EFFECTS OF ETHANOL EXTRACT OF TETRACERA ALNIFOLA ON SODIUM LEVELS AND SUPEROXIDE DISMUTASE ACTIVITY IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

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CERTIFICATION

We the undersigned hereby certify that ADEBISI SAMUEL ADEOLUWA (BMS2001075) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

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DEDICATION

To the Hands that Shaped Me

With deepest gratitude and love, I dedicate this project to those who have profoundly impacted my life and academic journey. To my loving parents, Mr. and Mrs. ADEBISI, your unwavering support and guidance have been a constant source of strength. To the Department of Medical Biochemistry and the National Association of Medical Biochemistry Students (NAMBS), Uniben Chapter, I appreciate the knowledge, mentorship, and camaraderie that have shaped me into a better biochemist. May this project be a testament to the love, hard work, and dedication that have brought me thus far.

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ABSTRACT

This study investigated the antidiabetic and electrolyte-modulating effects of ethanol extract of Tetracera alnifolia in streptozotocin-induced diabetic Wistar rats. The extract was administered at doses of 200 mg/kg, 500 mg/kg, and 800 mg/kg, and its effects on fasting blood sugar (FBS) levels and sodium levels were evaluated. The results showed that the 500 mg/kg dose exerted a significant hypoglycemic effect, maintaining lower FBS levels over time. Additionally, the extract at 200 mg/kg and 500 mg/kg maintained sodium levels closer to the normal range. These findings suggest that the ethanol extract of Tetracera alnifolia may have therapeutic potential in the management of diabetes, particularly in regulating blood glucose and electrolyte balance.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus, a cluster of endocrine disorders, is marked by persistently elevated blood glucose level (WHO,2022; Erika, 2023). The condition arises from insufficient insulin production by the pancreas or impaired cellular responsiveness to insulin (Shoback *et al.,* 2011). Typical symptoms include excessive thirst (polydipsia), frequent urination (polyuria), increased hunger (polyphagia), weight loss, and visual impairment. If left unaddressed, diabetes can lead to severe complications affecting the cardiovascular system, eyes, kidneys, and nervous system (Kitabchi *et al.,*, 2009) The disease is responsible for approximately 4.2 million annual fatalities, (IDF Diabetes Atlas, 2019) with around 1.5 million attributed to inadequate or ineffective diabetes management (WHO, 2022)

Various pharmaceuticals, including oral antidiabetics and preloaded insulins, contain chemical compounds that help regulate blood glucose levels. In addition to these conventional treatments, researchers are exploring the potential of natural products to manage this condition (Ripson et al., 2009). Natural products have been investigated for their role in controlling blood glucose levels, with plants like Bauhinia forficata, Cecropia obtusifolia, Equisetum myriochaetum, and Cucurbita ficifolia being studied for their antidiabetic properties (NIDDK,2014; Brutsaert, 2017). The use of natural products for diabetes treatment often has cultural and regional significance, as different countries and communities favor specific plants and remedies. For instance, Bauhinia forficata is commonly used in Latin America, while Senna auriculata is more frequently used in Sri Lanka (IDF Diabetes Atlas, 2019). Other natural products, such as alfalfa, Ginkgo biloba,

ginseng, and turmeric, are also widely used. Understanding the properties of these plants and the scientific evidence supporting their use is crucial for harnessing their potential in the treatment of chronic diseases like diabetes (WHO,2022).

Plants from the genus Tetracera have been traditionally used to treat various ailments, including diabetes, due to their medicinal properties (Ogunlakin *et al.,* 2022). The root, stem bark, and leaves of these plants exhibit diverse physiological activities, attributed to the presence of phytochemicals such as tannins, flavonoids, and terpenoids (Ogunlakin *et al.,* 2022). Ethanol extracts of Tetracera species have been found to contain flavonoids with significant antioxidant, anti-inflammatory, and antidiabetic activities (Ogunlakin *et al.,* 2022). While several compounds have been isolated from these plants, including pentacyclic lupane-type triterpene derivatives and flavonoids, betulinic acid remains the most extensively studied compound (Ogunlakin *et al.,* 2022). This study provides an overview of the folkloric uses, isolated compounds, and pharmacological activities of Tetracera species, highlighting their potential therapeutic applications. In this study, the antidiabetic effects of Tetracera alnifolia are investigated, exploring its potential as a natural remedy for diabetes management.

