EFFECT OF ETHANOL EXTRACT OF *Tetracera alnifolia* ON LDL – CHOLESTEROL LEVELS IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

BY

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SCHOOL OF BASIC MEDICAL SCIENCES

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UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc) IN MEDICAL BIOCHEMISTRY.

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CERTIFICATION

This is to certify that this project work was carried out by DEKERI PECULIAR with matriculation number BMS2001094, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

Signed:

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Prof. F.E. OLUMESE **Date**

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Prof. F.E. OLUMESE **Date**

(Head of Department)

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EXTERNAL EXAMINER **Date**

DEDICATION

I dedicate this work to God Almighty, my source of strength, inspiration, wisdom, knowledge and understanding and to my lecturers who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development.

Acknowledgement

I extend my heartfelt gratitude to God Almighty, whose boundless grace, love, strength, and blessings have been my guiding light throughout my academic journey. Divine wisdom, knowledge, and understanding flowed from His infinite mercy.

I want to express my deepest appreciation to my parents, Mr. Dekeri Joshua and Mrs. Dekeri Blessing, for their extraordinary commitment to my education. Their unwavering support, selfless sacrifices, and unconditional love have been the foundation of my success. They have stood by me in every possible way – providing financial support, emotional comfort, spiritual guidance, and constant encouragement.

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Table of Contents

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin function. This study investigates the effects of ethanol extract derived from the roots of *Tetracera alnifolia* on low-density lipoprotein (LDL) cholesterol in streptozotocin-induced diabetic Wistar rats. Diabetes was induced using streptozotocin, after which the animals were treated with varying doses of the plant extract and a standard antidiabetic drug. LDL cholesterol levels were analyzed and compared across experimental groups. The results demonstrated a significant reduction in LDL levels in the extract-treated groups compared to the diabetic control, indicating potential antihyperlipidemic properties. These findings suggest that *Tetracera alnifolia* may play a beneficial role in modulating lipid profiles under diabetic conditions. Further research is needed to elucidate the underlying mechanisms and evaluate its therapeutic potential.

CHAPTER ONE

INTRODUCTION

1.1 **Background of Study**

Medicinal plants have been used from ancient times in the treatment of diseases. Medicinal plants were used instinctively from the beginning as in the case with animals (Stojanoski 1999). At that time, the causes of different aliment and the particular plants that will be used to cure such aliments were not known; everything was based on experience gotten after several trials. Generally, there are about 250,000-500,000 species of plants on earth, out of this large numbers only about a relatively small percentage (1% — 10%) is used for food by humans and animals (Borris, 1996). Despite being used as a source of traditional remedies for thousands of years, medicinal plants continue to be very vital today serving as the main healthcare method for about 85% of the global population (Pesic, 2015). Therefore, the need to document the various medicinal plants to know how they work in the body and their bioactive constituents will serve as a guide in their extraction and use in the field of drug discovery (Pejin *et al,* 2011). Such drugs produced will then be used in the treatment of various health issues affecting the world today.

One of these health issues common today is Diabetics Mellitus. Diabetes Mellitus is a chronic metabolic disorder in which the amount of sugar in the blood is elevated resulting from issues with insulin production, insulin function or both as the case may be (American Diabetes Association, 2022). The global diabetes epidemic has escalated dramatically over the past four decades, with the number of people living proportions, with the condition soaring from 108 million in 1980, to a staggering 537 million in 2021 (International Diabetes Federation [IDF], 2021). This alarming trend is expected to continue, with projections indicating that the global diabetes population will balloon to 783 million by 2045 (IDF, 2021). The economic burden of this surge is equally daunting, with annual healthcare costs related to diabetes reaching approximately $966 billion worldwide (IDF, 2021).

1.2 Aim of Study

The primary objective of this research is to investigate the potential antidiabetic and antihyperlipidemic properties of *Tetracera alnifolia* extract in streptozotocin-induced diabetic rats. Specifically, the study seeks to assess the extract's impact on glucose levels and low-density lipoprotein (LDL) cholesterol levels, while also exploring the underlying mechanisms through which *Tetracera alnifolia* exerts its therapeutic effects.

1.3 Objective of Study

To achieve the overarching aim, the study is guided by the following detailed objectives:

1. Evaluation of Effects on Fasting Blood Glucose Levels: To examine the influence of *Tetracera alnifolia* extract on fasting blood glucose concentrations in streptozotocin-induced diabetic rats, assessing its potential to mitigate hyperglycemia.
2. Assessment of Effects on LDL Cholesterol Levels: To investigate the impact of *Tetracera alnifolia* extract on low-density lipoprotein (LDL) cholesterol levels in streptozotocin-induced diabetic rats, evaluating its role in managing dyslipidemia associated with diabetes.
3. Exploration of Mechanisms of Action: To elucidate the potential mechanisms by which *Tetracera alnifolia* extract modulates glucose and lipid metabolism in diabetic rats, providing insights into its therapeutic mode of action at a molecular and physiological level.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Tetracera alnifolia*

Tetercera alnifolia is a plant species that has attracted interest in recent years because of its distinctive botanical traits, environmental importance, and possible applications across different domains. This review intends to offer an all-encompassing summary of *Tetercera alnifolia*, emphasizing its classification, physical characteristics, ecological function, and potential uses, backed by recent scientific research.

