-18B)
- Freeze Dryer (BIOBASE Freeze Dryer, China; Model BK- FD10S)
- Oven (WINCOM Thermostat Oven, Model OV-A25SF)
- pH Meter (PHS-25 pH meter, China; Model PHS-25)

3.2 Animal Experimental Study

The aim of this biomedical investigation was to assess how the aqueous fruit extract of Picralima nitida influences cortisol levels in male Wistar rats induced with streptozotocin-mediated diabetes. A total of nineteen (19) healthy male Wistar rats, weighing between 107.6 g and 169.3 g, were obtained from the Department of Anatomy, University of Benin, Nigeria.

The animals were housed under standard laboratory conditions in the Department of Anatomy, in clean, well-ventilated plastic cages lined with soft wood shavings. Before the experiment began, the rats underwent a four-week acclimatization period in a controlled environment (22 ± 2°C, 50–60% relative humidity, and a 12-hour light/dark cycle).

Throughout acclimatization, the rats had free access to water and were fed a standard rat diet (Chicken Grower Feed, Top Feeds Ltd, Nigeria) containing approximately 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus. Ethical approval for the study was obtained from the Departmental Animal Ethics Committee, and all procedures followed internationally accepted guidelines for the humane treatment of laboratory animals.

After acclimatization, the nineteen rats were assigned into four (4) groups of five rats each, except Group 0, which had four rats. Grouping was done based on initial body weight ranges as follows:

- Group 0: 155–169.3 g
- Group 1: 107.6–120.3 g
- Group 2: 138.7–142.2 g
- Group 3: 148.3–154.5 g

To ensure easy identification, each rat was marked with coloured Gentian Violet on specific body parts (hand, leg, back, head, or tail), while a few remained unmarked. Each animal was assigned a unique identification number corresponding to the marked location.

During the experimental period, the rats were observed daily for signs of stress, behavioural changes, or any physical abnormalities. Their body weights were measured weekly, and fasting

blood glucose levels were also checked weekly between 7:30 am and 8:30 am to monitor any significant changes resulting from the treatments. All handling procedures were carefully carried out to minimize stress and ensure the well-being of the animals.

At the end of the 10-day experimental period, the rats were humanely sacrificed using chloroform anaesthesia, in accordance with established ethical standards. Blood samples were collected via cardiac puncture into appropriately labelled lithium heparin and plain sample containers and stored in a refrigerator to maintain sample integrity until analysis.

This study adhered strictly to the ethical guidelines of the University of Benin and emphasized humane treatment and proper handling of all animals throughout the research process.

3.3 Collection and Identification of Plant Material

Approximately 300 fresh fruits of *Picralima nitida*, weighing between 0.2 kg and 0.3 kg, with most unripe and a few fully ripe, were purchased from Oliha Market along Siloko Road in 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, where a voucher specimen was deposited for future reference. The assigned voucher number was UBH-P424.

3.4 Preparation of Picralima nitida Extract

The fruits were first carefully sorted to remove soil, stones, insects, and any rotten or moldy samples. Only fresh, clean, healthy, and mature fruits were selected for the study. The selected fruits were thoroughly washed with clean water to eliminate any remaining dirt or debris. The rind (outer covering) was manually peeled off, after which the seeds and pulp were cut into smaller pieces and divided into six (6) batches to allow for easier drying.

The cut pieces were air-dried under shade for 7–10 days to protect the bioactive compounds from photodegradation. After shade-drying, the material was further dried in an oven at 45°C for about four weeks to ensure complete removal of moisture. The oven-dried material weighed 2,202.19 g and was then ground into a fine powder using an electric grinder (Dry Herb Industrial Grinder, Model SYB-18B). Grinding was carried out thoroughly to obtain a smooth, fine powder suitable for efficient extraction of bioactive constituents.

A known quantity of the powdered material was weighed and soaked in distilled water at a 1:10 w/v ratio (100 g of powder to 1000 mL of water) for 72 hours, with intermittent stirring to enhance extraction. The mixture was filtered using muslin cloth to separate the filtrate (containing the extracted compounds) from the solid residue. The filtrate was then freeze-dried using a BIOBASE Freeze Dryer (Model BK-FD10S, China) to obtain a dry extract. Freeze-drying involved freezing the filtrate and removing water by sublimation under low temperature and pressure, yielding a stable powdered extract.

The final dried extract was weighed to determine percentage yield and stored in an airtight container at 40°C until required for the experiment. At the end of the process, the total extract obtained weighed 1,271.98 g, giving a percentage yield of 57.78%.

Phytochemical analysis of the freeze-dried aqueous extract confirmed the presence of important bioactive constituents. According to Ilenowa et al. (2024), the extract contains 39.24 mg GAE/g of total phenols and approximately 24.39 mg QE/g of flavonoids. These compounds are known for their potent antioxidant properties and contribute significantly to the therapeutic potential of the extract. The preparation process ensured that the extract retained its bioactive integrity while producing a safe, stable, and effective preparation suitable for experimental use.