1.2 AIM OF STUDY

The aim of this study is to investigate the potential antidiabetic effects of the ethanol extract of roots of Tetracera alnifolia in streptozotocin-induced diabetic Wistar rats and its antioxidant activity.

1.3 OBJECTIVES OF STUDY

The specific objectives of this study include

1. to determine the effects of the ethanol extract of roots Tetracera alnifolia on blood glucose levels in streptozotocin-induced diabetic Wistar rats.

2. to evaluate effect of this extract on superoxide dismutase activity in streptozotocin-induced diabetic Wistar rats.

3. to compare the effects of the ethanol extract of Tetracera alnifolia with the standard antidiabetic drugs, glibenclamide.

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes is a complex metabolic disorder characterized by disruptions in insulin secretion or sensitivity, leading to hyperglycemia and abnormalities in carbohydrate, lipid, and protein metabolism (Nacer *et al.,* 2020; Hacioglu *et al.,* 2021; Al-Brakati *et al.,* 2020). The prevalence of diabetes is increasing rapidly, affecting over 400 million people worldwide, regardless of age, sex, socioeconomic status, or ethnicity (Okoduwa *et al.,* 2017; Hassan *et al.,* 2021). By 2040, this number is expected to surpass 600 million, with the majority having type 2 diabetes (Chaudhary *et al.,* 2016). Type 2 diabetes is marked by impaired insulin secretion or signal transduction, accompanied by microvascular and macrovascular complications that affect various organs, including the kidneys, nerves, eyes, and cardiovascular system (Hacioglu *et al.,* 2021; Hassan *et al.,* 2021; Chaudhary *et al.,* 2016]. Hyperglycemia in diabetes promotes excessive reactive oxygen species (ROS) production, leading to oxidative stress, insulin resistance, lipid peroxidation, and cellular damage (Hacioglu *et al.,* 2021; Al-Brakati *et al.,* 2020). Elevated ROS levels and suppressed antioxidant defenses trigger pro-inflammatory mediators, modulating nuclear factor-κB (NFκB) and inducing local and systemic inflammation (Nacer *et al.,* 2020; Al-Brakati *et al.,* 2020). Furthermore, hyperglycemia accelerates lipolysis, increasing free fatty acid content and enhancing cytokine secretion, such as interleukin-6 and tumor necrosis factor-α (Nacer *et al.,* 2020). Tumor necrosis factor-α activates the caspase cascade, leading to apoptotic cell death (Giribabu *et al.,* 2017). Despite extensive research and the development of anti-diabetic medications, these treatments cannot reverse diabetes-associated complications and may

lead to drug resistance and adverse effects, such as acute kidney injury (Chaudhary *et al.,* 2016; Luo *et al.,* 2021).

2.1.1 INSULIN RESISTANCE AND PANCREATIC BETA CELL DYSFUNCTION

Insulin resistance is characterized by the impaired biological response of tissues to insulin stimulation, affecting tissues with insulin receptors, particularly the liver, skeletal muscle, and adipose tissue (Seong *et al.,* 2019; Brown *et al.,* 2019;Nolan *et al.,* 2019;Deacon *et al.,* 2019; Thomas *et al.,* 2019). This impairment leads to reduced glucose disposal, prompting a compensatory increase in beta-cell insulin production and hyperinsulinemia. Recent research has sparked debate about the relationship between hyperinsulinemia and insulin resistance, with some suggesting that hyperinsulinemia itself contributes to insulin (Seong *et al.,* 2019; Brown *et al.,* 2019;Nolan *et al.,* 2019;Deacon *et al.,* 2019; Thomas *et al.,* 2019). This concept has significant clinical implications, as it suggests that hyperinsulinemia resulting from excessive caloric intake may drive metabolic dysfunction associated with insulin resistance. The metabolic consequences of insulin resistance are far-reaching, including hyperglycemia, hypertension, dyslipidemia, hyperuricemia, elevated inflammatory markers, endothelial dysfunction, and a prothrombotic state (Seong *et al.,* 2019; Brown *et al.,* 2019;Nolan *et al.,* 2019;Deacon *et al.,* 2019; Thomas *et al.,* 2019).