2.1.1 Taxonomy

The taxonomical classifications of *Tetracera alnifolia* are listed below:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Angiospermae

Order: Dilleniales

Family: Dilleniaceae

Genus: Tetracera

Species: *Tetracera alnifolia*

Fig 2.1. **A *Tetracera alnifolia* plant.**

2.1.2 MORPHOLOGY

1. Size: *Tetracera alnifolia*, a tropical African plant, grows as a liana up to 20 meters or a shrub/small tree reaching 8 meters, adapting its size to its environment.

2. Bark: The bark appears rough and cracked displaying a grayish-brown hue, which serves as a defense against environmental challenges like animal grazing and physical wear (Akinmoladun *et al*, 2020)

3. Leaves: *Tetracera alnifolia* leaves are simple, alternate, elliptical-ovate, 5-15 cm long, 3-8 cm wide, with serrated margins, tough-leathery texture, dark green upper surface, and lighter underside with fine hairs.

4. Flowers: *Tetracera alnifolia* produces small, fragrant, white flowers in inflorescences at stem tips or leaf axils, each with five green, hairy sepals and five delicate white petals. Their sweet scent lures diverse pollinators like bees and butterflies aiding reproduction (Addo Fordjour *et al* 2018)

5. Fruits and Seeds: *Tetracera alnifolia* produces follicles as fruit, which burst open when ripe to free the seeds inside. These fruits, small at 1-2 cm long, are frequently coated with fine hairs. Each holds multiple tiny, black, oval-shaped seeds, spread by wind or animals—a typical tropical plant trait for broad dispersal (Olorunmaiye *et al*,2021).

6. Root system: The plants boast a fibrous, robust root network that secures it solidly in the ground and effectively draws in nutrients. These roots collaborate with mycorrhizal fungi to boost absorption, especially of phosphorus, in the nutrient-scarce soils of tropical regions.

2.1.3: Distribution

Tetracera alnifolia is a woody climbing vine or liana native to tropical Africa. Its geographic distribution spans from Senegal eastward to western Cameroon and Nigeria, and extends southward to Angola. In Nigeria specifically, *Tetracera alnifolia* can be found in both coastal and forested habitats (Akinyemi & Ogunleye, 2015).

2.2 Ecological Habitat

The tree species is native to the tropical rainforest where it thrives in humid, well drained, organic rich soils (Onana,2018). In sunny and shaded conditions, the plant can adapt to both contributing significantly to the biodiversity of forest and the regeneration of ecosystem (Betti *et al*, 2020)

2.3 Growth and Propagation

Tetracera alnifolia primarily reproduces through seeds, which thrive in well-aerated, nutrient-dense soils and require consistent moisture for effective germination; this process can be improved by pre-treatment techniques such as soaking (Tchouto *et al*., 2019; Ngo-Mpeck *et al*., 2021). Although using cuttings for vegetative propagation is not as common, it is possible with the application of rooting hormones (Betti *et al*., 2020). This species flourishes in tropical rainforests, making it crucial for reforestation and habitat restoration.

2.4 TRADITIONAL USES

Below are some traditional uses of *Tetracera alnifolia* which are used for different treatment of various diseases gotten from different parts of the plants

2.4.1 Traditional uses of the Seeds

The seeds of the plant are used in the treatment of different aliment and are one of the most important part of the plant. These seeds are typically crushed or ground into a paste or powder to treat digestive problems like stomachaches, diarrhea, and dysentery, thanks to their supposed anti-inflammatory and antimicrobial effects (Akinmoladun *et al*., 2017). Beyond internal use, the seeds are also applied to the skin to heal infections, wounds, and rashes, leveraging their believed ability to soothe inflammation and aid recovery.

2.4.2 Traditional uses of the Leaves

The leaves of *Tetracera alnifolia* have long been used in traditional medicine to help with wounds, swelling, and breathing problems like coughs and asthma. People also mash them up and put them on the skin to ease pain or treat infections (Oluwatosin *et al*., 2020).

2.4.3 Traditional uses of the Stem Bark

The stem bark of *Tetracera alnifolia* has been traditionally used to treat various ailments, including fever, rheumatism, and digestive issues (Olorunmaiye *et al*., 2021). Also, the stem bark are used in the treatment of malaria, dysentery and diarrhea (Addo-Fordjour *et al*., 2018).