3.5 Experimental Design

A total of nineteen (19) physiologically normal male Wistar rats weighing between 107.6 g and 169.3 g were used in this study. The animals were obtained from the Department of Anatomy, University of Benin, Nigeria, and housed under standard laboratory conditions in clean, well-ventilated plastic cages lined with soft wood shavings.

Before the experiment began, the rats underwent a four-week acclimatization period in a controlled environment maintained at 22 ± 2°C, with 50–60% relative humidity and a 12-hour light/dark cycle.

Throughout the acclimatization period, the rats had unrestricted access to clean water (ad libitum) and were fed a standard rat diet (Chicken Grower Feed, Top Feeds Ltd, Nigeria), which contains approximately 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus.

Ethical approval for the study was obtained from the Departmental Animal Ethics Committee, and all procedures followed international guidelines for the humane care and use of laboratory animals.

3.6 Experimental Grouping

The nineteen (19) rats were assigned into four (4) experimental groups, each containing five (5) rats except Group 0, which consisted of four (4) rats. The grouping was done based on the animals' mean body weight, which ranged from 107.69 g to 183.59 g. The groups were arranged as follows:

- Group 0: (Normal/Negative Control): Received only normal feed and water.
- Group 1: (Diabetic/Positive Control): Received streptozotocin (STZ) only, with no treatment.
- Group 2: Diabetic rats treated with *Picralima nitida* extract at 200 mg/kg.
- Group 3: Diabetic rats treated with *Picralima nitida* extract at 500 mg/kg

To ensure easy identification throughout the study, each rat was marked individually using coloured Gentian Violet on specific body parts such as the hand, leg, back, head, or tail, while a few remained unmarked. Each marking corresponded to a unique identification number.

After diabetes was confirmed in the appropriate groups, the animals were reorganized into the same treatment categories, but with only two (2) rats per group, except for the positive control group, which retained four (4) rats.

3.7 Administration of Picralima nitida

The aqueous extract of *Picralima nitida* fruit (pulp and seeds), prepared as previously described, was administered to the experimental groups (G2–G3). Group 0 (G0) served as the negative control and received only water, feed, and citrate buffer in volumes matched to those given to rats of similar weight in the other groups. This provided a baseline for assessing the effects of the extract.

Initial grouping was based on body weight, allocating the nineteen rats into four (4) groups of five (5) rats each. The aqueous extract was administered using the oral gavage technique as described by Turner *et al.,* (2011) and Diehl *et al.,* (2001). After diabetes was confirmed, diabetic rats were separated from non-diabetic animals and randomly reorganized into four (4)

experimental groups consisting of two (2) rats each, except the positive control group, which contained four (4) rats.

Diabetic rats in Groups 2 and 3 received the *Picralima nitida* extract via oral gavage at doses of 200 mg/kg, and 500 mg/kg respectively, alongside regular feed and water. Throughout the treatment period, no signs of toxicity or mortality were recorded.

3.8 Dosage Calculation of Picralima nitida

The required concentrations of the *Picralima nitida* extract were prepared according to the individual body weight of each rat. Dosages were calculated using the formula:

For a standard dose of 200 mg/kg:

200 mg of extract is required for every 1 kg (1000 g) of body weight.

For example, for a rat weighing 136.6 g, the exact dose was calculated as:

200 mg × 136.6/1000 g = 27.32mg

To determine the volume of extract solution required for this rat, a stock solution was prepared at a concentration of 1 g in 10 mL of distilled water:

1000 mg = 10 mL

Thus,

Volume = 27.32 mg × 10 mL/1000 mg = 0.27 mL

A stock solution of the extract was prepared by dissolving 1 g (1000 mg) in 10 mL of distilled water. This approach was applied to all diabetic rats in Groups G2–G3, ensuring that each animal received the correct dose based on its body weight.

The extract was administered orally once daily for 10 days using a calibrated syringe fitted with a stainless-steel oral gavage needle.

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

A. 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.

B. 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

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.

C. 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 Henderson–Hasselbalch equation as follows:

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

pH = 4.50; pKa3 = 6.50

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

10^-2 = [B]/[A]

10^-2 [A] = [B]

10^-2 [A]= [B]

[B] = 0.01[A]

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

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

[A] + 0.01[A] = M

Molar concentration of citrate buffer to be prepared is 0.1M

[A]+ 0.01[A) = 0.1M

1.01[A] = 0.1

[A]= 0.1/1.01

[A] = 0.099M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.099M

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

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.099M × 210.14g = 20.80g/L

Mass concentration of B = 0.001M × 294.10g= 0.29 g/L

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.