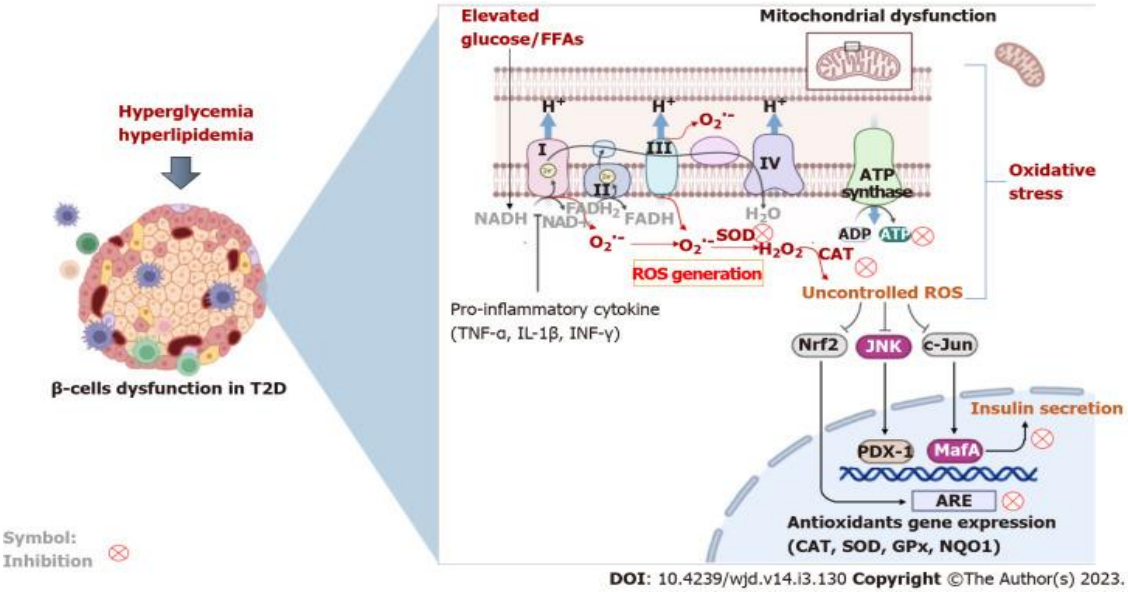

Figure 1: Showing the effects of pancreatic beta cell dysfunction on elevated glucose levels

Prolonged insulin resistance can progress to metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes. While insulin resistance is primarily an acquired condition linked to excess body fat, genetic factors also play a role (Hossan *et al.,* 2019;Bothou *et al.,* 2020). Despite its significance, there is no universally accepted test for insulin resistance, and its clinical definition remains elusive. Instead, clinicians rely on identifying metabolic consequences associated with insulin resistance, such as those seen in metabolic syndrome and insulin resistance syndrome (Hossan *et al.,* 2019;Bothou *et al.,* 2020). The hyperinsulinemic-euglycemic glucose clamp technique is considered the gold standard for measuring insulin resistance, but its clinical applicability is limited(Owei *et al* 2018). As a result, several surrogate measures, including HOMA-IR, HOMA2, QUICKI, serum triglyceride, and triglyceride/HDL ratio, are used to assess insulin resistance in clinical settings.