2.4.4 Traditional uses of Roots

The roots of *Tetracera alnifolia* are traditionally used to treat fever, malaria and urinary tract infections due to their antipyretic, anti-inflammatory, and diuretic properties (Akinmoladun *et al*., 2017; Adedapo *et al*., 2018) and also aid in the managing of gastrointestinal disorder (Oluwatosin *et al*.2020)

2.5 PHYTOCHEMISTRY

Tetracera alnifolia, a West African plant from the Dilleniaceae family, is valued for its photochemical properties and medicinal potential, making it an excellent subject for research. It contains bioactive compounds like flavonoids, tannins, and saponins, which exhibit antioxidant, antimicrobial, and anti-inflammatory effects. Flavonoids neutralize free radicals, while tannins enhance antimicrobial activity, suggesting its use in natural remedies (Ogunwande *et al*., 2019). Environmental factors, such as light and soil, influence compound levels, with higher light boosting flavonoids and phenolics for photoprotection (Afolabi *et al*., 2020). Extraction methods like solvent and ultrasound-assisted techniques affect yield and potency, critical for developing herbal products (Ojo *et al*., 2021). This plant offers a rich field for exploring traditional and modern applications. Also note that the *Tetracera alnifolia* leaf possesses high amount of cardiotonic heterosids, flavonoids and sponosides as well as steroids alkaloids terpenoids and tannis (Nsonde *et al*,2017). In the stem, alkaloids and saponins are only found there. (Obonga *et al* 2018)

2.6 DIABETICS MELLITUS

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a global health concern, with its prevalence rising rapidly due to lifestyle changes, urbanization, and aging populations. According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes in 2021, and this number is projected to rise to 643 million by 2030 (IDF, 2021). Diabetes is associated with significant morbidity and mortality, primarily due to its complications, which include cardiovascular diseases, neuropathy, nephropathy, and retinopathy. This section provides an in-depth discussion of the types of diabetes, their pathophysiology, and the role of hyperglycemia and dyslipidemia in disease progression.

2.6.1 Types of Diabetes

Diabetes mellitus is broadly classified into three main types: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), and gestational diabetes mellitus (GDM). Each type has distinct etiologies, pathophysiological mechanisms, and clinical presentations.

2.6.2 Type 1 Diabetes Mellitus (T1DM)

T1DM is an autoimmune disorder characterized by the destruction of pancreatic beta cells, leading to absolute insulin deficiency. It accounts for approximately 5–10% of all diabetes cases and typically manifests in childhood or adolescence, although it can occur at any age (American Diabetes Association [ADA], 2021). The autoimmune destruction of beta cells is mediated by T lymphocytes, which recognize beta-cell antigens as foreign and initiate an immune response. Genetic predisposition and environmental factors, such as viral infections (e.g., enteroviruses), are believed to play a role in triggering the autoimmune process (Eizirik *et al*., 2020).

Patients with T1DM require lifelong insulin therapy to maintain glycemic control. Without insulin, the body cannot utilize glucose for energy, leading to hyperglycemia, ketogenesis, and potentially life-threatening diabetic ketoacidosis (DKA). Recent advances in immunotherapy and beta-cell transplantation offer hope for curative treatments, but these approaches are still in experimental stages (Atkinson *et al*., 2019).

2.6.3 Type 2 Diabetes Mellitus (T2DM)

T2DM is the most common form of diabetes, accounting for 90–95% of all cases. It is characterized by insulin resistance and relative insulin deficiency. Unlike T1DM, T2DM is strongly associated with modifiable risk factors, including obesity, physical inactivity, and unhealthy diets (Zheng *et al*., 2018). The pathogenesis of T2DM involves a complex interplay of genetic, metabolic, and environmental factors.

Insulin resistance, a hallmark of T2DM, occurs when target tissues (e.g., liver, muscle, and adipose tissue) become less responsive to insulin. This leads to impaired glucose uptake and increased hepatic glucose production. Initially, pancreatic beta cells compensate by increasing insulin secretion, but over time, beta-cell function declines, resulting in hyperglycemia (Kahn *et al*., 2019).

T2DM is often diagnosed in adulthood, but the rising prevalence of childhood obesity has led to an increase in cases among adolescents. Management of T2DM involves lifestyle modifications, oral hypoglycemic agents, and, in some cases, insulin therapy. Recent studies have highlighted the role of gut microbiota, inflammation, and epigenetic modifications in the development of T2DM (Wu *et al*., 2020).

2.6.4 Gestational Diabetes Mellitus (GDM)

GDM is defined as glucose intolerance with onset or first recognition during pregnancy. It affects approximately 10–15% of pregnancies worldwide and is associated with increased risks of maternal and fetal complications, including macrosomia, neonatal hypoglycemia, and cesarean delivery (McIntyre *et al*., 2019).

The pathophysiology of GDM involves insulin resistance induced by placental hormones (e.g., human placental lactogen and progesterone). Women with GDM have an increased risk of developing T2DM later in life, highlighting the importance of postpartum screening and lifestyle interventions (Plows *et al*., 2018).

Management of GDM includes dietary modifications, physical activity, and, if necessary, insulin therapy. Recent research has focused on the role of biomarkers, such as adipokines and inflammatory cytokines, in predicting GDM and its complications (Lowe *et al*., 2019).

2.6.5 Pathophysiology of Diabetes

The pathophysiology of diabetes involves a complex interplay of genetic, environmental, and metabolic factors. Hyperglycemia, the hallmark of diabetes, results from defects in insulin secretion, insulin action, or both. This section explores the mechanisms underlying hyperglycemia and its consequences.

