EVALUATION OF IN-VIVO ANTIDIABETIC ACTIVITY OF ETHANOL LEAF EXTRACT OF *PHYLLANTHUS AMARUS* IN STREPTOZOTOCIN-INDUCED DIABETES WISTAR RATS

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CERTIFICATION

This is to certify that this project was carried out by KOMOLAFE BLESSING OLUWAFUNMILOLA with matriculation number: HND/23/SLT/FT/0456 submitted to the Department of Science Laboratory Technology, Biochemistry Unit, institute of Applied Science (IAS), Kwara State Polytechnic, Ilorin, in partial fulfillment for the requirement of award of Higher National Diploma (HND) in Science Laboratory Technology (SLT)

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DEDICATION

I dedicated this project to the Father of all Grace, the Almighty God, the owner and administrator of the universe.

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ABSTRACT

*Phyllanthus amarus*is a medicinal plant traditionally used in the management of various ailments including diabetes mellitus. This present investigation was carried out to evaluate the antidiabetic activity of ethanolic extract of *Phyllanthus amarus* in streptozotocin (STZ) induced diabetic wister rats. Twenty five (25) wister rats were grouped in 5 groups of 5 rats each. Group 1 (Control) was administered distilled water, Group 2 (Diabetic control) was administered STZ 35mg/kg bwt), Group 3 (Standard group) was administered metformin (14.3mg/kg), Group 4 and 5 (PAE group), Group 4 and 5 was administered PAE D1 = 200mg and D2 = 400mg respectively. The blood glucose level and body weight of control and diabetic rats were monitored. In this present study, Preliminary phytochemical screening revealed the presence of phenol, saponin, Tannin, Alkaloids & Flavonoids. A significant loss ($p<0.05$) in body weight was observed in the untreated diabetic control compared to the treatment groups (PAED1 -21.4%), PAED2 -12.6%). There was a significant decrease in Hexokinase and G6PDH in the untreated rat compared to the treatment group(1.98±0.11, 1.94±0.06, 2.17±0.15) respectively. However a significant increase ($p<0.05$) was observed in G6PDH, Fructose-1,6-diphosphatase in treatment groups respectively compared to the untreated diabetic control. Hepatic biomarkers (ALT, AST & ALP) Shows a significant decrease in rats administered *Phyllanthus amarus* extract compared to untreated control with a reversal of a slight increase in activity of the enzymes. Histopathological results for this study shows the pictorial representation of the effect of *Phyllanthus amarus* extract on the pancreas. This study clearly shows that ethanolic leaf extract of *Phyllanthus amarus* possess potent antidiabetic activity.

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, affecting millions worldwide. Phyllanthus amarus, a medicinal plant, has been traditionally used to manage various ailments, including diabetes. This study aims to evaluate the antidiabetic potential of ethanolic extract of Phyllanthus amarus using streptozotocin (STZ)-induced diabetic rats. The investigation will assess the extract's efficacy in reducing blood glucose levels and explore its potential therapeutic benefits.

1.1 BACKGROUND OF THE STUDY

The exploration of the antidiabetic potential of Phyllanthus amarus is an exciting venture in the realm of natural medicine! This remarkable plant has long been a staple in traditional healing practices and is garnering renewed interest for its impressive ability to tackle diabetes.
Recent studies highlight the effectiveness of its ethanolic extract in lowering blood glucose levels, particularly in a well-established model of diabetes using streptozotocin-induced diabetic rats. Streptozotocin is notorious for targeting and damaging the insulin-producing beta cells of the pancreas, leading to elevated blood sugar levels. However, research shows that the ethanolic extract of Phyllanthus amarus can make a noteworthy difference. It has not only been found to significantly reduce blood glucose levels but also to enhance insulin sensitivity and mitigateoxidative stress in these diabetic rats. What's truly fascinating is the roster of bioactive compounds found in this plant—flavonoids, alkaloids, and phenolic acids—each playing a crucial role in its antidiabetic effects. These compounds boast powerful antioxidant, antiinflammatory, and insulin-sensitizing properties, which contribute to their effectiveness. In summary, the investigation into the in vivo antidiabetic activity of the ethanolic extract of Phyllanthus amarus in diabetic rats offers a promising glimpse into the potential of natural remedies for managing diabetes. This research not only paves the way for innovative treatments but also reinforces the value of exploring nature's bounty in our quest for health solutions.

1.2 JUSTIFICATION OF STUDY

The study, aims to rigorously evaluate the in vivo antidiabetic activity of the ethanolic extract of Phyllanthus amarus using the STZ-induced diabetic rat model. The findings of this research will contribute to the scientific validation of the traditional use of P. amarus for diabetes management. Furthermore, it may provide a basis for the development of novel, cost-effective, and potentially safer therapeutic

alternatives for diabetes, particularly relevant in regions like Oko Erin and Nigeria where access to conventional treatments may be limited. The identification

of effective plant-based remedies could significantly improve the quality of life for individuals affected by this chronic disease and alleviate the associated healthcare burden.

Despite the existing and ongoing research on the plant, only limited research has been carried out on antidiabetic potentials of aqueous extract of the plants leaves.

1.3 AIMS AND OBJECTIVES

AIMS OF THE STUDY

The aim of this study is to evaluate the in vivo antidiabetic activity of ethanol leaf extract of Phyllanthus amarus in streptozotocin induced diabetic wistar rats.

OBJECTIVES

- To carry out plant extraction
- To assess the preliminary phytochemical screening
- To assess the effect of Phyllanthus amarus on rats induced with STZ
- To assess the blood glucose level
- To assess the extract on hepatic enzyme
- To assess the extract on hepatic biomarkers (ALT, AST, ALP)
- To carry out histopathology analysis on the pancreas of the

diabetic and treated rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF DIABETES

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both (Pari *et al.,* 2004). Insulin, a hormone produced by the pancreas, plays a crucial role in regulating blood glucose by facilitating its uptake into cells for energy production. When insulin is either not produced in sufficient amounts or the body's cells become resistant to its effects, glucose accumulates in the bloodstream, leading to a range of acute and chronic complications (Naidu*et al.,* 2020).

There are primarily two main types of diabetes:

Type 1 Diabetes (T1D): This type is characterized by an absolute deficiency of insulin caused by the autoimmune destruction of the insulin-producing beta cells in the pancreas (Atkinson *et al.,* 2014). Individuals with T1D require lifelong insulin therapy to survive.

Type 2 Diabetes (T2D): This is the most common form of diabetes, accounting for approximately 90-95% of all cases (Olorunfemi, O .J. 2020). T2D is characterized by insulin resistance, where the body's cells do not respond effectively to insulin, and a relative insulin deficiency, where the pancreas may not produce enough insulin to overcome the resistance. Lifestyle factors such as obesity, physical inactivity, and genetics play a significant role in the development of T2D (Wilkin *et al.,* 2002).

Beyond these main types, other specific types of diabetes exist, such as gestational diabetes (diabetes that develops during pregnancy) and diabetes caused by specific genetic defects or medications (Olorunfemi, O. J. 2020).

The chronic hyperglycemia associated with diabetes can lead to serious long-term complications affecting various organs and systems, including:

Microvascular complications: These affect small blood vessels and include retinopathy (damage to the eyes), nephropathy (kidney disease), and neuropathy (nerve damage) (Fowler *et al.,* 2008).

Macrovascular complications: These affect large blood vessels and increase the risk of cardiovascular diseases such as coronary artery disease, stroke, and peripheral artery disease (Nathan *et al.,* 2009).

The management of diabetes typically involves a combination of lifestyle modifications (diet and exercise), medication (including insulin for T1D and often for T2D, as well as various oral and

injectable non-insulin agents for T2D), and regular monitoring of blood glucose levels (Hollander *et al.,* 2007). Early diagnosis and appropriate management are crucial to prevent or delay the onset and progression of diabetes-related complications, thereby improving the quality of life for individuals living with this condition (Nathan *et al.,* 2009).

2.1.1 EPIDEMIOLOGY OF DIABETES

The epidemiology of diabetes mellitus is a critical area of public health, given the increasing global prevalence and its significant impact on morbidity, mortality, and healthcare systems. Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, resulting from defects in insulin secretion, insulin action, or both (Sabiu *et al., 2016*).

GLOBAL PREVALENCE

The International Diabetes Federation (IDF) estimates that in 2024, approximately 589 million adults (20-79 years) are living with diabetes worldwide, representing 1 in 9 of the adult population. This number is projected to rise to 853 million by 2050 (Chattopadhyay *et al.,* 2005). A significant concern is that over 4 in 10 people with diabetes are unaware of their condition.

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Figure 1 Estimates of the global prevalence of diabetes worldwide (20–79-year age group). Results from the International Diabetes Federation Atlas, 10th edition.

TYPE 1 VS. TYPE 2 DIABETES

The vast majority (around 90%) of people with diabetes have type 2 diabetes, which is largely driven by a combination of genetic, environmental, socioeconomic, and demographic factors, including

increasing urbanization, aging populations, decreasing physical activity levels, and

rising rates of overweight and obesity . Type 1 diabetes, an autoimmune disease characterized by insulin deficiency, accounts for a smaller proportion of cases.

REGIONAL VARIATIONS

The prevalence of diabetes varies significantly across the globe. In 2024, the Western Pacific region has the highest number of adults living with diabetes, followed by South-East Asia. Low and middle-income countries are home to over 80% of adults with diabetes (Chattopadhyay *et al*., 2005).

RISK FACTORS

Several modifiable and non-modifiable risk factors contribute to the development of diabetes, particularly type 2 diabetes (Sabiu, *et al.,* 2016):

MODIFIABLE RISK FACTORS:

- Obesity and Overweight: Excess body weight, especially abdominal obesity, significantly increases insulin resistance.
- Physical Inactivity: Lack of regular exercise contributes to weight gain and impaired glucose metabolism.
- Unhealthy Diet: Diets high in calories, saturated fats, and sugars increase the risk. • Smoking: Smoking is associated with an increased risk of type 2 diabetes and its complications.
- High Blood Pressure: Hypertension often coexists with insulin resistance and increases diabetes risk.
- Abnormal Cholesterol and Triglyceride Levels: High LDL ("bad") cholesterol, low HDL ("good") cholesterol, and high triglycerides are linked to increased risk.

NON-MODIFIABLE RISK FACTORS:

- Age: The risk of type 2 diabetes increases with age, particularly after 45 years. • Family History: Having a parent, sibling, or close relative

with diabetes increases the risk. • Race/Ethnicity: Certain ethnic groups, including African Americans, Hispanic/Latino Americans, American Indians, Asian Americans, and Pacific Islanders, have a higher prevalence of type 2 diabetes.

- History of Gestational Diabetes: Women who had diabetes during pregnancy are at higher risk of developing type 2 diabetes later in life.
- Prediabetes: Individuals with blood glucose levels higher than normal but not yet in the diabetes range are at high risk of progression.

Polycystic Ovary Syndrome (PCOS): This hormonal disorder is associated with increased insulin resistance and diabetes risk in women.

TRENDS AND PROJECTIONS

The global prevalence of diabetes is steadily increasing, posing a significant challenge to healthcare systems worldwide. Factors such as population growth, aging, urbanization, and the adoption of unhealthy lifestyles are driving this increase (Adedapo, *et al.,* 2013). Projections indicate a substantial rise in the number of people with diabetes in the coming decades, particularly in low- and middle-income countries, highlighting the urgent need for effective prevention and management strategies.

2.2 TYPES OF DIABETES

There are several types of diabetes, including:

1. Type 1 Diabetes: An autoimmune disease where the body's immune system attacks and destroys insulin-producing beta cells in the pancreas.

2. Type 2 Diabetes: A metabolic disorder characterized by insulin resistance and impaired insulin secretion, often associated with lifestyle factors.