Type 2 diabetes (T2D) is a leading cause of mortality worldwide, affecting approximately one in ten adults globally, with over 90% of cases attributed to T2D (Seong *et al.,* 2019; Brown *et al.,*

2019). Insulin resistance and β-cell dysfunction are the primary pathophysiological mechanisms underlying T2D. While insulin resistance is commonly associated with T2D, individuals with type 1 diabetes can also develop insulin resistance due to genetic or lifestyle factors (Brown *et al.,* 2019; Nolan *et al.,* 2019). β-cell dysfunction refers to impaired insulin secretion, whereas insulin resistance describes the reduced responsiveness of target organs to insulin (Nolan *et al.,* 2019). The interplay between insulin resistance and β-cell dysfunction contributes to elevated blood glucose levels, exacerbating T2D pathogenesis (Deacon *et al.,* 2019). β-cell dysfunction occurs early in diabetes development and worsens with disease progression (Thomas *et al.,* 2019; Hossan *et al.,* 2019). Elucidating the mechanisms driving β-cell dysfunction is crucial for mitigating T2D complications, including those involving inflammation and oxidative stress. Chronic inflammation is a key component of diabetes, triggered by elevated blood glucose levels and exacerbated by inflammatory mediators produced by adipocytes and macrophages in adipose tissue (Bothou *et al.,* 2020; Matthews *et al.,* 1985; Levy *et al.,* 1998). This process induces pancreatic β-cell injury, leading to inadequate insulin production and hyperglycemia (Levy *et al.,* 1998). Uncontrolled inflammation is a major contributor to T2D pathogenesis (Bothou *et al.,* 2020;Matthews *et al.,* 1985). Several reviews have examined the role of inflammation in β-cell dysfunction during T2D. For instance, research has highlighted the importance of immune cells, including helper T cells, cytotoxic T cells, and regulatory T cells, in pancreatic β-cell failure (Katz *et al.,* 2000). Additionally, studies have shown that epigenetic alterations, such as DNA methylation and histone modification, contribute to β-cell malfunction during embryonic development and postnatal growth, leading to β-cell dysfunction in T2D (Kim-Dorner *et al.,* 2010; Tobin *et al.,* 2012; Abdul-Ghani *et al.,* 2021).

2.1.2 ROLE OF OXIDATIVE STRESS IN DIABETES MANAGEMENT AND PROGRESSION

Oxidative stress is a critical factor consistently linked to β-cell destruction during the development of type 2 diabetes (T2D) (Giacco *et al.,* 2010; Asmat *et al.,* 2016). This stress arises from an overproduction of free radicals, particularly reactive oxygen species (ROS), which overwhelm the neutralizing capacity of intracellular antioxidants (Giacco *et al.,* 2010; Asmat *et al.,* 2016). Oxidative stress can exert destructive effects by damaging DNA, proteins, and lipids. Moreover, in T2D patients with dyslipidemia, uncontrolled oxidative stress can cluster with plasma lipid and lipoprotein anomalies, exacerbating diabetic complications (Asmat *et al.,* 2016).