D. 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.

E. 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 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.

F. 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 0.1L(100mL).

pH and Weight Calculations

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

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

pH = 4.50; pKa3 = 6.50

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

10^-2 = [B]/[A]

10^-2 [A] = [B]

10^-2 [A]= [B]

[B] = 0.01[A]

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

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

[A] + 0.01[A] = M

Molar concentration of citrate buffer to be prepared is 0.05M

[A]+ 0.01[A] = 0.05M

1.01[A] = 0.05

[A]= 0.05/1.01

[A] = 0.0495M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.0495M

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

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.0495M × 210.14g= 10.40g/L

Mass concentration of B = 0.0005M × 294.10g= 0.147 g/L

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

A = 10.40 g/L × 0.1L = 1.04g

B = 0.147 g/L × 0.1L = 0.0147g

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

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

G. 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.

H. 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.

3.10 Weight and Blood Glucose Evaluation

The rats were administered their respective doses of the extract once daily for ten (10) consecutive days. During this period, both body weight and blood glucose levels were monitored at the start and end of the experiment. Blood glucose measurements were taken after an overnight fast using a Newspring Glucose Meter (Model KF-B12, China). Blood samples were collected from the tail tip using a sterile lancet, and the glucose readings were recorded accordingly.

Body weights were measured using a high-precision NEWSpring digital weighing scale (Model NS-790, China).

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

Table 2: Weight and Blood Glucose Evaluation

Grou	Label	Treatment	Ra	Baselin	Baselin	FBG	Weight	FBG

p			t ID	e FBG (mg/dl)	e Weight (g)	Before Induction (mg/dl)	Before Induction (g)	After Induction (mg/dl)
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 only	S1 S2 S3 S4 S5	72 76 59 86 81	117.0 117.4 120.3 107.6 117.3	65 74 77 90 79	123.9 138.5 154.0 134.9 127.8	365 127 86 272 61
G3	Low P.Nitida	200 ml	L1 L2 L3 L4 L5	61 59 68 79 68	129.0 129.9 130.1 134.7 136.9	74 104 72 77 79	141.6 152.6 156.2 161.0 161.1	52 135 43 279 130
G6	High P.Nitida	500 ml	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

3.11 Blood Collection

Good hygiene was consistently maintained in the animal house by routinely cleaning the cages and replacing the sawdust bedding. The rats were closely monitored throughout the study to assess their health and general well-being, and they were provided with fresh food and water every day.

On the tenth (10^{th}) day, the animals were fasted overnight, weighed again, and then sacrificed. Anesthesia was induced using chloroform to minimize pain and distress. The rats were euthanized in a chloroform chamber, after which a lateral incision was made in the abdominal cavity. Blood samples were collected via cardiac puncture and transferred into appropriately labeled containers depending on the type of analysis required:

- Plain bottles: For biochemical assays
- Heparin bottles: To preserve plasma integrity

The collected blood samples were stored at 4°C until analysis. Before biochemical evaluation, the samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was later used to determine cortisol levels using standard biochemical techniques.

3.12 Biochemical Analysis

The cortisol assay uses a competitive ELISA method, which works on the principle that the cortisol in the patient's sample competes with a labeled form of cortisol for a limited number of antibody binding sites.

Core Principle: There is an inverse relationship between the cortisol concentration in the sample and the color produced. More cortisol in the sample = Less color.

Steps Involved

1. **Add**: Patient sample (serum, plasma, or saliva) and an enzyme-linked cortisol (conjugate) are added to a well pre-coated with cortisol.
2. **Compete**: An anti-cortisol antibody is added. The cortisol from the patient and the enzyme-linked cortisol compete to bind to the antibody.
3. **Wash**: The well is washed. This removes all unbound materials. If the patient's cortisol level is high, it will have occupied the antibodies, preventing the enzyme-linked cortisol from binding, so it will be washed away.

4. **Develop**: A colorless substrate solution is added. Any enzyme-linked cortisol that did bind to an antibody (which is more likely when patient cortisol is low) will convert the substrate into a colored product.
5. **Stop and Read:** A stop solution is added, and the intensity of the color is measured. The darker the color, the lower the patient's cortisol concentration.

The test measures the inhibition of color development, which is directly proportional to the amount of cortisol in the original sample.

3.13 Determination of Serum Cortisol Activity

Table 3: Cortisol level in experimental rats

Group	Rat ID	Cortisol (µg/ml)
Group 0 (Control)	C3	15.8
	C4	10.9
Group 1 (Diabetic)	D5	18.6
	S4	18.4
Group 3 (Low p. nitida)	M1	8.9
	M4	11.7
Group 6 (High p. nitida)	A1	8.7
	A4	11.6

CHAPTER FOUR

4.1 RESULT

This chapter presents the effects of aqueous *Picralima nitida* extract, administered via oral gavage, on serum cortisol levels in male Wistar rats. Initially, the rats were distributed into four groups, each comprising five rats, except the control group, which consisted of four rats. During the course of treatment, some rats did not survive, leaving a total of ten rats. Consequently, the surviving rats were regrouped into four experimental groups containing two rats each, while the control group remained unchanged, as no deaths were recorded in that group.