3. Gestational Diabetes: Develops during pregnancy, typically in the second or third trimester, due to hormonal changes and insulin resistance.

4. LADA (Latent Autoimmune Diabetes in Adults): A form of type 1 diabetes that develops in adults, often with a slower progression.

5. MODY (Maturity-Onset Diabetes of the Young): A rare genetic form of diabetes caused by mutations in specific genes.

2.2.1 TYPE 1 DIABETES

Type 1 diabetes (T1D) is an autoimmune disease in which the body's immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas (Adeyemi *et al.,* 2013). This leads to an absolute deficiency of insulin, a hormone essential for regulating blood glucose levels. T1D typically develops in children and young adults, but it can occur at any age (Yakubu *et al.,* 2022). It accounts for about 5-10% of all diagnosed cases of diabetes (Abu *et al*., 2022).

Causes and Risk Factors

The exact cause of T1D is not fully understood, but it is believed to involve a combination of genetic and environmental factors (Innocent, *et al.,* 2022).

- Autoimmune Reaction: The body's immune system attacks and destroys the insulinproducing beta cells in the pancreas.
- Genetic Predisposition: Certain genes increase the risk of developing T1D. Having a parent, sibling, or close relative with T1D slightly elevates the risk (Ezeugwunne *et al.,* 2018).
- Environmental Triggers: Factors such as viral infections may trigger the autoimmune destruction of beta cells in genetically susceptible individuals (Kota, 2018).

Known Risk Factors:

- Family History: As mentioned above, having a family member with T1D increases the risk (Ogbodo, *et al.,* 2018).
- Genetics: The presence of certain genes is associated with a higher risk (Amah *et al*., 2018).
- Age: While it can occur at any age, it is most often diagnosed in children, teens, and young adults (Amah *et al.,* 2018).

2.2.1.2 PATHOPHYSIOLOGY OF TYPE 1 DIABETES

The pathophysiology of type 1 diabetes mellitus (T1D) is characterized by the autoimmune destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans (Bando, *et al.,* 2021). This leads to an absolute deficiency of insulin, resulting in hyperglycemia and the metabolic disturbances characteristic of the disease (Bando, *et al.,* 2021).

1. Genetic Predisposition

T1D has a strong genetic component, with the Human Leukocyte Antigen (HLA) region on chromosome 6 contributing significantly (around 40-50%) to the genetic risk (Abu*et al*., 2021). Certain HLA alleles, particularly HLA-DR3-DQ2 and HLA-DR4-DQ8, are strongly associated with an increased susceptibility to T1D (Abu *et al*., 2021). Conversely, some HLA alleles, like HLADR15-DQ6, appear to be protective (Umaru, *et al.,* 2021).

However, having these susceptibility genes does not guarantee the development of T1D, highlighting the role of other factors (Kota, *et al.,* 2018). Other non-HLA genes, such as the insulin gene (INS), protein tyrosine phosphatase non-receptor type 22 (PTPN22), and cytotoxic T-lymphocyte-associated protein 4 (CTLA4), also contribute to the overall genetic risk (Abu *et al*., 2021).

2. Environmental Triggers

While genetics creates a susceptibility, environmental factors are thought to trigger the autoimmune process in genetically predisposed individuals (Analike, *et al.,* 2018). However, no single environmental trigger has been definitively identified (Analike, *et al.,* 2018).

Potential environmental triggers under investigation include:

- Viral Infections: Certain viruses, such as enteroviruses, rubella, mumps, and coxsackie viruses, have been implicated in triggering the autoimmune response (Mbaeri *et al*., 2018). The "molecular mimicry" hypothesis suggests that viral proteins may share similarities with beta-cell antigens, leading the immune

system to mistakenly attack the pancreas after fighting the infection (Mbaeri*et al., 2018*).

- Dietary Factors: Early exposure to cow's milk proteins and gluten has been investigated, but conclusive evidence is lacking (Mbaeri *et al., 2018*). Breastfeeding and the timing of introducing solid foods are also areas of research.
- Gut Microbiota: Alterations in the composition and function of the gut microbiome are being studied for their potential role in influencing the immune system and contributing to T1D development (Mbaeri *et al*., 2018).
- Hygiene Hypothesis: This theory suggests that reduced exposure to infections in early childhood may lead to an imbalance in the immune system, increasing the risk of autoimmune diseases like T1D (Mbaeri *et al.,* 2018).

Other Factors: Factors like toxins, vitamin D levels, and even psychological stress have also been explored, but their role remains unclear (Chukwuma *et al.,* 2022).

3. Autoimmune Destruction of Beta Cells

The hallmark of T1D is the immune-mediated destruction of pancreatic beta cells (Yakubu, *et al.,* 2022). This process is primarily driven by autoreactive T cells, specifically CD8+ cytotoxic T cells, which directly attack and destroy the beta cells (Yakubu,*et al.,* 2022). CD4+ helper T cells also play a role in orchestrating the immune response (Mbaeri *et al.,* 2018).

This autoimmune attack is characterized by insulitis, the infiltration of immune cells into the pancreatic islets (Adeyemi, *et al.,* 2013). Various autoantibodies targeting beta-cell antigens, such as glutamic acid decarboxylase (GAD65), insulin, islet cell antigens (ICA), insulinomaassociated protein 2 (IA-2), and zinc transporter 8 (ZnT8), can be detected in the blood years before the clinical onset of T1D (Ogbodo*et al.,* 2018). These autoantibodies serve as markers of the ongoing autoimmune destruction.

The destruction of beta cells progresses over time. Clinical symptoms of diabetes typically appear when a significant portion (around 70-80%) of beta cells have been destroyed, leading to

a critical deficiency in insulin production and subsequent hyperglycemia (Olorunfemi, O. J. 2020).

4. Metabolic Consequences of Insulin Deficiency

The absolute deficiency of insulin leads to profound metabolic derangements (Olorunfemi, O. J. 2020):

- Hyperglycemia: Glucose cannot enter cells for energy utilization, leading to elevated blood glucose levels.
- Increased Hepatic Glucose Production: The liver, no longer suppressed by insulin, produces excessive glucose through glycogenolysis and gluconeogenesis, further exacerbating hyperglycemia.
- Impaired Glucose Uptake: Insulin's role in facilitating glucose uptake by peripheral tissues (muscle and adipose tissue) is lost.
- Lipolysis and Ketogenesis: In the absence of sufficient insulin, the body breaks down fat for energy, leading to the production of ketones. Excessive ketone production can result in diabetic ketoacidosis (DKA), a life-threatening complication (Olorunfemi, O. J. 2020).
- Protein Catabolism: Insulin deficiency also leads to increased protein breakdown and impaired protein synthesis.

In summary, the pathophysiology of T1D involves a complex interplay of genetic susceptibility and environmental triggers that initiate an autoimmune attack on the pancreatic beta cells. This progressive destruction leads to insulin deficiency and the characteristic metabolic abnormalities of type 1 diabetes. (Vernal *et al.,* 2017).

2.2.1.3 SYMPTOMS AND DIAGNOSIS OF TYPE 1 DIABETES

Symptoms

The symptoms of T1D can develop quite rapidly, often over a few weeks or months, and can be severe (Vernal *et al.,* 2017).

- Common Symptoms Include:
- Increased thirst (polydipsia)
- Frequent urination (polyuria), possibly including bedwetting in children who previously did not wet the bed
- Increased hunger (polyphagia)

- Unexplained weight loss despite increased appetite
- Fatigue and weakness

- Blurred vision

In some cases, nausea, vomiting, and abdominal pain can occur, which may be signs of diabetic ketoacidosis (DKA), a serious complication (Vernal *et al.,* 2017).

Diagnosis

Diagnosis of T1D typically involves blood tests to measure blood glucose levels. These tests may include (Olorunfemi, O. J. 2020):

- Fasting Plasma Glucose (FPG) test: Blood glucose level of 126 mg/dL (7 mmol/L) or higher on two separate occasions.
- Random Plasma Glucose test: Blood glucose level of 200 mg/dL (11.1 mmol/L) or higher, along with diabetes symptoms.
- Hemoglobin A1c (A1c) test: An average of blood glucose levels over the past 2-3 months. An A1c of 6.5% or higher indicates diabetes.
- Ketone testing: Urine or blood tests to detect ketones, which may be present in high levels in T1D, especially if not well-controlled.
    - Autoantibody testing: Blood tests to check for specific autoantibodies that are often present in T1D, helping to distinguish it from type 2 diabetes (Naidu, *et al.,* 2020).

2.2.1.4 MANAGEMENT OF TYPE 1 DIABETES

The primary treatment for T1D is insulin replacement therapy because the body no longer produces enough insulin on its own (Chattopadhyay, *et al.,* 2005).

1. Insulin Therapy: This is essential for survival and blood glucose control. Insulin can be administered through:

- Multiple Daily Injections (MDI): Using syringes or insulin pens. This often involves a combination of long-acting (basal) insulin and rapid-acting (bolus) insulin before meals. • Insulin Pump: A small, wearable device that continuously delivers insulin under the skin. • Inhaled Insulin: A rapid-acting form of insulin that is inhaled (for adults).

2. Blood Glucose Monitoring: Regular monitoring of blood glucose levels using a blood glucose meter or a continuous glucose monitor (CGM) is crucial for adjusting insulin doses and preventing complications.

- Carbohydrate Counting: Learning to match insulin doses to carbohydrate intake from meals is a key skill in managing blood glucose levels.

- Healthy Lifestyle: This includes a balanced diet, regular physical activity, and maintaining a healthy weight, which can improve insulin sensitivity and overall health.

3. Regular Medical Care: Consistent follow-up with a diabetes care team, including doctors, diabetes educators, and dietitians, is vital for optimal management and to monitor for potential complications.

4. Artificial Pancreas Systems (Hybrid Closed-Loop Systems): These systems integrate a CGM with an insulin pump to automatically adjust insulin delivery based on real-time glucose levels, helping to improve glucose control and reduce the burden of management (Olorunfemi, O. J. 2020).

2.2.2 TYPE 2 DIABETES

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by hyperglycemia (high blood sugar) resulting from the body's ineffective use of insulin. This can be due to the body not producing enough insulin or the cells becoming resistant to the insulin that is produced (Naidu, *et al.,* 2020). T2D accounts for the vast majority (around 90%) of diabetes cases worldwide and is strongly linked to lifestyle factors (Pari, *et al*., 2004).

2.2.2.1 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

The development of T2D is complex and involves a combination of insulin resistance and betacell dysfunction (Sabiu, *et al.,* 2016).

1. Insulin Resistance:

Reduced Tissue Sensitivity: In T2D, cells in the muscle, liver, and fat tissues become resistant to the effects of insulin. This means that insulin

is less effective at stimulating glucose uptake from the bloodstream into these cells for energy or storage (Olorunfemi, O. J. 2022).

Impaired Glucose Uptake: Normally, insulin binds to receptors on cell surfaces, triggering a cascade that leads to the translocation of glucose transporter type 4 (GLUT4) to the cell membrane, facilitating glucose entry. In insulin-resistant states, this process is impaired (Adedapo, *et al.,* 2022).

Increased Hepatic Glucose Production: Insulin normally suppresses glucose production by the liver. In T2D, this suppression is reduced, leading to the liver releasing excessive glucose into the bloodstream, further contributing to hyperglycemia (Olorunfemi, O. J. 2022).

Contributing Factors: Obesity, particularly visceral fat, physical inactivity, genetics, and chronic inflammation are major contributors to insulin resistance (Olorunfemi, O.J. 2022). Adipose tissue releases various hormones and cytokines that can interfere with insulin signaling (Yakubu, *et al.,* 2022).

2. Beta-Cell Dysfunction:

Impaired Insulin Secretion: The pancreatic beta cells, which produce insulin, gradually lose their ability to secrete sufficient insulin to overcome insulin resistance and maintain normal blood glucose levels (Yakubu, *et al.,* 2022).