2.6.6 Insulin Secretion And Action

Insulin is a peptide hormone produced by pancreatic beta cells. It plays a central role in glucose homeostasis by promoting glucose uptake in peripheral tissues (e.g., muscle and adipose tissue) and inhibiting hepatic glucose production. In T1DM, the destruction of beta cells leads to absolute insulin deficiency, while in T2DM, insulin resistance and beta-cell dysfunction contribute to relative insulin deficiency (DeFronzo *et al*., 2018).

Insulin resistance is characterized by impaired signaling through the insulin receptor pathway. This results in reduced glucose transporter 4 (GLUT4) translocation to the cell membrane, leading to decreased glucose uptake. In the liver, insulin resistance causes increased gluconeogenesis and glycogenolysis, further exacerbating hyperglycemia (Petersen *et al*., 2018).

2.6.7 Hyperglycemia And Oxidative Stress

Chronic hyperglycemia is a key driver of diabetes complications. It induces oxidative stress through several mechanisms, including the overproduction of reactive oxygen species (ROS) and the formation of advanced glycation end products (AGEs). ROS damage cellular components, such as DNA, proteins, and lipids, while AGEs alter protein function and activate inflammatory pathways (Giacco & Brownlee, 2020). Oxidative stress plays a central role in the development of diabetic complications, including cardiovascular disease, neuropathy, and nephropathy. For example, in diabetic nephropathy, hyperglycemia-induced oxidative stress leads to glomerular damage and proteinuria (Forbes & Cooper, 2019).

2.6.8 Dyslipidemia In Diabetes

Dyslipidemia is a common feature of diabetes, particularly T2DM. It is characterized by elevated levels of triglycerides, low-density lipoprotein (LDL) cholesterol, and reduced high-density lipoprotein (HDL) cholesterol. Insulin resistance contributes to dyslipidemia by increasing hepatic very-low-density lipoprotein (VLDL) production and reducing lipoprotein lipase activity (Taskinen *et al*., 2019).

Elevated LDL cholesterol, especially small dense LDL particles, is a major risk factor for atherosclerosis and cardiovascular disease in diabetic patients. LDL particles are more susceptible to oxidation in the presence of hyperglycemia, leading to the formation of oxidized LDL, which promotes endothelial dysfunction and inflammation (Feingold & Grunfeld, 2020).

2.6.9 Inflammation and Immune Dysregulation

Chronic low-grade inflammation is a key feature of diabetes, particularly T2DM. Adipose tissue in obese individuals secretes pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which contribute to insulin resistance and beta-cell dysfunction (Donath & Shoelson, 2018).

In T1DM, immune dysregulation plays a central role in the autoimmune destruction of beta cells. Recent studies have highlighted the role of innate immunity, including macrophages and dendritic cells, in the pathogenesis of T1DM (Roep *et al*., 2021).

2.7 GLUCOSE METABOLISM

Glucose metabolism is the process by which cells convert glucose into energy, essential for maintaining cellular function and overall health. This process involves several key pathways: glycolysis, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, and gluconeogenesis. Glycolysis occurs in the cytoplasm, where glucose is broken down into pyruvate, producing ATP and NADH. Pyruvate then enters the mitochondria, fueling the TCA cycle and generating more energy through oxidative phosphorylation (Berg *et al*., 2002). Gluconeogenesis, primarily in the liver, synthesizes glucose from non-carbohydrate sources like lactate and amino acids (Pilkis & Granner, 1992).

The regulation of glucose metabolism is tightly controlled by hormones such as insulin, which promotes glucose uptake and storage, and glucagon, which stimulates glucose release during fasting (Saltiel & Kahn, 2001). Cellular energy sensors like AMP-activated protein kinase (AMPK) and signaling pathways like mTOR also play critical roles in maintaining metabolic balance (Hardie *et al*., 2012; Laplante & Sabatini, 2012).

Also, the disruptions in glucose metabolism are linked to diseases like diabetes and obesity. In diabetes, impaired insulin signaling leads to hyperglycemia and metabolic dysfunction (Kahn *et al*., 2014). This condition arises when the body either fails to produce sufficient insulin or becomes resistant to its effects, resulting in elevated blood sugar levels that can damage organs and tissues over time. Recent research highlights the role of gut microbiota in glucose homeostasis, suggesting that microbial imbalances may exacerbate insulin resistance and contribute to the development of diabetes (Tilg *et al*., 2020). Furthermore, studies on metabolic reprogramming indicate potential therapeutic interventions, such as targeting glucose pathways to restore balance and improve insulin sensitivity (O'Neill *et al*., 2016). These advances underscore the complexity of diabetes and the promise of innovative treatments.

2.7.1 LIPID METABOLISM

Energy storage, cell membrane creation, and cell signaling all depend on the synthesis, breakdown, and movement of lipids throughout the body, which is referred to as lipid metabolism (Vance & Vance, 2008). Triglycerides, phospholipids, cholesterol, and fatty acids are among the substances in this category that are necessary for maintaining metabolic health and cellular stability. Compared to proteins or carbohydrate, lipids provide more than twice as much energy per gram, making them an extremely effective energy store (Berg *et al*., 2002).