Blood samples were collected and analyzed in the laboratory to determine serum cortisol concentrations. Statistical analyses were performed using SPSS, and the level of significance was set at $p < 0.05$, meaning that differences were considered statistically significant if the probability of the observed effect occurring by chance was less than 5%.

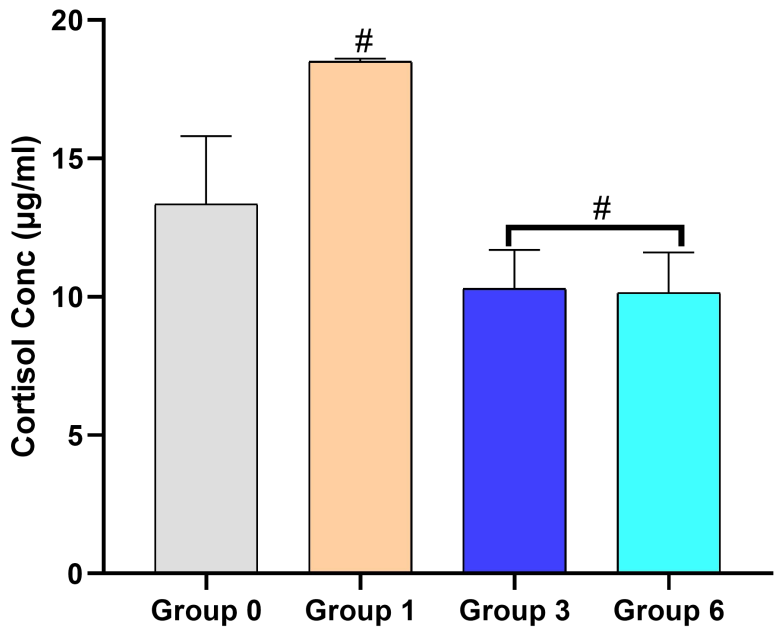


Figure 4.1: Effect of *Picralima nitida* on cortisol concentrations of male Wistar rats. Values are expressed as mean ± SEM. # represent statistical significant at P < 0.05 when compared to group 0.

CHAPTER FIVE

5.1 DISCUSSION

The results of this study offer valuable insight into how *Picralima nitida* modulates cortisol levels in male Wistar rats experiencing streptozotocin-induced oxidative stress. Cortisol, the primary glucocorticoid hormone, is essential for regulating metabolic activity, immune function, and stress responses. Disturbances in its circulating levels are commonly linked to metabolic disorders such as diabetes mellitus. In this investigation, rats in the diabetic untreated group (Group 1) showed a marked elevation in cortisol concentration (18.5 ± 0.1 µg/ml) compared with the normal control group (13.35 ± 2.45 µg/ml). This significant rise corresponds with established research showing that streptozotocin induces diabetes by destroying pancreatic β-cells, which leads to hyperglycemia, enhanced oxidative stress, and subsequent stimulation of the hypothalamic–pituitary–adrenal (HPA) axis. Persistent activation of the HPA axis increases adrenocorticotropic hormone (ACTH) release, which then drives the adrenal cortex to secrete higher amounts of cortisol. Elevated cortisol levels are frequently reported in diabetic states and are associated with poor glucose regulation, compromised immune function, and heightened systemic inflammation.

In contrast, rats treated with the *Picralima nitida* extract showed a considerable decrease in cortisol levels when compared with both the diabetic untreated and the normal control groups. The low-dose group (Group 3) recorded a cortisol level of 10.3 ± 1.4 µg/ml, while the high-dose group (Group 6) showed a comparable value of 10.15 ± 1.45 µg/ml. The fact that both treatment groups demonstrated significantly lower cortisol levels—despite being subjected to the same diabetogenic stressor as Group 1 strongly indicates that *Picralima nitida* contains bioactive compounds capable of reducing oxidative stress and downregulating excessive HPA axis activity. These results support previous findings that highlight the antioxidant, anti-inflammatory, and adaptogenic properties of the alkaloid-rich constituents of *Picralima nitida*. By attenuating oxidative stress and restoring cellular redox balance, the extract may have lowered the overall physiological stress load, thereby reducing the demand for elevated cortisol secretion.

Additionally, the pattern observed where both low and high doses produced nearly identical cortisol-lowering effects may point to a saturation response, implying that even relatively small amounts of the extract are sufficient to influence endocrine regulation. The ability of *Picralima nitida* to counter cortisol dysregulation in a diabetic model underscores its potential therapeutic value in conditions characterized by chronic stress and disrupted metabolic function. Lower cortisol levels are particularly advantageous, as persistent hypercortisolemia contributes to insulin resistance, muscle wasting, delayed wound healing, and several cardiovascular complications often associated with diabetes. Thus, the extract's ability to

modulate cortisol may reflect not only biochemical improvement but also a broader contribution to metabolic stability.