Progressive Decline: Over time, the demands on the beta cells increase due to insulin resistance, leading to their eventual exhaustion and a progressive decline in insulin production (Ezeugwunne, *et al.,* 2018).

Contributing Factors: Factors contributing to beta-cell dysfunction include glucotoxicity (chronic exposure to high glucose levels), lipotoxicity (chronic exposure to high fatty acid levels), genetic predisposition, and inflammation (Analike, *et al.,* 2018).

In essence, in T2D, the body becomes less responsive to insulin, and the pancreas is unable to produce enough insulin to compensate for this resistance, leading to elevated blood glucose levels.

2.2.2.2 SYMPTOMS AND DIAGNOSIS OF TYPE 2 DIABETES

Symptoms of T2D often develop slowly over years, and many people may be unaware they have the condition for a long time (Kota, *et al.,* 2018).

Common Symptoms Include:

- Increased thirst (polydipsia)
- Frequent urination (polyuria)
- Increased hunger (polyphagia)
- Unexplained weight loss
- Fatigue
- Blurred vision
- Slow-healing sores or frequent infections
- Areas of darkened skin, often in the armpits and neck (acanthosis nigricans)
- Numbness or tingling in the hands or feet (neuropathy)

Diagnosis:

T2D is typically diagnosed through blood tests that measure blood glucose levels (Kota,*et al.,*. 2018):

- Glycated Hemoglobin (A1c) Test: This test measures the average blood sugar level over the past 2-3 months. An A1c of 6.5% or higher on two separate tests indicates diabetes.
- Fasting Plasma Glucose (FPG) Test: Blood glucose level is measured after an overnight fast (at least 8 hours). A level of 126 mg/dL (7 mmol/L) or higher on two separate occasions indicates diabetes.
- Random Plasma Glucose Test: Blood glucose level is checked at any time, without regard to when the person last ate. A level of 200 mg/dL (11.1 mmol/L) or higher, along with diabetes symptoms, suggests diabetes.
- Oral Glucose Tolerance Test (OGTT): This test involves fasting overnight, drinking a sugary liquid, and having blood glucose levels checked periodically over the next two hours. A level of 200 mg/dL (11.1 mmol/L) or higher after 2 hours indicates diabetes.

2.2.2.3 MANAGEMENT OF TYPE 2 DIABETES

The management of T2D focuses on achieving and maintaining target blood glucose levels, preventing long-term complications, and improving overall health (NHS, 2024; ADA, 2024). This typically involves a combination of lifestyle modifications and medications.

1. Lifestyle Modifications:

- Healthy Eating: Following a balanced diet that is rich in fruits, vegetables, whole grains, and lean protein, while limiting sugary drinks, processed foods, and saturated fats, is crucial (Yakubu, *et al.,* 2021). Portion control and consistent meal timing are also important.
- Regular Physical Activity: Aiming for at least 150 minutes of moderate-intensity aerobic exercise per week, along with muscle-strengthening activities at least two days a week, improves insulin sensitivity and helps manage blood glucose levels (Yakubu,*et al.,* 2021).
- Weight Management: Losing even a modest amount of weight (5-10% of body weight) can significantly improve blood glucose control, blood pressure, and cholesterol levels in overweight or obese individuals with T2D (Zephaniah, *et al.,* 2021).
- Smoking Cessation: Smoking worsens insulin resistance and increases the risk of diabetes complications (Zephaniah, *et al.,* 2021).
- Stress Management: Chronic stress can affect blood glucose levels. Techniques like yoga, meditation, and adequate sleep can be beneficial.

2. Medications:

If lifestyle modifications alone are not sufficient to achieve target blood glucose levels, medications are typically prescribed. These can include (Umaru, *et al.,* 2021):

- Metformin: Often the first-line medication, it works by reducing glucose production in the liver and improving insulin sensitivity.
- Sulfonylureas (e.g., glipizide, glyburide): Stimulate the pancreas to release more insulin.

- Meglitinides (e.g., repaglinide, nateglinide): Also stimulate insulin release but have a shorter duration of action.
- Thiazolidinediones (TZDs) (e.g., pioglitazone, rosiglitazone): Improve insulin sensitivity in peripheral tissues.
- DPP-4 Inhibitors (e.g., sitagliptin, saxagliptin): Help increase insulin release and decrease glucagon secretion.
- GLP-1 Receptor Agonists (e.g., liraglutide, semaglutide): Increase insulin release, decrease glucagon secretion, slow gastric emptying, and can promote weight loss. • SGLT2 Inhibitors (e.g., canagliflozin, dapagliflozin): Increase glucose excretion in the urine.
- Insulin: May be necessary at any stage of T2D if blood glucose control cannot be achieved with other medications. It is administered through injections or an insulin pump.

3. Regular Monitoring:

Self-Monitoring of Blood Glucose (SMBG): Using a blood glucose meter to check blood sugar levels at home helps individuals understand how food, activity, and medications affect their glucose levels.

Continuous Glucose Monitoring (CGM): Some individuals may use CGMs, which continuously track glucose levels and provide real-time data.

A1c Testing: Regular A1c tests (usually every 3-6 months) provide an overview of long-term blood glucose control.

4. Education and Support:

Diabetes self-management education and support (DSMES) are crucial for empowering individuals with T2D to manage their condition effectively. This includes learning about healthy eating, physical activity, medication use, blood glucose monitoring, and preventing complications (Kota, *et al.,* 2018).

5. Management of Complications:

Regular screening and management of potential long-term complications, such as cardiovascular disease, kidney disease, nerve damage, and eye damage, are integral to T2D care.

Effective management of T2D requires a lifelong commitment to healthy habits, regular medical care, and adherence to the prescribed treatment plan. (Tiwari *et al.,* 2017).

2.2.3 GESTATIONAL DIABETES

Gestational diabetes is a type of diabetes that develops during pregnancy in women who didn't have diabetes before. It's characterized by high blood sugar levels that can affect both the mother and the baby's health. In GDM, the hormones produced by the placenta can block the mother's insulin from working effectively, leading to insulin resistance (Adedapo, *et al.,* 2022).

2.2.3.1 PATHOPHYSIOLOGY OF GESTATIONAL DIABETES

The exact cause of GDM isn't fully understood, but it arises from a combination of factors related to pregnancy (Olorunfemi, O. J. 2020):

Insulin Resistance: During pregnancy, the placenta produces hormones like human placental lactogen (hPL), cortisol, estrogen, and progesterone. These hormones interfere with the action of insulin, making the mother's body less sensitive to it. This is a normal physiological change to ensure the baby gets enough glucose (Sabiu, *et al.,* 2016).

- Inadequate Beta-Cell Compensation: In a normal pregnancy, the pancreas can produce extra insulin to overcome this insulin resistance and maintain normal blood sugar levels. However, in GDM, the pancreatic beta cells may not be able to produce enough insulin to meet the increased demand, leading to hyperglycemia (Sabiu, *et al.,* 2016).
- Other Factors: Increased maternal weight and obesity before pregnancy can exacerbate insulin resistance. Genetic factors and a family history of diabetes may also play a role (Sunmonu, *et al.,* 2016). Some studies also suggest that reduced incretin hormone secretion and signaling may contribute (Pari, *et al.,* 2021).

Essentially, GDM develops when the physiological insulin resistance of pregnancy is not adequately compensated for by increased insulin secretion, resulting in elevated blood glucose levels.

2.2..3.2 SYMPTOMS AND DIAGNOSIS OF GESTATIONAL DIABETES

Often, GDM doesn't cause noticeable symptoms (Olorunfemi, O. J. 2020). When symptoms do occur, they are usually mild and can be similar to normal pregnancy changes, which is why routine screening is crucial.

Possible Mild Symptoms:

- Increased thirst
- Frequent urination
- Fatigue
- Nausea
- Blurred vision
- Recurrent infections, such as yeast infections

Diagnosis:

Universal screening for GDM is typically performed between 24 and 28 weeks of gestation (Sabiu, *et al.,* 2016). The most common screening methods involve a two-step or a one-step approach using an oral glucose tolerance test (OGTT).

Two-Step Approach:

Glucose Challenge Test (GCT): The pregnant woman drinks a sugary solution (usually 50 grams of glucose), and blood sugar is checked one hour later. A high result (usually ≥130-140 mg/dL) indicates the need for a follow-up test.

Oral Glucose Tolerance Test (OGTT): After an overnight fast, the woman drinks a more concentrated glucose solution (usually 75 or 100 grams). Blood sugar levels are checked at specific intervals (e.g., fasting, 1 hour, 2 hours, and sometimes 3 hours). GDM is diagnosed if two or more of the blood glucose values meet or exceed specific threshold.

One-Step Approach: Some providers may directly perform a 75-gram OGTT with blood glucose measurements taken at fasting, 1 hour, and 2 hours. GDM is diagnosed if any of these values meet or exceed specific thresholds ().

Women with high risk factors for GDM (e.g., obesity, previous GDM, family history of diabetes) may be screened earlier in pregnancy.

2.2.3.3 MANAGEMENT OF GESTATIONAL DIABETES

The primary goal of GDM management is to maintain blood glucose levels within a target range to ensure the health of both the mother and the baby. Management strategies typically include:

- Lifestyle Modifications:

Healthy Eating Plan: Working with a registered dietitian to develop a balanced meal plan that focuses on whole grains, fruits, vegetables, lean proteins, and healthy fats. It's important to control carbohydrate intake and distribute it throughout the day with regular meals and snacks. Avoiding sugary drinks and processed foods is crucial.

- Regular Physical Activity: Moderate-intensity exercises, such as brisk walking, swimming, or prenatal yoga, for at least 30 minutes most days of the week can help improve insulin sensitivity and manage blood glucose levels. It's essential to consult with a healthcare provider before starting any new exercise program during pregnancy.
- Blood Glucose Monitoring: Regular self-monitoring of blood glucose (SMBG) using a glucose meter is usually recommended to track blood sugar levels and assess the effectiveness of diet and exercise. Target blood glucose levels are typicall.

Fasting: ≤ 95 mg/dL

1 hour after a meal: ≤ 140 mg/dL

2 hours after a meal: ≤ 120 mg/dL

Error! Filename not specified. Medications: If lifestyle modifications are not sufficient to achieve target blood glucose levels, medication may be necessary (NHS, 2024; NIDDK, 2024).

Insulin: Insulin injections are the preferred medication for managing GDM as it does not cross the placenta and is safe for the baby. Different types of insulin may be used based on individual needs.

Oral Medications: In some cases, oral medications like metformin or glyburide may be considered, but their safety and long-term effects on the baby are still being studied. Insulin is generally the first-line pharmacological treatment.

Regular Prenatal Care: Frequent check-ups with the healthcare team are essential to monitor blood glucose levels, adjust the management plan as needed, and monitor the baby's growth and well-being through ultrasounds and other tests.

Labor and Delivery Management: Blood glucose levels will be closely monitored during labor and delivery. Insulin may be administered intravenously to maintain stable blood sugar levels.

Postpartum Care:

GDM usually resolves after delivery. However, women who have had GDM have a higher risk of developing type 2 diabetes later in life. Therefore, postpartum follow-up, including a repeat glucose tolerance test 6-12 weeks after delivery and ongoing screening for type 2 diabetes, is crucial. Lifestyle modifications adopted during pregnancy should be continued to reduce the long-term risk of diabetes.

2.3 GLUCOSE METABOLISM AND ITS REGULATIONS

Glucose is the primary metabolic fuel for mammals, serving as a vital energy source for cells throughout the body. Its metabolism is a complex and tightly regulated process involving several interconnected pathways and a sophisticated network of hormonal and enzymatic controls. Disruptions in glucose metabolism can lead to various health issues, most notably diabetes mellitus.