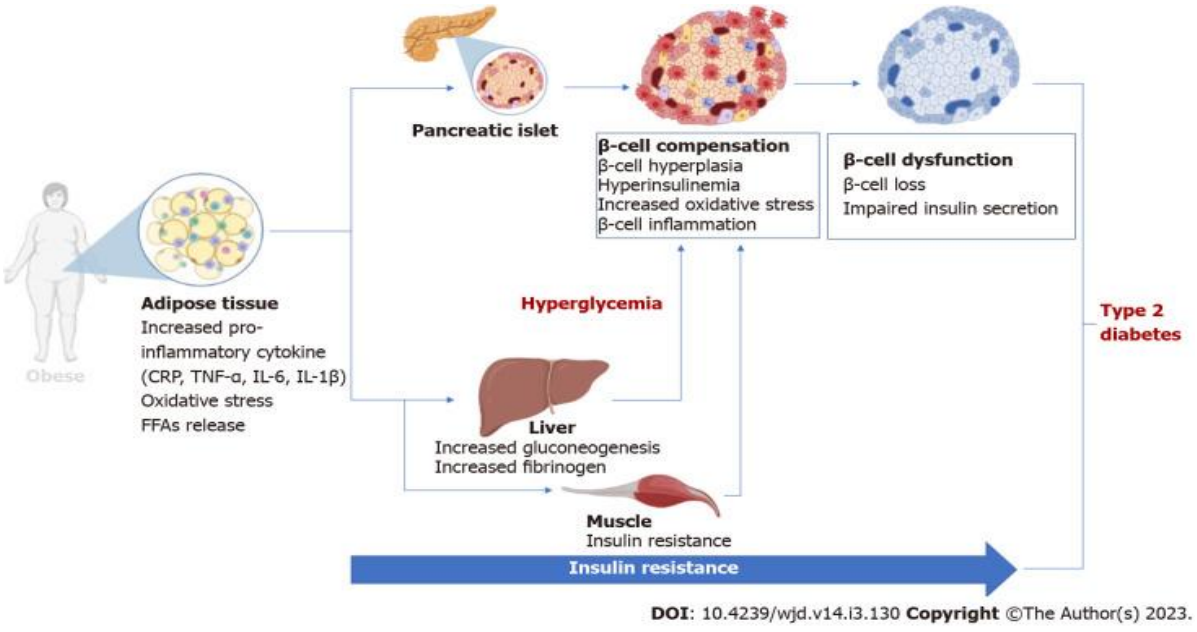

Figure 2: Showing the effects of oxidative stress on diabetes progression

Notably, the inherently low expression of antioxidant enzymes in pancreatic islets makes β-cells vulnerable to oxidative stress, leading to devastating effects on β-cell function and diabetes development (Lenzen *et al.,* 1996;Drews *et al.,* 2010). Obesity and excessive pancreatic fat

accumulation are key mechanisms promoting oxidative stress, insulin resistance, and β-cell dysfunction in T2D (Rohm *et al.,* 2022; Singh *et al.,* 2017). Enhancing intracellular antioxidants may alleviate oxidative stress and improve β-cell function, helping to combat diabetes-related complications (Lei *et al.,* 2011; Wang *et al.,* 2017). The pathological relationship between inflammation and oxidative stress can have severe consequences, contributing to T2D progression and worsening related abnormalities, such as retinopathy, neuropathy, nephropathy, and tissue damage (Halim *et al.,* 2019).

2.1.3 SUPEROXIDE DISMUTASE AND DIABETES

Oxidative stress (OS) is a significant risk factor for early-onset type 2 diabetes (T2D) and the development of diabetic complications (Pejin *et al.,* 2019; Darenskaya *et al.,* 2021). OS is characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular damage (Hou *et al.,* 2021). T2D is strongly associated with OS, and chronic hyperglycemia can result in excessive ROS formation, exacerbating the condition (Ghasemi *et al.,* 2020). The body's defense against ROS involves enzymatic antioxidants, such as superoxide dismutase (SOD) and catalase (CAT), as well as non-enzymatic antioxidants like vitamins A and E (Haddad *et al.,* 2016). Evaluating antioxidant defenses in blood can help predict the risk of developing T2D and related complications (Pieme *et al.,* 2017). SOD plays a crucial role in mitigating ROS-induced damage by converting superoxide anions into hydrogen peroxide and oxygen, which are then broken down by CAT (Omoruyi *et al.,* 2020). Research suggests that SOD is associated with reduced OS in T2D patients, while decreased SOD concentrations may increase susceptibility to OS (Fei *et al.,* 2021; Darenskaya *et al.,* 2021). CAT, another essential antioxidant enzyme, degrades hydrogen peroxide into oxygen and water (Darenskaya *et al.,* 2021). Deficiencies in CAT have been linked to an increased risk of T2D,

contributing to β-cell dysfunction and damage from ROS (Darenskaya *et al.,* 2021). Studies have consistently shown that low CAT activity in T2D patients is associated with disease development and that hyperglycemia downregulates CAT expression (Darenskaya *et al.,* 2021;Nwakulite *et al.,* 2021). However, previous research has yielded conflicting results regarding antioxidant enzyme status in T2D patients, with some studies reporting reduced, unchanged, or increased SOD and CAT concentrations compared to controls (Pejin *et al.,* 2019; Ghasemi *et al.,* 2020).