Overall, these findings highlight the protective influence of *Picralima nitida* against the endocrine and oxidative imbalances caused by streptozotocin. The substantial reduction in cortisol levels in treated rats demonstrates the extract's promising adaptogenic and stress-moderating properties, suggesting that it may serve as a beneficial complementary therapy in diabetes-associated oxidative stress. Future research should focus on clarifying the exact molecular mechanisms involved whether through direct antioxidant effects, modulation of adrenal signaling pathways, or enhancement of glucose metabolism and identifying the optimal dosage needed to maximize its therapeutic potential.

CONCLUSION

The present study demonstrates that *Picralima nitida* possesses significant cortisol-modulating properties in Sprague Dawley rats exposed to streptozotocin-induced oxidative stress. The marked elevation of cortisol observed in the untreated diabetic group confirms the profound physiological stress and HPA axis activation associated with streptozotocin-induced diabetes. However, treatment with *Picralima nitida* at both low and high doses effectively reversed this hormonal imbalance, producing a significant reduction in cortisol levels compared with both the control and diabetic groups. These findings indicate that the extract exerts protective, antioxidant, and adaptogenic effects capable of attenuating stress-related endocrine disturbances. Overall, the results highlight the therapeutic potential of *Picralima nitida* in managing oxidative stress and cortisol dysregulation associated with diabetic conditions. Further research exploring its molecular mechanisms, long-term efficacy, and optimal dosing is recommended to support its development as a complementary intervention in metabolic and stress-related disorders.

REFERENCES

Adeola, O. E., Akpor, O. A., Adamolekun, M. M., Adewale, O. B., and Akpor, O. B. (2024). In vitro antioxidant and antimicrobial potentials of aqueous extract of Picralima nitida seeds. *Vegetos, 37*(2), 486-493.

American Diabetes Association. (2013). Diagnosis and classification of diabetes mellitus. *Diabetes care, 36*(Supplement_1), S67-S74.

Akabassi, G. C., Padonou, E. A., Gbesso, G. H. F., Assogbadjo, A. E., and Guede, N. Z. (2023). Effect of climate and habitat on morphological characteristics and fruit production of Picralima nitida (Stapf) in West Africa. *Journal of Environmental Geography, 16*(1-4), 95-101.

Al-Hussaini, A., Almutairi, A., Mursi, A., Alghofely, M., and Asery, A. (2012). Isolated cortisol deficiency: a rare cause of neonatal cholestasis. *Saudi Journal of Gastroenterology, 18*(5), 339-341.

Al-Mahmood, S., Razak, T. A., Abdullah, S. T., Fatnoon NA, N. N., Mohamed, A. H., and Al-Ani, I. M. (2016). A comprehensive study of chronic diabetes complications in streptozotocin-induced diabetic rat. *Makara Journal of Health Research, 20*(2), 4.

Alves Abrantes, J.J.P., Veríssimo de Azevedo, J.C., Fernandes, F.L., Duarte Almeida, V., Custódio De Oliveira, L.A., Ferreira de Oliveira, M.T., Galvão De Araújo, J.M., Lanza, D.C.F., Bezerra, F.L., Andrade, V.S. and Araújo de Medeiros Fernandes, T.A. (2024). Viruses as a potential environmental trigger of type 1 diabetes mellitus. Biomedical Reports, 20(5), 81.

Azhar, S., Dong, D., Shen, W. J., Hu, Z., and Kraemer, F. B. (2020). The role of miRNAs in regulating adrenal and gonadal steroidogenesis. *Journal of molecular endocrinology, 64*(1), R21-R43.

Balaji, R., Duraisamy, R., and Kumar, M. P. (2019). Complications of diabetes mellitus: A review. *Drug Invention Today, 12*(1).

Bauer, B. M., Bhattacharya, S., Bloom-Saldana, E., Irimia-Dominguez, J. M., and Fueger, P. T. (2023). Dose-dependent progression of multiple low-dose streptozotocin-induced diabetes in mice. *Physiological genomics, 55*(9), 381-391.

Carvalho, B., Marques, C. J., Santos-Silva, R., Fontoura, M., Carvalho, D., and Carvalho, F. (2021). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: an update on genetic analysis of CYP21A2 gene. *Experimental and Clinical Endocrinology & Diabetes, 129*(07), 477-481.

Chandrasekaran, P., and Weiskirchen, R. (2024). The role of obesity in type 2 diabetes mellitus—An overview. *International journal of molecular sciences, 25*(3), 1882.

Creed, S.M., Gutridge, A.M., Argade, M.D., Hennessy, M.R., Friesen, J.B., Pauli, G.F., van Rijn, R.M. and Riley, A.P. (2020). Isolation and pharmacological characterization of six opioidergic picralima nitida alkaloids. *Journal of natural products, 84*(1), 71-80.