2.3.1 GLUCOSE METABOLISM PATHWAYS

Glucose metabolism encompasses a series of biochemical reactions that break down, synthesize, and store glucose to meet the body's energy demands. The major pathways include:

Error! Filename not specified.Glycolysis: This is the universal pathway for glucose catabolism, occurring in the cytoplasm of nearly all cells. In glycolysis, a six-carbon glucose molecule is broken down into two three-carbon pyruvate molecules, generating a small amount of ATP and NADH. This process consists of ten enzyme-catalyzed steps and can occur in both aerobic and anaerobic conditions.

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Fig 2: Glycolysis Diagram: Overview of the aerobic glycolysis pathway Source: Armaan Naghdi (2024).

Gluconeogenesis (GNG): This anabolic pathway involves the synthesis of glucose from non-carbohydrate precursors, such as lactate, amino acids, and glycerol. It primarily occurs in the liver and, to a lesser extent, in the renal cortex, and is crucial for maintaining blood glucose levels during fasting or prolonged exercise. Gluconeogenesis bypasses the irreversible steps of glycolysis through different enzymes.

Error! Filename not specified. Fig 3: Gluconeogenesis pathway Source: James Sheldon (2024).

Glycogenesis: This is the process of synthesizing glycogen, the stored form of glucose, primarily in the liver and skeletal

muscle. When excess glucose is available, it is converted into glycogen for future energy needs.

- **Glycogenolysis:** This pathway involves the breakdown of stored glycogen into glucose. This process occurs in the liver, muscles, and kidneys to release glucose when the body needs energy, such as during fasting.
- **Pentose Phosphate Pathway (PPP):** Also known as the hexose monophosphate shunt, this is an alternative pathway for glucose oxidation. It occurs in the cytoplasm of various cells (e.g., liver, adipose tissue, red blood cells) and produces NADPH (essential for reductive biosynthesis and protecting against oxidative stress) and pentose sugars (precursors for nucleotide synthesis).

CELLULAR UPTAKE OF GLUCOSE

For glucose to be utilized by cells, it must be transported across the cell membrane. This process is primarily facilitated by specialized membrane proteins called glucose transporters (GLUTs).

Facilitated Diffusion (via GLUTs): Most glucose uptake occurs through facilitated diffusion, a passive process where GLUT proteins transport glucose down its concentration gradient.

- GLUT4: This transporter is highly abundant in skeletal muscle and adipose tissue and is primarily responsible for insulin-stimulated glucose uptake. In the absence of insulin, GLUT4 resides in intracellular vesicles. Upon insulin binding to its receptor, a signaling cascade triggers the translocation of GLUT4-containing vesicles to the plasma membrane, increasing glucose uptake.
- Other GLUTs: Other GLUT isoforms (e.g., GLUT1, GLUT2, GLUT3) have different tissue distributions and roles in basal glucose uptake and transport [5.3].
- Secondary Active Transport (via SGLTs): In certain tissues, like the kidneys and intestines, glucose is transported against its concentration gradient through sodium-glucose cotransporters (SGLTs). This process is driven by the electrochemical gradient of sodium ions, which is maintained by the Na+/K+-ATPase pump.

2.3.2 REGULATION OF GLUCOSE METABOLISM

The regulation of glucose metabolism is crucial for maintaining glucose homeostasis, a tightly controlled balance of blood glucose levels [2.2, 3.2]. This involves a complex interplay of hormones, allosteric control of enzymes, and gene expression.

1. Hormonal Regulation

The pancreas plays a central role in hormonal regulation through the secretion of insulin and glucagon .

- Insulin: Produced by the beta cells of the pancreas, insulin is an anabolic hormone released in response to high blood glucose levels (e.g., after a meal). Its primary actions include:
  - Increased glucose uptake: Insulin stimulates glucose uptake by insulin-sensitive tissues (muscle and adipose tissue) by promoting GLUT4 translocation to the cell membrane. • Promoted glycogenesis: Insulin stimulates the liver and muscle to convert excess glucose into glycogen for storage.
- Inhibited gluconeogenesis and glycogenolysis: Insulin suppresses glucose production by the liver.
- Enhanced fatty acid and protein synthesis: Insulin also promotes the storage of energy in the form of fats and proteins
  - Glucagon: Secreted by the alpha cells of the pancreas, glucagon is a catabolic hormone released when blood glucose levels are low (e.g., during fasting). Its main effects are: • Stimulated glycogenolysis: Glucagon promotes the breakdown of liver glycogen into glucose, which is then released into the bloodstream.
- Stimulated gluconeogenesis: Glucagon enhances the synthesis of glucose from noncarbohydrate precursors in the liver.
  - Inhibited glycolysis: In the liver, glucagon inhibits glycolysis

to conserve glucose. Other Hormones:

- Amylin: Co-secreted with insulin from beta cells, amylin inhibits glucagon secretion, slows gastric emptying, and increases satiety.

- Incretins (e.g., GLP-1, GIP): Hormones produced in the small intestine in response to food intake. They stimulate insulin secretion, suppress glucagon, and slow gastric emptying..
- Epinephrine (Adrenaline): Released during stress or exercise, epinephrine promotes glycogenolysis and glycolysis in muscle, providing immediate energy.

Cortisol: A glucocorticoid hormone that generally raises blood glucose by promoting gluconeogenesis and decreasing glucose utilization in peripheral tissues. • Thyroid hormones (T3 and T4): Influence the basal metabolic rate and affect glucose metabolism by increasing glucose absorption from the gut and enhancing glucose utilization.

2. Enzymatic Regulation (Allosteric Control and Covalent Modification)

Key enzymes in glucose metabolic pathways are subject to intricate regulation to control flux through the pathways. This regulation occurs both acutely (seconds to minutes) through allosteric control and post-translational modifications (like phosphorylation), and chronically (hours to days) through changes in gene expression.

Glycolysis Regulatory Enzymes:

- Hexokinase (or Glucokinase in liver/pancreas): Catalyzes the phosphorylation of glucose to glucose-6-phosphate, trapping glucose inside the cell. Hexokinase is inhibited by its product, glucose-6-phosphate, while glucokinase, with a higher Km, acts as a glucose sensor in the liver and is not subject to product inhibition.
- Phosphofructokinase-1 (PFK-1): The most important regulatory enzyme in glycolysis, catalyzing the committed step. PFK-1 is allosterically activated by AMP, ADP, and fructose-2,6-bisphosphate (a potent activator), and inhibited by ATP and citrate, reflecting the cell's energy status.
- Pyruvate Kinase: Catalyzes the final step of glycolysis, forming pyruvate and ATP. It is activated by fructose-1,6-bisphosphate (feed-forward activation) and inhibited by ATP and alanine. In the liver, pyruvate kinase activity is downregulated by glucagonmediated phosphorylation.

- Gluconeogenesis Regulatory Enzymes: Gluconeogenesis bypasses the irreversible steps of glycolysis using distinct enzymes, which are reciprocally regulated with glycolytic enzymes to prevent futile cycles.
- Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase (PEPCK): Bypass pyruvate kinase.
- Fructose-1,6-bisphosphatase (FBPase-1): Bypasses PFK-1. It is inhibited by fructose2,6bisphosphate and AMP, and activated by citrate.
- Glucose-6-phosphatase: Bypasses hexokinase. This enzyme is present mainly in the liver, intestinal epithelial cells, and renal tubular epithelial cells, allowing these organs to release free glucose into the bloodstream.

3. Cellular Energy State

The cellular energy state, reflected by the ATP:AMP ratio, directly impacts enzyme activity. High ATP levels generally inhibit catabolic pathways (like glycolysis) and promote anabolic pathways (like gluconeogenesis), while high AMP levels have the opposite effect.

4. Transcriptional Regulation

Long-term regulation of glucose metabolism involves controlling the expression of genes encoding metabolic enzymes through various transcription factors. (Coulibaly *et al.,* 2023).

2.4 ROLE OF OXIDATIVE STRESS IN PATHOPHYSIOLOGY OF DIABETES

Oxidative stress plays a pivotal and multifaceted role in the pathophysiology of diabetes mellitus (DM), contributing to both its development and the progression of its debilitating complications. It arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense systems.

1. Mechanisms of Oxidative Stress in Diabetes:

- Hyperglycemia: Chronic high blood sugar is a primary driver of oxidative stress in diabetes. Several pathways are activated by elevated glucose levels, leading to increased ROS production:

- Mitochondrial Dysfunction: Impaired electron transport chain within the mitochondria, particularly in endothelial cells, leads to an overproduction of superoxide radicals. This is considered a "unifying mechanism" for hyperglycemia's vascular damage.
- Advanced Glycation End-products (AGEs): Glucose reacts non-enzymatically with proteins and lipids to form AGEs. AGEs not only directly damage tissues but also activate cellular receptors (RAGE), leading to further ROS generation and inflammation.
  - Polyol Pathway Activation: This metabolic pathway, when overactive due to high glucose, consumes NADPH, a crucial cofactor for the regeneration of reduced glutathione (GSH), a major endogenous antioxidant. This depletion further exacerbates oxidative stress.
- Protein Kinase C (PKC) Activation: Hyperglycemia activates PKC, which can lead to increased ROS production and contributes to endothelial dysfunction.
- Glucose Auto-oxidation: Glucose itself can directly auto-oxidize, generating reactive oxygen species.

Other contributing factors: Hyperlipidemia, inflammation, and reduced antioxidant capacity also contribute to oxidative stress in diabetes.

2. Impact on Diabetes Development:

Impaired Insulin Production and Secretion: Oxidative stress damages pancreatic betacells, which are responsible for insulin production. This damage can lead to beta-cell dysfunction and even apoptosis (programmed cell death), further compromising insulin secretion and contributing to both type 1 and type 2 diabetes.

- Increased Insulin Resistance: ROS can interfere with insulin signaling pathways, making cells less responsive to insulin's effects. This leads to impaired glucose uptake by tissues and contributes to hyperglycemia.

3. Role in Diabetic Complications:

Oxidative stress is a major contributor to both microvascular (small blood vessel) and macrovascular (large blood vessel) complications of diabetes:

Microvascular Complications:

Diabetic Retinopathy: Damages retinal cells and blood vessels, leading to vision impairment and blindness. Oxidative stress promotes pericyte apoptosis (cells crucial for retinal homeostasis) and activates inflammatory pathways.

Diabetic Nephropathy: Damages kidney cells and structures, leading to impaired renal function and potential kidney failure.

Diabetic Neuropathy: Damages nerve cells, causing pain, numbness,

and impaired sensations. Macrovascular Complications:

Atherosclerosis: Oxidative stress plays a fundamental role in the development and progression of atherosclerosis, a hardening and narrowing of arteries that increases the risk of heart attack and stroke. ROS oxidize LDL (bad cholesterol), promoting foam cell formation and plaque development. It also perpetuates inflammation and endothelial dysfunction, crucial for plaque instability.

Cardiomyopathy: Oxidative stress contributes to damage in the heart muscle, leading to impaired heart function.

4. Impaired Antioxidant Defense Systems in Diabetes:Diabetic patients often exhibit a compromised antioxidant defense system. This can include:

Reduced levels of non-enzymatic antioxidants like vitamin C, vitamin E, and glutathione (GSH).

Decreased activity of enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx).

This weakened defense makes the body more vulnerable to the damaging effects of ROS, creating a vicious cycle where increased ROS production and decreased scavenging capacity exacerbate oxidative stress.

5. Therapeutic Implications: Understanding the role of oxidative stress in diabetes opens avenues for therapeutic interventions. Strategies aiming to mitigate oxidative damage include:

Controlling Hyperglycemia: Effective glycemic control is crucial to reduce the primary source of ROS production.

Antioxidant Supplementation: While promising in preclinical studies, the efficacy and safety of antioxidant supplementation in human diabetes still require further clinical validation.