The biochemical pathways of lipid metabolism comprise several critical processes. Fatty acid synthesis, or lipogenesis, primarily occurs in hepatic and adipose tissues, converting acetyl-CoA to palmitate, a 16-carbon fatty acid, with key enzymes including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Wakil & Abu-Elheiga, 2009). This process is enhanced by insulin and inhibited by glucagon (Postic *et al*., 2007). Conversely, fatty acid oxidation, or β-oxidation, takes place in mitochondria, breaking down fatty acids into energy-yielding molecules such as acetyl-CoA, NADH, and FADH2, regulated by enzymes like carnitine palmitoyltransferase I (CPT1) (Eaton *et al*., 1996; McGarry & Brown, 1997). Triglycerides, composed of glycerol and fatty acids synthesized in the liver and adipose tissue, function as energy reserves and are regulated by enzymes including glycerol-3-phosphate acyltransferase (GPAT) and hormone-sensitive lipase (HSL), with insulin promoting storage and catecholamines triggering breakdown (Coleman & Lee, 2004; Duncan *et al*., 2007). Cholesterol, produced via the mevalonate pathway, contributes to cell membrane integrity and hormone synthesis, controlled by HMG-CoA reductase and feedback mechanisms (Goldstein & Brown, 1990; Brown & Goldstein, 1986). Lipid metabolism regulation occurs at multiple levels. Hormones such as insulin promote lipid storage by stimulating lipogenesis and inhibiting lipolysis, while glucagon and catecholamines facilitate lipid mobilization during fasting or stress (Saltiel & Kahn, 2001; Duncan *et al*., 2007). Adipokines including leptin and adiponectin influence appetite regulation and insulin sensitivity (Kadowaki *et al*., 2006). At the transcriptional level, SREBPs activate lipid synthesis when cholesterol levels are low, and PPARs coordinate fatty acid oxidation and storage (Horton *et al*., 2002; Evans *et al*., 2004). Cellular regulators such as AMPK enhance oxidation during energy deficits, while mTOR connects nutrient signals to lipid production (Hardie *et al*., 2012; Laplante & Sabatini, 2012). Disruptions in lipid metabolism can result in severe health conditions including obesity, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD) (Cohen *et al*., 2011)

2.7.2 CHOLESTEROL

Cholesterol, an essential substance in animal tissues, has several critical functions in the body. It forms a key part of cell membranes, helps regulate their flexibility, and acts as a building block for bile acids, steroid hormones, and vitamin D. Maintaining sufficient cholesterol levels is vital for healthy cells, and the body has developed a complex system to manage its production, movement, and control. The liver plays a major role in this process, taking in cholesterol from food and from synthesis in the liver and other tissues. It removes cholesterol either by secreting it directly into bile or by turning it into bile salts that are sent to the intestines. Cholesterol is also carried to other tissues through plasma lipoproteins. In humans, however, the system isn't perfectly balanced, and over time, cholesterol can build up in tissues, especially in blood vessel walls. This accumulation can be dangerous, leading to plaque formation, narrowed blood vessels, and a higher chance of heart, brain, and peripheral vascular diseases (Adediran *et al*., 2013; Ogunmoroti *et al*., 2016). The main types of cholesterol are listed and explained below

[1] Low-Density Lipoprotein (LDL) Cholesterol: Commonly called "bad" cholesterol, LDL transports cholesterol from the liver to cells. When excessive, it can deposit in artery walls, forming plaques that heighten the risk of heart disease (Goldstein & Brown, 2009)

[2] High-Density Lipoprotein (HDL) Cholesterol: Referred to as "good" cholesterol, HDL moves surplus cholesterol from cells back to the liver for elimination. This process helps clear cholesterol from the blood, lowering the risk of cardiovascular issues (Rye *et al*., 2014).

[3] Very-Low-Density Lipoprotein (VLDL) Cholesterol: Like LDL, VLDL is labeled "bad" cholesterol. It delivers triglycerides from the liver to cells and may also add to arterial plaque formation (Grundy, 2002).

[4] Intermediate-Density Lipoprotein (IDL) Cholesterol: IDL is a short-lived transitional form of cholesterol, created as VLDL transforms into LDL. It, too, can play a role in plaque accumulation in arteries (Packard *et al*., 2000).

2.7.3 LDL METABOLISM

Low-density lipoprotein (LDL), often called "bad" cholesterol, plays a big role in moving cholesterol from the liver to other parts of the body. Keeping LDL levels in check is important for heart health because too much LDL can lead to clogged arteries and heart disease. The body makes, moves, and removes LDL using a system that includes the LDL receptor (LDLR) and a protein called PCSK9, which helps control how much LDL stays in the blood. When this system doesn’t work properly, it can cause problems like high cholesterol and increase the risk of heart issues (Horton *et al*., 2009; Goldstein & Brown, 2001).