2.1.4 EFFECTS OF DIABETES ON BODY ORGANS

KIDNEY

The global burden of diabetes mellitus (DM) has been escalating over the past two decades, with a staggering 387 million individuals worldwide affected by this condition (Aziz *et al*., 2015) The comorbidities associated with DM, particularly cardiovascular complications such as coronary artery disease, cerebrovascular disease, and peripheral artery disease, significantly exacerbate morbidity and mortality rates among diabetic patients. Furthermore, DM has a profound impact on renal function, accounting for a substantial proportion of end-stage renal disease cases in Western Europe and the United States(Mima *et al.,* 2013). Approximately 40% of patients undergoing regular dialysis therapy have DM as the underlying cause (Mima *et al.,* 2013) The progression to chronic renal insufficiency in diabetic patients is attributed to both extrarenal and intrarenal atherosclerosis, as well as diabetes-related glomerular damage, manifested as diabetic nephropathy (Hoshino *et al*., 2015). Additionally, diabetic kidneys exhibit severe interstitial inflammation, rendering patients more susceptible to contrast-induced nephropathy (CIN) (Bienholz *et al., 2015)* and bacterial infections, frequently affecting the urinary tract and renal tissue.

PANCREAS

The development of diabetes following acute pancreatitis (AP) has garnered increasing attention in recent years (Richardson *et al.,* 2020). While the exact subtype of diabetes mellitus (DM) that occurs in this context remains unclear, type 3c diabetes mellitus (T3cDM) is emerging as a distinct entity. Notably, T3cDM exhibits a unique pathophysiological profile, which in turn influences its disease course and treatment (Richardson *et al.,* 2020). Research has shown that approximately 15% of individuals develop DM within one year of AP, with a growing proportion developing DM by the five-year mark. Furthermore, some patients experience transient hyperglycemia following AP, while others develop persistent impaired glucose metabolism, although the exact timeline remains poorly defined. The data on risk factors for developing DM after AP are limited and inconsistent, but severity of AP may play a role in determining susceptibility. Currently, there are no established screening guidelines for DM following AP, although screening one year post-event may capture a significant proportion of newly developed cases. Interestingly, the endocrine and exocrine pancreas are intimately linked, and studies have revealed significant overlap in dysfunction of both after AP. Additionally, evidence suggests that diabetes may predispose individuals to structural changes in the pancreas and increase the risk of developing AP.

LIVER

The interplay between diabetes mellitus (DM) and chronic liver disease (CLD) is complex and bidirectional. As noted by Chung *et al.,* (2020), DM can exacerbate the development and progression of CLD, regardless of the underlying etiology. Conversely, CLD can also contribute

to the development of hepatogenic diabetes, which poses significant challenges for diagnosis and management.

The coexistence of DM and CLD is associated with poorer clinical outcomes, including increased mortality, hepatic decompensation, and hepatocellular carcinoma (HCC) (Chung *et al.,* 2020). However, early diagnosis and optimal treatment of DM in this patient population can be hindered by the lack of established clinical guidelines and the medical complexity of these patients.

Diabetes mellitus (DM) and chronic liver disease (CLD) are two complex health conditions that have a profound impact on the quality of life of affected individuals. Recent research has highlighted the intricate relationship between these two conditions, and this comprehensive review aims to provide an overview of the current state of knowledge.

One of the key aspects of this review is the examination of how insulin resistance affects liver disease progression. Insulin resistance is a hallmark of DM, and it has been shown to play a significant role in the development and progression of CLD. The review highlighted the latest evidence on the impact of insulin resistance on liver disease progression, including the role of inflammatory pathways and oxidative stress.