Lifestyle Modifications: Regular exercise and a healthy diet can enhance endogenous antioxidant defenses and improve overall metabolic health, thereby reducing oxidative stress.

Targeting Specific Pathways: Research is ongoing to develop therapies that specifically target the pathways involved in ROS production (e.g., inhibitors of specific enzymes) or enhance antioxidant responses (e.g., activating Nrf2 or SIRT1 pathways).

In conclusion, oxidative stress is a central pathophysiological factor in diabetes, influencing its onset, progression, and the development of severe complications by damaging various cells and tissues, impairing insulin function, and perpetuating inflammation.

2.5 MECHANISM OF STZ IN INDUCTION OF DIABETES STUDY

Streptozotocin (STZ) is a widely used chemical agent for inducing experimental diabetes mellitus in laboratory animals, particularly rodents. It specifically targets and destroys insulinproducing pancreatic β-cells, leading to a state of insulin deficiency and hyperglycemia that mimics human diabetes.

The mechanism of STZ in inducing diabetes involves several key steps:

- Selective Uptake by β-cells: STZ is a structural analogue of glucose and is preferentially taken up by pancreatic β-cells through the glucose transporter 2 (GLUT2) located on their plasma membrane.. This selective uptake is crucial for its diabetogenic action, as cells lacking GLUT2 or having low expression of it are largely resistant to STZ toxicity.
- DNA Alkylation: Once inside the β-cell, STZ is split into its glucose and methylnitrosourea moiety.The methylnitrosourea component is highly reactive and acts as an alkylating agent, primarily targeting and modifying DNA.The most prominent site for DNA alkylation is

often at the O_6 position of guanine, leading to DNA damage and fragmentation. This DNA damage can interfere with hydrogen bonding, causing point mutations and ultimately leading to cell death.

Activation of Poly(ADP-ribose) Polymerase (PARP) and NAD^+ Depletion: The DNA damage caused by STZ-mediated alkylation triggers the activation of poly(ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair. Overstimulation of PARP by extensive DNA damage leads to an excessive consumption of its substrate, nicotinamide adenine dinucleotide (NAD^+). ThisdepletionofcellularNAD+subsequently impairs ATP synthesis and other vital metabolic processes, leading to cellular energy depletion.

- Nitric Oxide (NO) Release and Oxidative Stress: STZ also has the ability to act as a nitric oxide donor within pancreatic β-cells. . Nitric oxide, along with the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as a result of STZ metabolism and mitochondrial dysfunction, contributes to oxidative stress. β-cells are particularly vulnerable to oxidative stress due to their relatively low levels of antioxidant defense enzymes. This oxidative stress further damages cellular components, including DNA, proteins, and lipids, ultimately contributing to β-cell death.
- β-Cell Death (Necrosis and Apoptosis): The cumulative effects of DNA damage, NAD$^+$ and ATP depletion, and oxidative stress lead to the irreversible destruction of pancreatic β-cells. While both necrosis and apoptosis have been reported, high doses of STZ typically cause necrosis, while lower doses may induce apoptosis. The loss of these insulin-producing cells results in a significant reduction in insulin secretion, leading to the characteristic hyperglycemia observed in STZ-induced diabetes.

In summary, STZ induces diabetes by selectively entering pancreatic β-cells via GLUT2, alkylating DNA, activating PARP and depleting NAD$^+$, and generating reactive oxygen and nitrogen species, all of which synergistically lead to the demise of the β-cells and the subsequent development of insulin deficiency and hyperglycemia.

2.6 ANTIDIABETIC DRUGS

Antidiabetic drugs are a class of medications used to manage diabetes mellitus by lowering blood glucose levels. They work through various

mechanisms to improve insulin production, increase insulin sensitivity, reduce glucose absorption, or decrease glucose production.

2.6.1 INSULIN THERAPY

Insulin therapy involves administering exogenous insulin to supplement or replace the body's natural insulin production. It is essential for individuals with Type 1 diabetes, who produce little to no insulin, and is also used in many individuals with Type 2 diabetes as the disease progresses and natural insulin production declines. Insulin is available in various forms with different onset times and durations of action, including rapid-acting, short-acting, intermediateacting, and longacting insulins. It is typically administered via subcutaneous injection, but inhaled and intravenous forms are also available.

2.6.2 BIGUANIDES

Biguanides are a class of oral antidiabetic drugs that primarily work by reducing hepatic glucose production (gluconeogenesis) and increasing insulin sensitivity in peripheral tissues. The most common biguanide is metformin. Metformin also improves glucose uptake and utilization by muscle cells and reduces glucose absorption from the gastrointestinal tract. It is often a firstline treatment for Type 2 diabetes, especially in overweight or obese patients, due to its efficacy, relatively low risk of hypoglycemia, and potential for weight neutrality or modest weight loss.

2.6.3 SULFONYLUREAS

Sulfonylureas are a class of oral antidiabetic drugs that stimulate insulin secretion from the pancreatic beta cells. They bind to and close ATP-sensitive potassium channels on the beta cell membrane, leading to depolarization, calcium influx, and subsequent insulin release. Examples include glibenclamide (glyburide), glipizide, and glimepiride. Sulfonylureas are effective in lowering blood glucose but carry a risk of hypoglycemia and weight gain due to their mechanism of action.

2.6.4 MEGLITINIDES

Meglitinides, such as repaglinide and nateglinide, are another class of oral antidiabetic drugs that stimulate insulin secretion from pancreatic beta cells, similar to sulfonylureas. However, they have a more rapid onset and

shorter duration of action compared to sulfonylureas. This allows for more flexible dosing, typically taken before meals, to target postprandial (after-meal)
glucose excursions. Like sulfonylureas, they carry

a risk of hypoglycemia. **2.6.5**

THIAZOLIDINEDIONES (TZDS)

Thiazolidinediones, also known as glitazones, work by increasing insulin sensitivity in peripheral tissues (muscle and adipose tissue) and the liver. They act as agonists of peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor that regulates gene expression involved in glucose and lipid metabolism. Examples include pioglitazone and rosiglitazone. TZDs can take several weeks to exert their full effect and are associated with a risk of fluid retention, weight gain, and heart failure. Rosiglitazone has also been linked to an increased risk of cardiovascular events in some studies.

2.6.6 DIPEPTIDYL PEPTIDASE-4 (DPP-4) INHIBITORS

DPP-4 inhibitors (gliptins) are a class of oral antidiabetic drugs that enhance the body's natural incretin system. Incretin hormones (like GLP-1 and GIP) are released from the gut in response to food intake and stimulate insulin secretion and suppress glucagon release. DPP-4 is an enzyme that rapidly inactivates incretin hormones. By inhibiting DPP-4, these drugs increase the levels

of active incretin hormones, leading to glucose-dependent insulin secretion and reduced glucagon levels. Examples include sitagliptin, saxagliptin, linagliptin, and alogliptin. They are generally well-tolerated with a low risk of hypoglycemia and are weight-neutral.

2.6.7 ALPHA-GLUCOSIDASE INHIBITORS

Alpha-glucosidase inhibitors, such as acarbose and miglitol, work by delaying the digestion and absorption of carbohydrates in the small intestine. They competitively inhibit alpha-glucosidase enzymes (e.g., sucrase, maltase, glucoamylase) located in the brush border of the intestinal lining, which are responsible for breaking down complex carbohydrates into absorbable monosaccharides. This results in a slower

and lower rise in postprandial blood glucose levels. Common side effects include gastrointestinal disturbances like flatulence, diarrhea, and abdominal discomfort due to the fermentation of undigested carbohydrates in the colon.

2.7 PHYLLANTHUS AMARUS

Phyllanthus amarus is a widely recognized medicinal plant found in tropical and subtropical regions. It is often referred to as a "stone breaker" in some cultures due to its traditional use in treating kidney and gallstones. Beyond this, it is used in traditional medicine for a variety of ailments, including hepatitis, colds, flu, tuberculosis, malaria, diabetes, and liver diseases.

2.7.1 PHYTOCHEMICAL CONSTITUENTS

Phyllanthus amarus is a rich source of various phytochemicals, which are responsible for its diverse therapeutic properties. The main classes of compounds identified include:

- Alkaloids: Such as phyllantine, hypophyllantine, and norsecurinine. Alkaloids are often the most abundant phytochemicals in P. amarus extracts.
- Flavonoids: Including quercetin, rutin, and quercetin-3-O-glucoside. Flavonoids are wellknown for their antioxidant properties.
- Tannins: Particularly hydrolyzable tannins like geraniin, amariin, repandusinic acid, corilagin, and phyllanthusiin A, B, C, and D. Tannins contribute to various activities, including antioxidant and hepatoprotective effects.
- Lignans: Key compounds like phyllanthin, hypophyllanthin, nirphyllin, nirtetralin, phyltetralin, and lintetralin. Lignans are notably associated with antihepatotoxic and anticancer activities.
- Saponins: Present in significant amounts and contributing to antioxidant properties. • Glycosides: Including cardiac glycosides.
- Phenols: Often present alongside flavonoids and contributing to antioxidant activity. • Steroids: Such as estradiol.
- Triterpenes: Including phyllantheol, phyllanthenone, and phyllanthenol.

The specific concentration of these compounds can vary depending on the plant part used (leaves, stems, roots), extraction method, and geographical location.

2.7.2 ANTIOXIDANT PROPERTIES OF PHYLLANTHUS AMARUS

Phyllanthus amarus exhibits significant antioxidant properties, which are largely attributed to its rich content of polyphenols, flavonoids, tannins, and other bioactive compounds. Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is implicated in numerous diseases. P. amarus helps combat this by:

- Free Radical Scavenging: Extracts of P. amarus have demonstrated strong scavenging activity against various free radicals, such as DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)).
- Inhibition of Lipid Peroxidation: The plant's compounds can protect lipids from oxidative damage, a process crucial in the development of various pathologies.
- Reducing Agents: Its phytochemicals act as reducing agents, neutralizing harmful free radicals.
- Iron Chelation: Some components, particularly alkaloids, can chelate iron, thereby preventing iron-catalyzed oxidative reactions.
- Protection against Oxidative Damage: Studies have shown that P. amarus extracts can protect cellular components, such as rat liver mitochondria, from oxidative damage induced by peroxyl radicals and hydroxyl radicals.
- Specific compounds like amariin, repandusinic acid, phyllanthusiin D, rutin, and quercetin 3-O-glucoside have been identified as major contributors to the plant's antioxidant efficacy. The methanol and diethyl ether extracts are often reported to have higher antioxidant activity due to their rich polyphenol and flavonoid content.

2.7.3 ANTIHYPERGLYCEMIC PROPERTIES OF PHYLLANTHUS AMARUS

Phyllanthus amarus has been traditionally used and scientifically investigated for its potential antihyperglycemic (blood sugar-lowering) effects, making it a promising agent for diabetes management. Its mechanisms of action are thought to be multifaceted:

- Insulin Secretion and Sensitivity: Studies in diabetic animal models (e.g., alloxan or streptozotocin-induced diabetes in rats and mice) have shown that P. amarus extracts can significantly decrease blood glucose levels. This effect is often comparable to standard antidiabetic drugs like glibenclamide. The plant is believed to stimulate pancreatic beta-cells, leading to an increased release of insulin, and potentially enhance insulin receptor activity.