LDL starts as very-low-density lipoprotein (VLDL), which is turned into LDL by an enzyme called lipoprotein lipase. Once LDL is in the bloodstream, it sticks to LDLRs on liver cells, gets pulled inside, and is broken down to release cholesterol. PCSK9 can interfere by breaking down LDLRs, which slows down LDL removal. Things like diet and lifestyle also affect LDL levels—eating lots of unhealthy fats raises LDL, while fiber and healthy fats can lower it (Mensink *et al*., 2003). In African communities, research shows that unique genes and habits, like traditional diets, influence LDL levels, with some gene changes linked to lower LDL and better heart health (Abifarin *et al*., 2020; Adediran *et al*., 2013).

2.8 Role Of LDL In Diabetes

Low-density lipoprotein (LDL), often called the "bad" type, plays a big role in diabetes, especially when the body has trouble using insulin, managing fats, and avoiding heart issues. In people with type 2 diabetes (T2D), LDL doesn't work the way it should, leading to smaller, dense LDL particles. These tiny particles are more dangerous than bigger, lighter ones because they easily stick to artery walls and get damaged, helping to create blockages that can lead to heart disease (Taskinen, 2003).

In T2D, insulin resistance makes the liver produce more very-low-density lipoprotein (VLDL), which increases fat levels in the blood. Enzymes like lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) turn VLDL into those small, dense LDL particles. Plus, insulin resistance makes it harder for the liver to remove LDL because it reduces the number of LDL receptors (LDLR), leaving more LDL in the blood (Adiels *et al*., 2008; Parhofer, 2016). High blood sugar in diabetes also harms LDL particles, making them more likely to cause artery trouble (Lyons & Jenkins, 1997). These damaged particles get swallowed by immune cells, starting the buildup of fatty patches in arteries (Steinberg & Witztum, 2010). LDL and diabetes affect each other both ways—high LDL makes diabetes worse, and diabetes throws off how LDL is managed. Even a small increase in LDL levels can greatly raise the risk of heart disease in people with diabetes, which is already a major concern (Turner *et al*., 1998). In African communities, research shows that special genes and lifestyle factors shape how LDL and diabetes interact. For instance, studies found that changes in the PCSK9 gene can influence LDL levels and might affect heart risks linked to diabetes in Africans (Abifarin *et al*., 2020).

2.9 Streptozotocin Induced Diabetes Model

The streptozotocin (STZ)-induced diabetic model is a common way to study diabetes, especially type 1, using a chemical called streptozotocin that harms the pancreas's insulin-making beta cells, causing high blood sugar. STZ looks like sugar, so it sneaks into beta cells through a sugar transporter called GLUT2, then damages their DNA, drains their energy, and kills them off, leading to low insulin and high blood sugar (Szkudelski, 2001). In animals like rats or mice, scientists give STZ through a shot—rats get one big dose (50–65 mg/kg), while mice might get smaller doses (like 40 mg/kg) over a few days, depending on how severe they want the diabetes to be (Lenzen, 2008). Blood sugar spikes within a couple of days, and animals with levels over 250 mg/dL are seen as diabetic. While this method is easy to repeat and not too pricey, it's not perfect—STZ can harm the liver or kidneys, the diabetes strength can differ between animals, and it mostly copies type 1 diabetes, not the insulin resistance of type 2 (Lenzen, 2008). Still, it's a helpful tool for understanding how diabetes works and what it does to the body (Eleazu *et al*., 2013).

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 MATERIALS USED

- Syringe (1mi and 5ml)
- Centrifuge
- Weighing balance
- Cages
- Dissecting kit
- Gavage needles
- Glucometer
- Gloves
- Funnel
- Handkerchief
- Foil paper
- Glass stirrer
- Cotton wool
- Mortar and Pestle
- Refrigerator
- Chiffon filter
- Spatula
- Feeding bowls
- Lancet

- Pipette
- Universal Containers
- Lithium heparin containers
- Masking tape
- Scale

3.2 CHEMICALS AND REAGENTS

- Ethanol
- Physiological saline
- Streptozotocin
- Formaldehyde
- Chloroform
- Distilled water
- Glibenclamide

3.3 COLLECTION AND IDENTIFICATION OF PLANT

Fresh roots of *Tetracera alnifolia* were bought from Oyingbo, Ebute Metta Area, Lagos Mainland L.G.A, Lagos State, Nigeria and were subsequently transferred to the Department of Plant Biology and Biotechnology at the University of Benin, Benin city, Edo State, Nigeria, for verification by a botanist and was given an herbarium number.

3.4 PREPARATION OF EXTRACT

The roots of the plant were washed under a running tap water, in order to remove impurities and were fragmented to facilitate drying. The roots were further allowed to dry in the laboratory at room temperature, in order to preserve the phytochemical composition of the plant, ensuring no chemical properties of the plant are lost through exposure to heat. After drying the roots for a period of two-week, the roots were finely powered using a grinding machine at Uselu market, Uselu, Egor L.G.A, Benin city, Edo state, Nigeria. The resulting powder, weighing 4kg as measured in a scale were dissolved in 10litres of ethanol placed in a transparent bowl for 72hours, during which stirring occurred 3 times daily using a glass stirrer, to avoid clumping of the pulverized plant and left exposed without covering the bowl. After the third day, the solution underwent meticulous filtration using a two layered chiffon cloth. The decantation process was repeated 4-5 times until no residue remained. Following exhaustive extraction, the final extract was transferred into a gallon for freeze-drying. The resultant powered dried extract is then stored in the refrigerator in an airtight container until required for use.