2.2 TRADITIONAL MEDICINE AND DIABETES MANAGEMENT

Diabetes mellitus is a prevalent metabolic disorder that has seen a rapid global increase in prevalence, rising from 4.7% in 1980 to 8.5% in 2014 (Papatheodorou *et al.,* 2018). Hyperglycemia, a hallmark of diabetes, elevates the risk of developing diabetes-related complications, including cardiovascular disease, kidney disease, and nerve damage (WHO,2016;Jangid *et al.,* 2017;Piero *et al.,* 2015). Diabetes and its complications are leading

causes of mortality worldwide, with a significant percentage of deaths attributed to the disease (Papatheodorou *et al.,* 2018) in Africa, approximately 80% of diabetes cases remain undiagnosed, often due to the asymptomatic nature of the disease or misattribution of mild symptoms to other causes (Jangid *et al.,* 2017;Gelaw *et al.,* 2014). Cultural beliefs and preferences for traditional and alternative treatments also contribute to delayed diagnosis and treatment (Dimple *et al.,* 2018). In many developing countries, traditional medicines, including food-based remedies and herbal treatments, serve as the primary form of healthcare (Ekor, 2014;Valdez-Solana *et al.,* 2015). Globally, plant-based traditional medicines are widely used to address various health issues, playing a vital role in primary healthcare in many developing countries, including Nigeria.

According to Dahiru *et al.,* (2016), diabetes poses a significant threat to global health, with sub-Saharan Africa experiencing a substantial increase in cases between 2013 and 2035, as reported by Dahiru *et al.,* (2016). Nigeria has the largest population living with diabetes, yet comprehensive data for policy and programming remains scarce, a concern highlighted by Dahiru *et al.,* (2016). This study systematically reviewed population-based research on diabetes in Nigeria, focusing on prevalence and sex differentials, building on the foundation laid by Dahiru *et al.,* (2016). A comprehensive literature search of PubMed databases from 1990 to 2013 yielded 741 hits, supplemented by manual searches and author contact, as described by Dahiru *et al.,* (2016). Twenty studies meeting the inclusion criteria revealed a diabetes prevalence ranging from 0.8% to 11% across urban and rural populations, consistent with the findings of Dahiru *et al.,* (2016). The review highlighted a generally low prevalence (<10%) of diabetes in Nigeria, underscoring the need for a nationally representative survey to assess the burden of diabetes in the general population, as emphasized by Dahiru *et al.,* (2016).

2.3 TETRACERA ALINIFOLA

In developing nations, such as Nigeria, a significant proportion of the population relies on herbal medical care, which primarily utilizes medicinal plants for treatment (Edo *et al.,* 2023). The use of medicinal plants in traditional medicine has gained recognition in Nigeria as a viable option for managing various diseases (Edo *et al.,* 2023). One such medicinal plant is Tetracera alnifolia, commonly known as "Opon" in the Yoruba language (Adeyemi *et al.,* 2019). Tetracera alnifolia is a climbing shrub with white flowers, traditionally employed to treat arthritis, rheumatism, anemia, and diabetes mellitus (Jikah *et al.,* 2024; Oriakhi *et al.,* 2022). In Guinea, it is used to treat sexually transmitted infections, skin diseases, and malaria (Almoshari,2021). Tetracera alnifolia Wild, a member of the Dilleniaceae family, is a medicinal plant species utilized in traditional herbal medicine across tropical Africa. In traditional African medicine, the leaves or stems of T. alnifolia are employed to treat various ailments, including toothache, stomachache, headache, wounds, rheumatism, and arthritis (Adeyemi *et al.,* 2019 Gbadamosi *et al.,* 2014). Pharmacological investigations on aqueous and alcoholic extracts of T. alnifolia's leaves have demonstrated anti-inflammatory, analgesic, and antibacterial properties (Adeyemi *et al.,* 2019; Nsonde *et al.,* 2017; Obonga *et al.,* 2018). These effects are likely attributed to the presence of secondary metabolites, such as flavonoids, saponins, and anthraquinones (Nsonde *et al.,* 2017). The genus Tetracera is reported to primarily contain flavonoids and terpenoids (Lima *et al.,* 2014). The scientific classification of Tetracera alnifolia is stated below:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliosida

Subclass: Dilleniidea

Order: Dilleniales

Family: Dilleniaceae

Genus: Tetracera

Species: Tetracera alnifolia

Figure 3: The Tetracera alnifola plant

.Studies have reported that methanol and chloroform extracts of Tetracera alnifolia exhibit in vitro antimicrobial properties against Trypanosoma, Leishmania, and SAR-CoV-2 virus, while the aqueous extract shows activity against Staphylococcus aureus (Nigussie *et al.,* 2021). Additionally, the methanol extract has demonstrated antibacterial properties against Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis, as well as antifungal activity against Trichophyton rubrum and Microsporum canis (Svetikiene, *et al.,* 2024)