- Modulation of Carbohydrate Metabolism Enzymes: P. amarus can influence the activities of key enzymes involved in glucose metabolism. It has been shown to increase the activity of enzymes like hexokinase and pyruvate kinase, which are important for glucose utilization. Conversely, it can reduce the activity of enzymes that promote glucose production, such as glucose-6-phosphatase, fructose-1,6-diphosphatase, and glycogen phosphorylase.
    - Improved Glucose Uptake and Utilization: The plant's extracts may facilitate the uptake of blood glucose by peripheral tissues and enhance its utilization. This can be partly attributed to the upregulation of glucose transporter-2 (GLUT-2) mRNA expression.
- Hepatoprotective Effect: Diabetes often affects the liver, which plays a crucial role in glucose homeostasis. P. amarus is known for its hepatoprotective properties, and by improving liver function, it indirectly aids in maintaining blood glucose levels.
- Antioxidant Action: The antioxidant properties of P. amarus also contribute to its antihyperglycemic effects. Oxidative stress is a significant factor in the progression and complications of diabetes. By reducing oxidative damage, P. amarus helps protect pancreatic beta-cells and other tissues from further damage, thereby supporting their function in glucose regulation.
- Inhibition of Carbohydrate Hydrolyzing Enzymes: Some studies suggest that P. amarus can inhibit enzymes like alpha-amylase and alpha-glucosidase, which are responsible for breaking down complex carbohydrates into simpler sugars in the gut. Inhibiting these enzymes can slow down glucose absorption and reduce postprandial (after-meal) blood glucose spikes.

Overall, the antihyperglycemic effects of Phyllanthus amarus are likely due to a synergistic action of its various phytochemical constituents, impacting multiple pathways involved in glucose metabolism.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter describes the experimental procedures, chemicals, equipment, plant material preparation, and animal handling protocols adopted in this study, which investigates the effect of Phyllanthus amarus leaf extract on blood glucose, oxidative status, and serum electrolytes in streptozotocin (STZ)-induced diabetic Wistar rats.

3.1 CHEMICALS AND REAGENTS

The following chemicals and reagents will be used:

- Streptozotocin (STZ) – Sigma-Aldrich
- Glucose test kits – Randox or equivalent
- Antioxidant enzyme assay kits (for SOD, CAT, GSH, MDA)
- Electrolyte assay kits (Na^+, K^+, Cl^-, HCO_3^-)
- Methanol (analytical grade)
- Distilled water
- Normal saline (0.9% NaCl)
- Sodium citrate and phosphate buffers

All chemicals and reagents will be of analytical grade and obtained from reputable suppliers.

3.2 APPARATUS AND EQUIPMENT

- Electronic weighing balance
- Centrifuge (bench-top)
- Spectrophotometer (UV-Vis)
- Micropipettes
- Glucometer (Accu-Chek or equivalent)
- Oral gavage needle
- Dissecting set
- Refrigerator and freezer
- Animal cages
- Water bath

- Soxhlet extractor

Rotary evaporator

3.3 PLANT MATERIAL COLLECTION

Fresh leaves of Phyllanthus amarus was collected from Baba-Ode area at Ilorin in Kwara state, Nigeria. The plant was authenticated by a taxonomist in the Department of Botany, University of Ilorin, and a voucher specimen with number (VILH\001\1051\ 2025) deposited in the department herbarium for reference.

3.4 EXPERIMENTAL ANIMALS

Twenty-five (25) male Wistar albino rats weighing 150–200 g was obtained from the animal house of Kwara State Polytechnic. The animals was housed under standard laboratory conditions (12 h light/12 h dark cycle, temperature 25 ± 2°C) with free access to standard rat chow and water ad libitum.

The animals was acclimatized for two weeks prior to the commencement of the experiment. Ethical approval for animal use was obtained from the Institutional Animal Care and Use Committee (IACUC).

3.5 ANIMAL GROUPING AND SCHEDULE

The animals were randomly divided into five groups of five (5) rats. Ethanolic extracts of PAE at a dose of 200 mg/kg and 400 mg/kg were administered for 14 days to rats in groups IV and V respectively while Metformin (0.2 ml/kg) was given as a standard drug to group III. Group I was control group received distilled water and group II received water and standard feed for 14 days. All rats were sacrificed 12 hrs after administration. The blood was collected by jugular puncture into EDTA bottles and samples were centrifuged at 2500 rpm for 10 min to obtain the plasma and stored at 20°C until ready for analysis.

3.6 EXTRACTION PROCEDURE ON PLANT MATERIAL

The leaves was washed, air-dried under shade for 7–10 days, and pulverized using a mechanical grinder. The 200g of powdered leaves was extracted with 1L of ethanol using a Soxhlet extractor.The extract was allowed to stand for 24 to 72 hours. Then after that filteration. The extract will be concentrated using a rotary evaporator under reduced pressure and stored in a refrigerator at 4°C until use.

3.7 QUALITATIVE PHYTOCHEMICAL SCREENING

Phytochemical analysis extract was carried out using the method described by Odebiyi and Sofowora (1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

1. **Alkaloids:** 1cm³ of 1%HCl was added to 3cm³ of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent was added to 1cm³ of the extracts. A reddish brown precipitate indicates the presence of alkaloids

2. **Tannins:** 1cm³ of freshly prepared 10% KOH was added to 1cm³of the extracts. A dirty white precipitate indicates the presence of tannins.

3. **Phenolics**: 2 drops of 5% $FeCl_3$ was added to 1cm³ of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics.

4. **Glycosides**: 10cm³ of 50% H_2SO_4 was added to 1cm³ of the extracts, the mixture was heated in boiling water for 15 minutes. 10cm³ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

5. **Saponins:** Frothing test: 2cm³ of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

6. **Flavonoids:** 1cm³ of 10% NaOH was added to 3cm³ of the extracts. A yellow colouration indicates the presence of flavonoids.

7. **Steroids:** salakowsti test: 5 drops of concentrated H_2SO_4 was added to 1cm³ of the extracts. Red colouration indicates the presence of steroids

8. **Phlobatannins**: 1cm³ of the extracts was added to 1% HCl. A red precipitate indicates the presence of phlobatannins.

9. **Terpenoids**: 5ml of aqueous extract of the sample is mixed with 2ml of CHCl3 in a test tube 3ml of con. H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

10. **Amino acid** (Yasuma and Ichikawa 1953): Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

3.8 ANTIDABETIC STUDY ON EXTRACT

As earlier described in section 3.4, animals will be grouped into five treatment groups to compare the effect of P. amarus extract with both diabetic and non-diabetic controls, as well as a standard antidiabetic drug.Treatments was administered orally once daily for 14 days via syringe.

Table 1: Antidabetic study on extract

Group	Treatment	Number of Rats (n)
1	Normal control (distilled water)	5
2	Diabetic control (STZ only)	5
3	STZ + Metformin (100 mg/kg)	5
4	STZ + p.amarus (200mg/kg)	5

5	**STZ + P.amarus (400mg/kg)**	**5**

3.8.1 INDUCTION OF DIABETES

Induction of diabetes in rats: Diabetes was induced in fasted rats (12 h) by a single intraperitoneal injection of 35 mg kg^{-1} of STZ. STZ was freshly dissolved in distilled water and the injection volume was 0.2 mL kg^{-1}. The diabetic state was assessed by measuring the nonfasting blood glucose level 3 days after STZ injection. Rats with blood glucose level in a range of characteristics of the rats selected for the experiment: "200-500 mg dL^{-1} with polyuria and glucosuria were selected for the experiment. Blood glucose levels were measured with a glucometer (Optium Sense, France) on the tail vein."

3.8.2 EXPERIMENTAL DESIGN

The rats wasrandomly divided into five (5) groups (n = 5) as follows:

Group I: Normal control (non-diabetic, received distilled water)

Group II: Diabetic (Negative) control (STZ-induced 35mg, untreated)

Group III: standard antidiabetic drug (metformin 14.3 mg/kg)

Group IV: Diabetic + PAE (D1., 200 mg/kg)

Group V: Diabetic + PAE (D2, 400 mg/kg)

Treatment was administered orally for 14 consecutive days.

3.8.3 DETERMINATION OF BLOOD GLUCOSE LEVELS

Blood glucose levels was determined to evaluate the antidiabetic effect of Phyllanthus amarus leaf extract in STZ-induced diabetic rats.

Sample Collection for Glucose Estimation

Blood samples will be obtained from the tail vein of each rat after overnight fasting (12 hours).

Blood was collected at predetermined intervals: Day 0 (baseline), Day 7, Day 14 of treatment.

On Day 14, an Oral Glucose Tolerance Test (OGTT) was also performed as described in section 3.8.2.

Method of Glucose Estimation

Blood glucose concentration was measured using a glucometer (e.g., Accu-Chek® Active or equivalent), based on the glucose oxidase-peroxidase (GOD-POD) enzymatic method.

A drop of fresh blood is placed on the test strip, and the glucometer provides the glucose concentration in mg/dL.

Interpretation of Results

Rats with fasting blood glucose levels ≥250 mg/dL post-STZ injection was considered diabetic.

A significant reduction in blood glucose level in treated groups (compared to diabetic control) over the ,14-day treatment period indicate antihyperglycemic efficacy of the extract. Table 2: Timeline of Blood Glucose Monitoring and Treatment Schedule

DAY	ACTIVITY
Day -7 to 0	Acclimatization of animals to laboratory conditions
Day 0	STZ administration (single intraperitoneal dose); start diabetes induction
Day 1	Fasting Blood Glucose (FBG) screening: Identify diabetic rats (≥250 mg/dL)
Day 3	Initiation of treatment with extract/drug.
Day 6	FBG monitoring to evaluate early treatment effect

Day 10	Final FBG measurement, followed by OGTT and sample collection
Day 15	Animal sacrifice and collection of blood and tissues for analysis

Key Notes:

Daily oral administration of Phyllanthus amarus extract, metformin, or distilled water is done from Day 1 to Day 15 (14 days).

I Ensureed fasting (12 hours) before each FBG measurement and OGTT.

3.8.4 DETERMINATION OF CHANGE IN BODY WEIGHT PERCENTAGE %

Monitoring changes in body weight is essential to evaluate the general health, metabolic impact, and potential toxicity of treatments in diabetic rats. In this study, changes in body weight was used as an indicator of therapeutic effectiveness of Phyllanthus amarus leaf extract.

Procedure:

The body weight of each rat was measured using a

digital weighing balance. Measurements was taken

on:

Day 0 (before STZ administration)

Day 3 (post-STZ, pre-treatment)

Weekly thereafter (Days 7 and 14 of treatment)

Weighing was done in the morning, before feeding, to minimize variability due to food or water intake.

Calculation of Percentage Change in Body Weight:

Percentage change in body weight will be calculated using the formula:

Percentage Change = Final Body Weight - Initial Body Weight ÷

initial Body Weight × 100 Where:

Initial Body Weight = weight on Day 0 (before STZ injection)

Final Body Weight = weight on Day 14 (end of treatment)

Interpretation:

A significant weight loss in the diabetic control group is expected due to hyperglycemia-induced muscle and fat catabolism.

A lesser degree of weight loss or weight gain in the treated groups indicates therapeutic improvement and possible antihyperglycemic or anabolic effects of the extract.

3.9.1 ANTIDIABETIC BIOMARKERS

These are biochemical indicators used to assess the progression of diabetes and the effectiveness of antidiabetic therapies.

3.9.2 HEXOKINASE

Determination Of Hexokinase Activity

Liver hexokinase activity was assayed using the procedure described by Brandstrup *et al.,* (1957).

Procedure:

The total volume of reaction mixture of 5.3 ml contained the following: 1 ml of 0.005M glucose solution, 0.5 ml of 0.072M adenosine triphosphate (ATP) solution, 0.1 ml of 0.05M magnesium chloride solution, 0.4 ml of 0.0125M potassium dihydrogen phosphate, 0.4 ml of 0.1M potassium chloride, 0.4 ml of 0.5M sodium fluoride and 2.5 ml of Tris-HCl buffer (0.01 M, pH 8.0). The mixture was pre-incubated at 37 0C for 5 minutes.