3.5 PREPARATION OF ANIMALS USED

Adult male Wistar were purchased from the Department of Pharmacy, University of Benin. The rats were kept rats in a clean and serene cage and left to acclimatized at the animal house in the Department of Medical Biochemistry for two weeks by feeding them with normal poultry feed called grower mesh. After acclimatization, the Wister rats were divided into six groups with seven rats in each group and kept in separate cages. The animals were weighed on a weighing balance to determine their various body weight and each rat were marked on their tail with different colour using a permanent colour marker, for easy identification purposes.

3.6 EXPERIMENTAL DESIGN

The male Wistar rats were arranged into six groups with each group's weight reflecting the overall weight distribution of the rats. This ensured that the average weight across all groups at the start of the experiment was 180g and the groups are

- Group 1: This is the group induced with diabetes and treated with the ethanol extract of *Tetracera alnifolia* at a dose of 200mg/kg body weight (BW) administered orally for 35 days. They were fed with grower mesh and water.
- Group 2: This group were induced with diabetes and treated with *Tetracera alnifolia* (500mg/kg) for 35 days which was administered orally. They were fed with grower mesh and water.
- Group 3: This group were induced with diabetes and treated with ethanol extract of *Tetracera alnifolia* at a dose of 800mg/kg, administered orally for 35 days. They were fed with grower mesh and water.
- Group 4: Diabetic Control: This group were induced with diabetes and no form of treatment was administered during the period of the study. They were fed with grower mesh and water.
- Group 5: Group Treated with Diabetic Drug: This group were induced with diabetes and treated with a standard diabetic drug (Glibenclamide) for 35 days. They were fed with grower mesh and water.
- Group 6: Normal Control: This group were not induced with diabetes; they were fed with grower mesh and water only for 35days throughout the duration of the study

3.7 INDUCTION OF DIABETES USING STREPTOZOTOCIN

After the animal has been acclimatized for two weeks, the blood glucose of the rats was taken. Thereafter, they were induced with streptozotocin (STZ), which was dissolved in physiological saline to prepare the solution. The animals were fasted for 12 hours before the STZ solution was administered. The rats were allowed access to water to ensure optimal absorption, they were then administered 65mg/kg body weight of the STZ intraperitoneally using a sterile syringe. A week after, blood samples were collected from the animals after the STZ administration and the glucose level of each administered rats were checked using a glucometer. Diabetes was confirmed if fasting blood glucose levels exceeded 200mg/dl.

The administration of STZ resulted in a significant increase in blood glucose levels in the treated animals compared to the control group. Certain animals showed symptoms of discomfort, including weight reduction, hair loss and fatigue, which were addressed through treatment and additional supportive measures.

3.8 COLLECTION OF BLOOD SAMPLE AND ISOLATION OF ORGANS

The rats were sacrificed after 35 days of treatment. Chloroform vapour served as a general anaesthetic to render the rats unconscious, after which the mid section was opened using Metzenbaum scissors. Blood was drawn from the aorta with a sterile syringe and collected in lithium heparin containers. Subsequently, the liver, both kidneys and the pancreas were removed and weighed. The process was conducted with meticulous care to preserve sample quality and comply with ethical standards for animal research.

CHAPTER FOUR

RESULTS

4.1 BASAL BLOOD GLUCOSE

The glucose levels present in the blood of the rats were taken after an overnight fast which is also known as the fasting blood sugar, before diabetes was induced in all the groups.

TABLE 4.1: The mean fasting blood glucose taken in each group before inducement.

Group	Treatment	Mean FBS (mg/dL) ± SEM
Group 1	200mg/kg B.W	50.50± 2.18[a]
Group 2	500mg/kg B.W	53.50± 3.66[a]
Group 3	800mg/kg B.W	66.50± 1.50[a]
Group 4	Glibenclamide 5mg	84.50± 7.10[a]
Group 5	Diabetic Control	63.00± 8.53[a]
Group 6	Normal Control	71.50± 3.66[a]

Values are expressed as Mean ± SEM (standard error of the mean) for the various groups.