Czech, M. P. (2017). Insulin action and resistance in obesity and type 2 diabetes. *Nature medicine, 23*(7), 804-814.

Dapaah, G., Koffuor, G. A., Mante, P. K., and Ben, I. O. (2017). The possible mode of antitussive and expectorant activity of the ethanol seed extracts of Picralima nitida ((Stapf) Th. & H. Durand). *Journal of Traditional and Complementary Medicine, 7*(1), 133-140.

De Campos, O. C., Osaigbovo, D. I., Bisi-Adeniyi, T. I., Iheagwam, F. N., Rotimi, S. O., and Chinedu, S. N. (2020). Protective Role of Picralima nitida Seed Extract in High-Fat High-Fructose-Fed Rats. *Advances in Pharmacological and Pharmaceutical Sciences, 2020*(1), 5206204.

Dey, A., and Lakshmanan, J. (2013). The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver. *Food & function, 4*(8), 1148-1184.

Diehl, K-H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J-M. And van de Vorstenbosch, C. (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology, 21*(1), 15–23.

da Silva Rosa, S. C., Nayak, N., Caymo, A. M., and Gordon, J. W. (2020). Mechanisms of muscle insulin resistance and the cross-talk with liver and adipose tissue. *Physiological reports, 8*(19), e14607.

Elder, G. J., Wetherell, M. A., Barclay, N. L., and Ellis, J. G. (2014). The cortisol awakening response–applications and implications for sleep medicine. *Sleep medicine reviews, 18*(3), 215-224.

Fahrer, J., and Christmann, M. (2023). DNA alkylation damage by nitrosamines and relevant DNA repair pathways. *International Journal of Molecular Sciences, 24*(5), 4684.

Folorunso, I. M., Olawale, F., Olofinsan, K., and Iwaloye, O. (2022). Picralima nitida leaf extract ameliorates oxidative stress and modulates insulin signaling pathway in high fat-diet/STZ induced diabetic rats. *South African Journal of Botany, 148,* 268-282.

Furman, B.L., 2021. Streptozotocin-induced diabetic models in mice and rats. *Current Protocols, 1*(4), 1–21.

Gerber, P. A., and Rutter, G. A. (2017). The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. *Antioxidants & redox signaling, 26*(10), 501-518.

Ghasemi, A., and Jeddi, S. (2023). Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. *EXCLI journal, 22*, 274.

Hardy, R. S., Zhou, H., Seibel, M. J., and Cooper, M. S. (2018). Glucocorticoids and bone: consequences of endogenous and exogenous excess and replacement therapy. *Endocrine reviews, 39*(5), 519-548.

Hennessy, M.R., Creed, S.M., Gutridge, A.M., Rusali, L.E., Luo, D., Sepehri, B., Rhoda, E.S., Villegas, J.A., van Rijn, R.M. and Riley, A.P. (2024). Discovery of potent kappa opioid receptor agonists derived from akuammicine. *Journal of Medicinal Chemistry, 67*(23), 20842-20857.

Hossain, M. J., Al-Mamun, M., and Islam, M. R. (2024). Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. *Health Science Reports, 7*(3), e2004.

Keller-Wood, M. (2015). Hypothalamic-pituitary-adrenal Axis—feedback control. *Comprehensive Physiology, 5*(3), 1161-1182.

Knezevic, E., Nenic, K., Milanovic, V., and Knezevic, N. N. (2023). The role of cortisol in chronic stress, neurodegenerative diseases, and psychological disorders. *Cells, 12*(23), 2726.

Laryea, M. K., Abotsi, K. M. W., Woode, E., Dickson, R. A., Webster, L., and Ekuadzi, E. (2024). Antiplasmodial and anti-trypanosomial monoterpene indole alkaloids from Picralima nitida. *Natural Product Research,* 1-10.

Lightman, S. L., and Conway-Campbell, B. L. (2024). Circadian and ultradian rhythms: Clinical implications. *Journal of Internal Medicine, 296*(2), 121-138.

Lois, K., Valsamakis, G., Mastorakos, G., and Kumar, S. (2010). The impact of insulin resistance on woman's health and potential treatment options. *Annals of the New York Academy of Sciences, 1205*(1), 156-165.

Mauvais, F. X., and van Endert, P. M. (2025). Type 1 diabetes: A guide to autoimmune mechanisms for clinicians. *Diabetes, Obesity and Metabolism, 27*, 40-56.

Meimaridou, E., Hughes, C.R., Kowalczyk, J., Guasti, L., Chapple, J.P., King, P.J., Chan, L.F., Clark, A.J. and Metherell, L.A. (2013). Familial glucocorticoid deficiency: new genes and mechanisms. *Molecular and cellular endocrinology, 371*(1-2), 195-200.

Meyer, E. J., Nenke, M. A., Rankin, W., Lewis, J. G., and Torpy, D. J. (2016). Corticosteroid-binding globulin: a review of basic and clinical advances. *Hormone and Metabolic Research, 48*(06), 359-371.