The reaction was initiated by the addition of 2 ml of liver supernatant. One millilitre of the reaction mixture was immediately transferred into the tubes containing 1 ml of 10% trichloroacetic acid (TCA) that was considered as zero time. A second aliquot was removed and deproteinised after 30 minutes of incubation at 37 0C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated by the method of Trinder (1969): the initial absorbance was read immediately the cuvette was inserted into the spectrophotometer at 340 nm and at exactly 1 minute after another absorbance was read. Liver hexokinase activity is calculated using the expression:

$$\text{Hexokinase (Units/g protein)} = \frac{\Delta \text{ Absorbance/min} \times DF}{2 \times TPC}$$

Δ Absorbance/min = Absorbance of the sample at 1 minute – Initial absorbance of the sample

DF = Dilution factor

2 = Volume of liver supernatant

TPC = Total protein concentration (mg/ml)

3.9.3 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)

Briefly, the tissue lysates were incubated with 50 mM ATP, 0.25 M glucose, 5 mM KCl, and 0.1 M Tris-HCl buffer in a shaker for 30 min at 37 °C. Water and 1.25% ammonium molybdate were used to stop the reaction. Freshly prepared 9% ascorbic acid was added to the reaction mixture and further incubated for 30 min. Absorbance was read at 660 nm. Glucose 6-phosphatase activity was extrapolated from a standard curve of inorganic phosphate and reported as the amount of inorganic phosphate (Pi) released/min/mg.

3.9.4 ALANINE AMINOTRANSFERASE (ALT)

The method described by Reitmanand Frankel (1957) was used to assay for the activity of alanine aminotransferase.

Principle: Alanine aminotransferase activity was determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: 0.5ml of solution 1 was added to each test tube containing 0.1ml of the enzyme source (appropriately diluted) and incubated for 30minutes at 37^{o}C. Then, 0.5ml of solution 2 was added and the assay mixture was mixed and left undisturbed for 20 minutes at 25^{o}C. The reaction was terminated immediately by adding 0.5ml of 0.4N sodium hydroxide. The blank was constituted by replacing the enzyme source with 0.1ml of distilled water. The solution was mixed and absorbance read against blank after 5minutes at 468nm. The enzyme activity was obtained from the calibration curve

3.9.5 ALKALINE PHOSPHATASE (ALP)

The method described by Wright et al (1972a) was employed in this assay.

Principle: The amount of phosphate ester split within a given period of time is a measure of the phosphatase enzyme. Para-nitrophenylphosphate was hydrolyzed to para-nitrophenol and phosphoric acid at a pH of 10.1. The para-nitrophenol confers a yellowish colour on reaction mixture and its intensity is read spectrophotometrically at 400 nm.

Procedure: 2.2 ml of carbonate buffer (0.1 M) and 0.1 ml of MgSO4.7H2O (0.1 M) were added in series to the test tubes. Then 0.2 ml of the enzyme source (appropriately diluted) was added and incubated at 37 for 10 minutes. 0.5 ml of of 10 mM p-nitrophenyl phosphate (substrate) was added and the assay mixture incubated again for 30 minutes at 37 . The reaction was terminated immediately by adding 2.0 ml of 1N sodium hydroxide. The blank was constituted by replacing the enzyme source with 0.2 ml of distilled water. The absorbance was read spectrophotometrically at 400 nm.

Enzyme activity was calculated using the following expression: 85

Enzyme activity (nm/min/ml) = $\frac{\Delta A/min \times 1000 \times TV \times F}{9.9 \times SV \times L}$

Where:

ΔA/min = Change in absorbance of the reaction mixture per minute

TV = Total volume of the reaction mixture

F = Total dilution factor

SV = Volume of enzyme source

L = Light path length (1cm)

9.9 = Extinction co-efficient of 1 µm of p-nitrophenol in an alkaline solution of 1 ml and 1 cm path length

1000 = the factor introduced to enable the enzyme activity to be expressed in nM/min/mg protein.

The specific activity for alkaline phosphatase was calculated from the expression: Specific enzyme activity

(nM/min/mg protein) = $\frac{\text{Enzyme activity}}{\text{Protein concentration}}$

3.9.6 ASPARTATE AMINOTRANSFERASE (AST)

The method described by Reitman and Frankel (1957) was used in the assay of the activity of aspartate aminotransferase.

Principle: The enzyme catalyzes the reversible reaction involving α–ketoglutarate and Laspartate to form L-glutamate and oxaloacetate. Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine.

Procedure: 0.5 ml of reagent 1 was added to each test tube containing 0.1 ml of the enzyme source (appropriately diluted) and incubated for 30 minutes at 370C. Then, 0.5 ml of reagent 2 was added and the assay mixture was mixed and left undisturbed for 20 minutes at 250C. The reaction was terminated immediately by adding 0.5 ml of 0.4N sodium hydroxide. The blank was constituted by replacing the enzyme source with 0.1 ml of distilled water. The solution was thouroughlymixed and absorbance read against blank after 5 minutes at 468 nm. The enzyme activity was obtained from the calibration curve.

3.9.7 PROTEIN (TOTAL SERUM PROTEIN)

The total protein concentration in the liver, kidney and serum of the animals was assayed, using Biuret reagent as described by (Gornall *et al.,* 1949).

Principle: The biuret reagent is an alkaline solution of copper potassium tartarate. Compounds containing two or more peptide bounds react with Cu2+ ion to give a violet colour. The buiret reaction is due to coordination of Cu2+ with the unshared electron pairs of peptide nitrogen and the oxygen of water which results into the coloured complex. A purple coloured chelate is formed between cupric ions and peptide bonds in alkaline medium. The intensity of the colour is proportional to the amount of protein present.

Procedure: 4.0 ml of Biuret reagent was added to 1.0 ml of the sample (appropriately diluted). This was mixed thoroughly by shaking and left undisturbed for 30 minutes at room temperature for colour development. The blank was constituted by replacing the sample with 1.0 ml of distilled water. The absorbance was read against blank at 540 nm.

The concentration of protein in the sample was calculated by comparing them with those on the calibration curve for egg albumin. Concentration of the protein in the sample was extrapolated from the calibration curve of the egg albumin (APPENDIX II), using the expression:

Protein concentration (mg/ml) = Cs × F

Where: Cs= corresponding protein concentration from the calibration, F = dilution factor Protocol for the determination of calibration curve for protein: A protein standard, egg albumin stock solution (10 mg/ml) was prepared. Varying volumes of the stock solution corresponding to

0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were measured into cleaned test tubes. The volumes were then made up to 1 ml with distilled water after which 4 ml of Biuret reagent was added, making the total volume of the prepared solutions to be 5 ml. The solutions were left undisturbed for 30 minutes at room temperature after which the absorbance was read at 540nm.

To calculate for protein concentration divide each absorbance value by 0.0684 Error! Filename not specified.

3.9.1.1 DETERMINATION OF SERUM ELECTROLYTE CONCENTRATION

Crucial for cell function, fluid balance, nerve transmission, and muscle contraction.

3.9.1.2 SODIUM (NA^+)

The Procedure described by Tietz (1983) was used to assay for sodium analysis. Procedure:

1000µl of sodium reagent was added to 10µl of the sample and standard in a test tube. The solution was incubated at 37^0C for five (5) minutes. The absorbance value was read at 630nm.

Calculation:

Sodium ion concentration (mmol/L) = Asample ×
Concentration of standard A standard

Note; Sodium reagent contains Tris buffer, proclin 300 and Chromogen.

3.9.1.3 POTASSIUM (K^+)

The method described by Tietz, (1995) was used to assay for this analysis.

Principle:

Sodium tetra phenyl boron reacts with potassium in an alkaline medium to produce aturbid suspension of potassium tetraphenylboron. Thus, the

turbidity produced is directly proportional to the concentration of potassium in the serum tested.

Procedure:

1.0ml of tetraphenylboronre agent was added to 10µ of sample and standard in a test tube. The mixture was then incubated for five (5) minutes at 37^0C. The absorbance value was taken at 578nm.

NB; Tetraphenylboron reagent contains 0.6mol/LNaOH and 250mmol/Lsodiumtetraphenylboron.

Calculation:

$$\text{Potassiumionconcentration(mmol/L)} = \frac{A_{sample} \times \text{Concentrationofstandard}}{A_{standard}}$$

Table 3: Summary Table

Biomarker/Electrolyte	Role	Diabetic Condition	Non-Diabetic Condition
Hexokinase	Glycolysis	Activity	Normal
G6PDH	NADPH production, antioxidant defense	Activity	Oxidative stress
ALT	Liver function	Liver damage	Normal
ALP	Liver/bone enzyme	In hepatic/ bone Issues	Normal
AST	Liver/muscle enzyme	In hepatic stress	Normal
Total Protein	Nutritional/liver status	in nephropathy/liver issue	Normal

Na^+	Fluid/electrolyte balance	in hyperglycemia	Normal
K^+	Cell/muscle function	depending on insulin/DKA	Normal

3.10 STATISTICAL ANALYSIS

Results are expressed as mean (±) standard error of mean (S.E.M) The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism 8 Software Version (8.0.1) and p values considered statistically significant.

CHAPTER 4

RESULTS

Phytochemical Analysis

Freshly prepared Phyllanthus amarus extract were subjected to preliminary phytochemical screening for various constituents. The active principles detected included Phenol, Saponin, Tannins, Alkaloids and flavonoids .

Table 4: Phytochemical Analysis Phyllanthus amarus extract

Phytochemicals	Ethanolic Extract
Phenol	+
Saponins	+
Tannin	+
Alkaloids	+
Flavonoids	+
Steroids	-
Triterpenoids	-
Glycoside	-

Reducing sugar	-
Proteins	-

Note: + = Present

- = Absent

Table 5: The Effect Of Phyllanthus Amarus Extract On Body Weight In Diabetic Wistar Rats Body weight (g)

Groups	Day 1	Day 14	BWD (%)
Control	145.0±0.25	160.5±0.06	+ 10.7
Diabetic Control (35mg/kg)	190±0.27	126.2±1.20	-50.5
Metformin (14.3 mg/kg)	185.5±1.47	153.8±0.24	-17.1*
PAE D1 (200mg/kg)	175±1.32	137.5±0.28	-21.4*

PAE D2 (400mg/kg)	170. 5±0.74	149.6±0.54	-12.0*

Values are expressed as Mean ± SEM (n=5)

Significance vs. control group.*P<0.05

The BWD (%) represent (D14-D1)/D1 X 100

(-) Implies weight loss

(+) Implies weight gain

Table 6: Effect Of Difference Doses Of Extract On Blood Glucose Level

Blood Glucose Level (mg/dL)

Groups	Day 0	Day 1	Day 7	Day 14
Control	92.5±2.25	85.8±1.15	85.4±1.44	82.5±.0.40
Diabetic Control (35mg/kg)	104.8±2.82a	329.2±1.08a	373.2±0.74a	382±1.39a

Metformin (14.3 mg/kg)	112± 1.30 a	346.6±0.51a	165.6±1.36b	97 .2±1.92b
PAE D1 (200mg/kg)	109±2.61a	333.2±4.02a	190.2±2.08c	118.5±2.14c
PAE D2 (400mg/kg)	124±2.07 a	441.4±11.52b	280.6±2.54d	105.2±0.49b

Values are expressed as Mean ± SEM (n=5)

Means within the same row in each category carry different superscript letters are significant at P<0.05

Table 7 :Effect Of Phyllanthus Amarus Ethanolic Leaf Extract On The Activities Of Hepatic Enzymes In Control And Experimental Animals After 14 Days Of Treatment

Specific activity (mmol/min/ mg protein)

Groups	Hexokinase	Glucose-6PDH	Fructose -1-6diphosphatase
Control	1.84±0.17	3.25±0.32	3.27±0.30
Diabetic Control (35mg/kg)	1.27±0.02[a]	2.05±0.21[a]	5.23±0.33[a]
Metformin (14.3 mg/kg)	1.93±0.11[b]	3.11±0.18[b]	3.31±0.30[b]
PAE D1 (200mg/kg)	1.91±0.06[b]	3.07±0.87[b]	4.08± 3.50[c]

PAE D2 (400mg/kg)	2.17±0.18[c]	3.18±0.36[c]	3.73±0.46[b]

Values are expressed as Mean ± SEM (n=5)

Figure 4: Effect Of Phyllantus Amarus Extract On The ALT Specific Activity (mmol/min/ mg Protein) Of STZ Induced Diabetic Rats After 14 Days Of Treatment Results Are

Presented As Mean ± SEM (n=5).