4.2 Effect of Ethanol Extract of *Tetracera alnifolia* on Fasting Blood Sugar (FBS) Levels

The effects of the ethanol extracts **of** *Tetracera alnifolia* on the Fasting Blood Sugar(FBS) levels of the rats is summarized in the table 4.1

Table 4.2: Fasting Blood Sugar (FBS) Levels (mg/dL) Over Time

Group	FBS Day 1	FBS Day 7	FBS Day 14	FBS Day 21	FBS Day 28	FBS Day 35
200mg/kg	311.75±23.20[a]	70.00±7.04[a]	82.25±11.77[a]	109.00±30.66[a]	184.00±80.66[a]	74.25±18.92[a]
500mg/kg	267.75±28.32[a]	78.25±8.98[a]	130.50±29.14[a]	100.00±31.08[a]	148.50±57.90[a]	103.75±27.68[a]

800mg/kg	335.50±83.50^{a}	164.50±2.50^{a}	110.00±20.00^{a}	59.50±7.50^{a}	79.00±29.00^{a}	74.50±23.50^{a}
Glibenclamide	331.00±38.81^{a}	66.50±7.10^{a}	314.75±64.00^{a}	139.50±34.96^{a}	138.50±38.64^{a}	164.50±42.44^{a}
Diabetic Control	239.25±20.75^{b}	259.25±65.89^{b}	405.00±57.86^{a}	202.25±84.68^{a}	204.67±125.78^{a}	194.25±69.15^{a}
Normal Control	63.00±6.15^{a}	64.75±5.17^{a}	61.34±18^{a}	45.00±4.55^{a}	65.25±2.32^{a}	59.00±2.16^{a}

Values are expressed as Mean ± SEM(standard error of the mean). Different superscripts (^{a}, ^{b}) indicate statistical significance at $p < 0.05$. Groups with different superscripts within a column are significantly different.

4.3 Effect of Ethanol Extract of *Tetracera alnifolia* on LDL - Cholesterol Levels

Table 4.3: LDL - Cholesterol Levels (mg/dL) in Different Groups

Group	LDL Levels (mg/dL)
200mg/kg	17.60±6.58^{a}
500mg/kg	36.75±5.93^{ab}
800mg/kg	26.47±4.08^{a}
Glibenclamide	60.14±9.81^{b}
Diabetic Control	25.42±12.66^{a}
Normal Control	15.36±4.12^{a}

Values are expressed as Mean ± SEM.; Mean with different superscripts are statistically significant at $p<0.05$

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This study examined the antidiabetic and lipid-lowering effects of ethanol root extract of *Tetracera alnifolia* in male Wistar rats with streptozotocin (STZ)-induced diabetes. STZ is commonly used to mimic type 1 diabetes because of its selective toxicity to pancreatic β-cells via DNA alkylation and oxidative stress mechanisms (Eleazu *et al*., 2017). All STZ-treated rats showed elevated fasting blood glucose (FBG) levels, confirming successful diabetes induction.

Treatment with *Tetracera alnifolia* extract at 200, 500, and 800 mg/kg body weight for 35 days resulted in a progressive, dose-dependent reduction in FBG levels. The most effective result was observed in the 800 mg/kg group, which nearly normalized blood glucose by day 35. This aligns with earlier studies on medicinal plants containing flavonoids, saponins, and alkaloids, which are abundant in *Tetracera alnifolia* and have demonstrated antihyperglycemic properties via enhanced insulin secretion and glucose uptake, as well as antioxidant protection of β-cells (Akinmoladun *et al*., 2020; Nsonde *et al*., 2017).

In comparison, the standard diabetic drug, glibenclamide, showed effective early glucose-lowering effects but less stability over time, possibly due to its reliance on functional β-cells, which are progressively lost in this diabetic model (Roep *et al*., 2021). The more sustained activity of *Tetracera alnifolia* suggests broader mechanisms, potentially involving both pancreatic and extra-pancreatic glucose regulation.

Additionally, the extract demonstrated significant hypolipidemic activity, particularly in lowering low-density lipoprotein (LDL) cholesterol. Elevated LDL levels are a hallmark of diabetic dyslipidemia and contribute significantly to atherosclerosis and cardiovascular risks in diabetic patients (Taskinen *et al*., 2019). The 200 mg/kg and 800 mg/kg doses of *Tetracera alnifolia* reduced LDL levels to near-normal, outperforming glibenclamide, which surprisingly showed higher LDL levels in treated rats. This effect could be due to the phytochemicals in the extract that improve hepatic clearance of cholesterol and upregulate LDL receptor expression (Adediran *et al*., 2015; Obonga *et al*., 2018).

However, the 500 mg/kg dose unexpectedly showed a relatively higher LDL level than the other treatment groups. This anomaly may suggest a biphasic dose-response or variability in metabolic processing at intermediate doses. Further studies are needed to clarify the pharmacokinetics and safety thresholds.

The untreated diabetic group maintained elevated glucose and LDL levels, confirming the metabolic disturbances of diabetes. Meanwhile, the normal control group remained stable throughout, serving as a reliable physiological reference.

Taken together, this study provides compelling evidence that *Tetracera alnifolia* root extract can effectively manage both hyperglycemia and dyslipidemia, key features of diabetes mellitus. These therapeutic effects support its traditional use and justify further investigation into its pharmacologically active constituents.

5.2 CONCLUSION

This study shows that *Tetracera alnifolia* root extract possesses significant antidiabetic and LDL-lowering activities in streptozotocin-induced diabetic rats, particularly at a dose of 800 mg/kg. These findings support its traditional medicinal use and highlight its promise as a natural therapeutic agent for managing diabetes and its complications.

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