Mora-Ortiz, M., and Rivas-García, L. (2024). Gestational diabetes mellitus: unveiling maternal health dynamics from pregnancy through postpartum perspectives. *Open Research Europe, 4,* 164.

Nadagouda, V. G. (2025). Kidney Hormones and Hypertension. *Hypertension: Known & Less Known Facts,* 114.

Nava, R.G., Adri, A.S., Filgueiras, I.S., Nóbile, A.L., Barcelos, P.M., Corrêa, Y.L.G., de Oliveira, S.F., Cabral-Miranda, G., Dias, H.D., Schimke, L.F. and de Araújo Gleizer, R. (2025). Modulation of neuroimmune cytokine networks by antidepressants: implications in mood regulation. *Translational psychiatry, 15*(1), 314.

Newsholme, P., Cruzat, V. F., Keane, K. N., Carlessi, R., and de Bittencourt Jr, P. I. H. (2016). Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochemical Journal, 473*(24), 4527-4550.

Nowak, D., 2020. The Freeze-Drying of Foods — The Characteristics, Processes and Applications. *Foods, 9*(1), 87.

Ogbeide, O.K., Sajere, O., Omorefe, N., Aghedo, O.N., Isibor, J.I., Osarinmwian, O., Uadia, J.O. and Iyekowa, O. (2025). Selected Preclinical Studies on Picralima nitida Stem Bark Extract. *Tropical Journal of Drug Research, 2*(2), 29-37.

Olajide, O. A., Velagapudi, R., Okorji, U. P., Sarker, S. D., and Fiebich, B. L. (2014). Picralima nitida seeds suppress PGE2 production by interfering with multiple signalling pathways in IL-1β-stimulated SK-N-SH neuronal cells. *Journal of ethnopharmacology, 152*(2), 377-383.

Olumese, F. E., Aihie, P. A., and Oriakhi, K. (2023). Nutritional composition, phytochemical analysis and Antioxidant capacity of Ethanol extract of Picralima nitida fruit (bark and pulp). *Journal of Applied Sciences and Environmental Management, 27*(5), 1039-1046.

Ospina, N.S., Al Nofal, A., Bancos, I., Javed, A., Benkhadra, K., Kapoor, E., Lteif, A.N., Natt, N. And Murad, M.H. (2016). ACTH stimulation tests for the diagnosis of adrenal insufficiency: systematic review and meta-analysis. *The Journal of Clinical Endocrinology & Metabolism, 101*(2), 427-434.

Papadimitriou, A., and Priftis, K. N. (2009). Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation, 16*(5), 265-271.

Papadopoulos, V., and Miller, W. L. (2012). Role of mitochondria in steroidogenesis. *Best practice & research Clinical endocrinology & metabolism, 26*(6), 771-790.

Patani, A., Balram, D., Yadav, V. K., Lian, K. Y., Patel, A., and Sahoo, D. K. (2023). Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress. *Frontiers in Endocrinology, 14,* 1271521.

Qaid, M. M., and Abdelrahman, M. M. (2016). Role of insulin and other related hormones in energy metabolism—A review. *Cogent Food & Agriculture, 2*(1), 1267691.

Qamar, F., Sultana, S., and Sharma, M. (2023). Animal models for induction of diabetes and its complications. *Journal of Diabetes & Metabolic Disorders, 22*(2), 1021-1028.

Ruggiero, C., and Lalli, E. (2016). Impact of ACTH signaling on transcriptional regulation of steroidogenic genes. *Frontiers in endocrinology,* 7, 24.

Saisho, Y. (2015). B-cell dysfunction: Its critical role in prevention and management of type 2 diabetes. *World journal of diabetes, 6*(1), 109.

Sharma, V. K., and Singh, T. G. (2020). Chronic stress and diabetes mellitus: interwoven pathologies. *Current diabetes reviews, 16*(6), 546-556.

Sitorus, H. P., and Silitonga, M. (2025). The Role of cortisol in the stress response. *International Journal of Ecophysiology, 7*(1), 48-58.

Smith, C. M. (2014). Cholesterol Absorption, Synthesis, Metabolism, and Fate.

Strehl, C., Ehlers, L., Gaber, T., and Buttgereit, F. (2019). Glucocorticoids—all-rounders tackling the versatile players of the immune system. *Frontiers in immunology, 10,* 1744.

Subba, R., Sandhir, R., Singh, S. P., Mallick, B. N., and Mondal, A. C. (2021). Pathophysiology linking depression and type 2 diabetes: Psychotherapy, physical exercise, and fecal microbiome transplantation as damage control. *European Journal of Neuroscience, 53*(8), 2870-2900.

Sukhareva, E. V. (2021). The role of the corticotropin-releasing hormone and its receptors in the regulation of stress response. *Vavilov Journal of Genetics and Breeding, 25*(2), 216.