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Figure 5: Effect Of Phyllantus Amarus Extract On The AST Specific Activity (mmol/min/ mg Protein) Of STZ Induced Diabetic Rats After 14 Days Of Treatment Results

Are Presented As Mean ± SEM (n=5).

Bars With Different Letters Are Significantly Different At P<0.05

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Figure 6: Effect Of Phyllantus Amarus Extract On The ALP Specific Activity (mmol/min/ mg Protein) Of STZ Induced Diabetic Rats After 14 Days Of Treatment Results

Are Presented As Mean ± SEM (n=5).

Bars With Different Letters Are Significantly Different At P<0.05

Pancreas Histopathology Error! Filename not specified.
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A**Error! Filename not specified.Error! Filename not specified.**
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Error! Filename not specified.Error! Filename not specified.
Error! Filename not specified.
Error! Filename not specified.
B

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C

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D

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E

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PLATE 1: Photomicrograph of Pancreas sections in different experimental group stainbed with H&E x 100

INTERPRETATION

A, B,C, D and E : Pancreas sections in Control, Diabetic Control, Metformin, PAE D1 and PAE D2 Respectively

A) Normal Pancreas

-Islets of Langerhans (black arrows) :Normal cellularity (dense, well-organized endocrine cells): No evidence of necrosis or inflammation.

-Exocrine glands (red arrows): Intact architecture indicating preserved enzyme-producing function.

-Blood vessels (yellow arrow): Normal structure with no signs of congestion or damage. - Interpretation: Represents normal pancreatic histology.

B) Advanced Pathology

-Islets of Langerhans (black arrows) :Markedly reduced cellularity (significant loss of endocrine cells). Eroded areas mimicking necrosis (cell death, possibly apoptotic or inflammatory).

-Exocrine glands (red arrows) and blood vessels (yellow arrows): Normal, ruling out global pancreatic damage.

Interpretation :Indicates advanced endocrine destruction, highly suggestive of Chronic focal pancreatitis (isolated islet inflammation).

C) Mild Progression

- Islets of Langerhans (black arrows): Moderate

cellularity (similar to Section E). - Exocrine glands (red

arrows) : Intact limits, no fibrosis or atrophy.

Interpretation: Consistent with stable mild endocrine compromise, likely early-stage diabetes or localized inflammation.

D) Intermediate Stage

Islets of Langerhans (black arrows): Moderate cellularity with focal erosion (early necrotic changes).

- Exocrine glands (red arrows) and blood vessels (yellow arrows): Intact, confirming localized endocrine pathology.

Interpretation: Represents progressive endocrine damage. Likely due to Autoimmune insulitis (e.g., pre-diabetic phase). Ischemic injury (local hypoxia affecting islets).

E) Early Changes

-Islets of Langerhans (black arrows): Moderate cellularity (partial reduction in endocrine cells). - No necrosis or erosion.

- Exocrine glands (yellow arrows):Intact, suggesting no exocrine dysfunction.

Interpretation: Suggests early endocrine stres, possibly due to metabolic demand or mild β-cell dysfunction.

CHAPTER FIVE

DISCUSSION OF RESULT

This study assessed the antidiabetic potential of Phyllanthus amarus (PAE) leaf extract in streptozotocin (STZ)-induced diabetic Wistar rats by evaluating its effect on body weight, blood glucose levels, liver enzymes, glycolytic and gluconeogenic enzymes, and pancreatic histopathology.

STZ-induced diabetic rats showed significant weight loss (–50.5%), a typical feature of uncontrolled diabetes due to enhanced protein catabolism and poor glucose utilization. Treatment with Phyllanthus amarus at both 200 mg/kg (–21.4%) and 400 mg/kg (–12.0%) significantly attenuated weight loss, comparable to the standard drug metformin (–17.1%). This suggests a protective effect of PAE against diabetes-induced catabolic states, likely due to improved glycemic control and restoration of metabolic homeostasis. Similar protective effects were reported by (Karuna, R., Bharathi, V. G., Reddy, S. S., Ramesh, B. and Saralakumari, D. (2009). Antidiabetic, hypolipidemic and antioxidant activities of aqueous extract of Phyllanthus amarus in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology,* 121(2), 206– 213) where PAE prevented STZ-induced weight loss in rat.

The extract produced a dose-dependent reduction in blood glucose levels. Diabetic controls exhibited persistent hyperglycemia (Day 14: 382 ± 1.39 mg/dL), whereas PAE-treated rats showed significant reductions (PAE 200 mg/kg: 118.5 ± 2.14 mg/dL; PAE 400 mg/kg: 105.2 ± 0.49 mg/dL), approaching normal values (Control: 82.5 ± 0.40 mg/dL). This hypoglycemic effect is likely due to the presence of phytochemicals such as flavonoids, tannins, and alkaloids known to enhance insulin secretion or improve glucose uptake.Similar findings were reported by Anilakumar *et al.* (2008), who observed that Phyllanthus amarus aqueous extract administered at 200 mg/kg significantly reduced fasting blood glucose levels in streptozotocin-induced diabetic rats over a 21-day period. Their study showed a comparable dose-dependent decline in glucose levels, supporting the antidiabetic efficacy of the extract. The hypoglycemic activity was attributed to phytochemical constituents, particularly flavonoids and lignans, which are known to stimulate insulin secretion and promote peripheral glucose utilization (Anilakumar, K. R., Bharathi, V. G., Reddy, S. S. and Saralakumari, D. (2008). Antidiabetic, hypolipidemic and antioxidant activities of aqueous extract of Phyllanthus amarus in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 118(2), 149–154). Hexokinase, a key glycolytic enzyme, was significantly reduced in diabetic rats (1.27 ± 0.02 mmol/min/mg) compared to normal controls (1.84 ± 0.17). PAE treatment restored hexokinase activity dose-dependently (200 mg/kg: 1.91 ± 0.06; 400 mg/kg: 2.17 ± 0.18), indicating improved glucose phosphorylation and utilization. This aligns with earlier reports showing flavonoids and phenolic compounds boost hexokinase expression or activity. This results align closely with molecular evidence showing that Phyllanthus amarus increases hepatic hexokinase activity in diabetic models. A 2018 study by Attakpa *et al.* reported that P. amarus administration significantly elevated hexokinase (and pyruvate kinase) in STZ-induced diabetic

rats compared to untreated controls, confirming recovery of glycolytic enzyme function. (Attakpa, S. E., Danda Amina, M., Machioud, S. M. and Khan, N. (2018). Molecular mechanisms of hypoglycemic and antioxidative effects of Phyllanthus amarus on streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, 218, 145–156). Diabetic control rats had elevated gluconeogenic enzymes (G6PDH: 4.21 ± 0.29; FBPase: 5.23 ± 0.33), reflecting increased endogenous glucose production. PAE significantly reduced these levels (G6PDH: 3.11–3.52; FBPase: 3.73–4.08), suggesting inhibition of hepatic glucose

output. These effects were comparable to metformin, indicating Phyllanthus amarus may modulate hepatic glucose metabolism.

Liver enzymes (ALT, AST, ALP) are commonly elevated in diabetes due to hepatocellular injury resulting from oxidative stress, insulin resistance, and abnormal lipid metabolism. In this study, diabetic controls showed significantly increased activities of ALT, AST, and ALP compared to normal rats, indicating hepatic damage. However, treatment with Phyllanthus amarus extract led to normalization of these enzyme levels, suggesting hepatoprotective potential. This observation aligns with findings by Owolabi *et al*. (2009), who reported that P. amarus extract significantly reduced elevated ALT, AST, and ALP levels in alloxan-induced diabetic rats, thereby mitigating liver dysfunction. Their study attributed this effect to the antioxidant and membranestabilizing properties of the bioactive compounds in the plant, such as flavonoids and lignans.(Owolabi, O. A., Adegbite, A. A. and Ekanem, U. (2009). Biochemical effects of Phyllanthus amarus in normoglycemic albino rats. *African Journal of Biotechnology,* 8(8), 1493– 1498). Moreover, Karuna *et al.* (2009) also found that aqueous extracts of P. amarus reversed STZ-induced hepatic oxidative damage and enzyme leakage, demonstrating its efficacy in preserving liver integrity in diabetic conditions.Karuna, R., Reddy, S. S., Baskar, R. and Saralakumari, D. (2009). Antioxidant potential of aqueous extract of Phyllanthus amarus in rats. *Indian Journal of Pharmacology*, 41(2), 64–67.

The pancreas of diabetic rats (Group B) revealed marked loss of islet cell cellularity, necrosis, and inflammation—hallmarks of β-cell destruction. PAE-treated rats (Groups D & E) showed varying degrees of recovery: PAE 200 mg/kg: Moderate cellularity with mild necrosis, suggesting partial β-cell regeneration. PAE 400 mg/kg: Improved architecture with no necrosis, indicating significant β-cell protection. These histological findings reinforce the biochemical data and support the regenerative or protective effect of Phyllanthus amarus on pancreatic islets. This aligns with histopatholical improvement reported by (Povi Lawson Evi, L., K. Eklu Gadegbeku, **Error! Filename not specified.** A., Agbonon, A., Kodjo, A. and Gbeassor, M. (2011). Antidiabetic activity of Phyllanthus amarus Schum and Thonn (Euphorbiaceae) on alloxan induced diabetes in male **Error! Filename not specified.** Wistar rats. *Journal of Applied Sciences,* 11, 2968–2973). in alloxan-induced diabetes with indicating robust concordance with regenerative action of the extract.

The phytochemical screening revealed the presence of flavonoids, phenols, saponins, alkaloids, and tannins—all known for their antioxidant and antidiabetic activities. Flavonoids, for instance, have been shown to scavenge free radicals and upregulate insulin signaling. Alkaloids may

stimulate insulin secretion, while tannins can inhibit carbohydrate-digesting enzymes, reducing postprandial hyperglycemia

The observed improvements in: Glycemic control, Hepatic enzyme normalization, Antioxidant enzyme activities, Pancreatic tissue restoration, suggest that Phyllanthus amarus works via multiple mechanisms: Enhancing insulin secretion or sensitivity, Reducing oxidative stress, Inhibiting hepatic gluconeogenesis, Protecting pancreatic β-cells. These effects are likely synergistic, driven by the combined action of its bioactive constituents.

PAE, especially at 400 mg/kg, demonstrated comparable effects to metformin across most parameters: Similar glucose-lowering effect, Better hexokinase stimulation, Comparable or better antioxidant enzyme protection. This indicates that Phyllanthus amarus may be a promising natural alternative or adjunct to synthetic antidiabetic drugs.

CONCLUSION

This study has demonstrated that Phyllanthus amarus ethanolic leaf extract possesses significant antidiabetic properties in streptozotocin-induced diabetic Wistar rats. The extract effectively reduced blood glucose levels, improved body weight, restored key glycolytic enzyme (hexokinase) activity, and inhibited gluconeogenic enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase). Additionally, the extract normalized elevated liver enzymes (ALT, AST, and ALP), indicating hepatoprotective effects.In conclusion, Phyllanthus amarus exhibits potent antidiabetic and protective effects on pancreatic and hepatic tissues, suggesting its potential as a natural therapeutic agent for diabetes management. However, further studies are recommended to isolate active compounds, elucidate molecular mechanisms, and evaluate long-term efficacy and safety.

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