Turner, P.V., Brabb, T., Pekow, C. And Vasbinder, M.A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science, 50*(5), 600–613.

Udi, O. A. (2025). Sleep, Neuroendocrine Disorders, And the Bidirectional Relationship between the Hypothalamic-Pituitary-Adrenal Axis: A Mini-Review. *Journal of Applied Sciences and Environmental Management, 29*(4), 1217-1227.

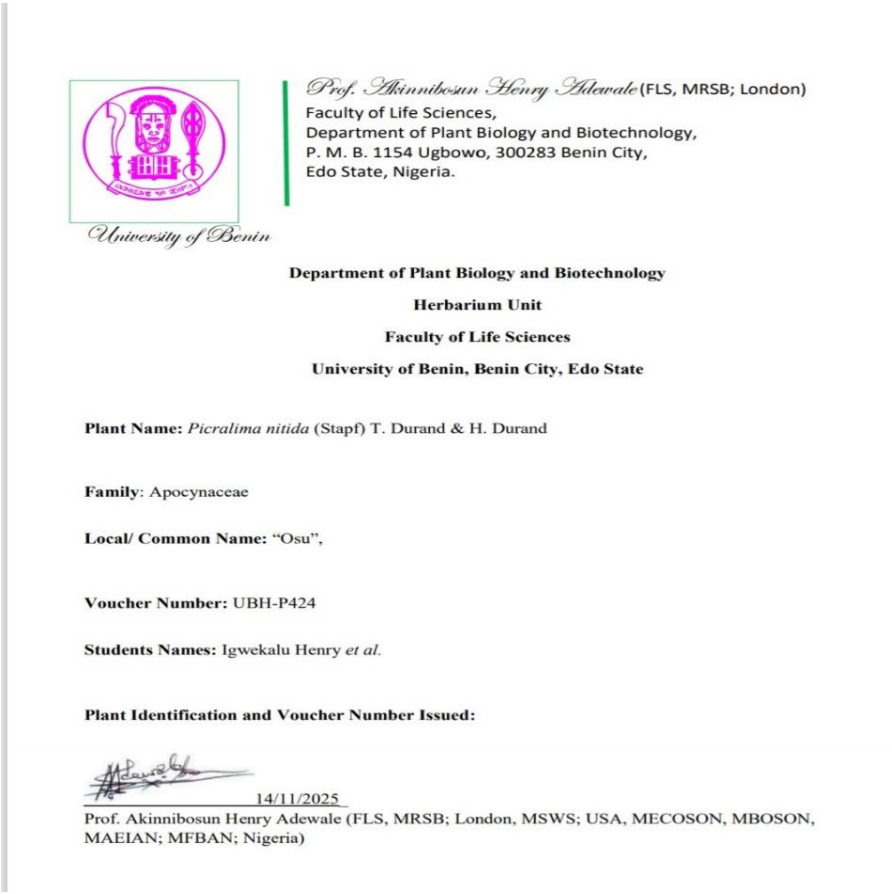
Zhu, B. T. (2022). Pathogenic mechanism of autoimmune diabetes mellitus in humans: potential role of streptozotocin-induced selective autoimmunity against human islet β-cells. *Cells, 11*(3), 492.

APPENDIX

Appendix I: Grouping and Identification of Rats

Groups	No of Rats	Weight Range (g)	Identification (specific body parts labelled with Genital Violet (GV)
G0	4	155.0 – 169.3	C1- Head C2- Head & Back C3- Leg & Back C4- Leg
G1	5	107.6 – 120.3	A1- Back & Tail A2- Tail A3- Plain A4- Two Legs A5- Head
G3	5	129.0 – 136.9	L1- Two legs L2- Tail & Back L3- Head & Tail L4- Two legs & Back L5- Back
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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Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
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*

Dried *Picralima* fruits

Grounded *Picralima nitida*

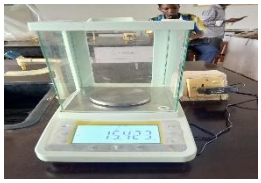
Extraction of *Picralima nitida*

Extract of *Picralima nitida*

Freeze-dried *Picralima. nitida*

Freeze-drying machine

Oven



Weighing Scale

Figure IV b: Acclimation of rats

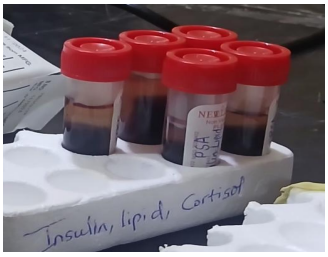
Figure IV c: Effect of *Picralima nitida* on cortisol concentrations in STZ induced diabetic male Wistar rats.

Parameters (µg/ml)	Group 0	Group 1	Group 3	Group 6
Cortisol	13.35 ± 2.45	18.5 ± 0.1$^{\#}$	10.3 ± 1.4$^{\#}$	10.15 ± 1.45$^{\#}$

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**