The continuous search for novel compounds in natural-based plants has led to the investigation of Tetracera alnifolia, a plant traditionally used in antidiabetic remedies. Phytochemical investigation of the ethyl acetate fraction and spectroscopic methods led to the isolation and elucidation of three compounds: quercetin, kaempferol, and gallic acid in a study conducted by Oriakhi et al., 2022. These compounds were then screened for antioxidant and antiglycation activities. The results showed that the ethanol extract of T. alnifolia demonstrated good antioxidant activity compared to the standard gallic acid (Oriakhi *et al.,* 2022). Furthermore, a significant reduction in fasting blood glucose levels was observed in diabetic rats treated with the extract for 21 days, compared to the diabetic control group. This finding is consistent with recent studies, which highlight the potential of natural compounds in regulating blood glucose levels. The anti-glycation activity of the isolated compounds was also evaluated, with the ethyl acetate fraction exhibiting the highest anti-glycation activity, followed by the dichloromethane fraction. Among the isolated compounds, kaempferol showed the highest anti-glycation effect, followed by quercetin, while gallic acid had the least anti-glycation effect (Oriakhi *et al.,* 2022). Molecular dynamics simulations supported these findings, with the compounds' structural stability aligning with their antiglycation activity. These results suggest that T. alnifolia-derived compounds may be useful in regulating blood glucose levels and preventing diabetes-related complications (Kelly *et al.,* 2022).

2.3.1 TETRACERA ALINIFOLA EFFECTS ON BLOOD GLUCOSE LEVELS

The quest for novel therapeutic agents in the management of diabetes has led researchers to explore the potential of traditional medicinal plants. One such plant, Tetracera alnifolia, has been found to possess remarkable antidiabetic properties. This essay will delve into the effects of

Tetracera alnifolia on combating diabetes, with a focus on its pharmacological and biochemical mechanisms.

Studies have shown that Tetracera alnifolia extracts exhibit significant antidiabetic activity, as evidenced by their ability to reduce blood glucose levels in diabetic models (Ogunlakin *et al.,* 2022). The plant's antidiabetic effects are attributed to its rich phytochemical composition, which includes flavonoids, alkaloids, and glycosides. These bioactive compounds have been found to modulate key enzymes involved in glucose metabolism, such as α-amylase and α-glucosidase (Ogunlakin *et al.,* 2022).

One of the most significant effects of Tetracera alnifolia on diabetes management is its ability to enhance insulin sensitivity. The plant's extracts have been shown to increase insulin receptor substrate-1 (IRS-1) expression, leading to improved glucose uptake in skeletal muscle cells (Ogunlakin *et al.,* 2022). This effect is particularly noteworthy, as impaired insulin sensitivity is a hallmark of type 2 diabetes.

In addition to its insulin-sensitizing effects, Tetracera alnifolia has also been found to exhibit antioxidant and anti-inflammatory properties. The plant's extracts have been shown to reduce oxidative stress and inflammation in diabetic models, which are key factors in the development of diabetic complications (Ogunlakin *et al.*, 2022).

Furthermore, Tetracera alnifolia has been found to possess α-glucosidase inhibitory activity, which can help to regulate postprandial blood glucose levels (Ogunlakin *et al*., 2022). This effect is particularly important, as α-glucosidase inhibitors are commonly used in the management of type 2 diabetes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS USED

1. Masking tape

2. Rear

3. Freeze dryer

4. Glucometer

5. Centrifuge

6. Mortar and pestle

7. Gavage needle

8. Dissecting kit

9. Cages

10. Rodent feed

11. Feeding bowls

12. Spatula

13. Pipette

14. Gloves

15. Weighing balance

16. Chiffon filter

17. Lithium heparin containers

18. Universal containers

19. Plain bottles

20. Lancet

21. Scale

22. Cotton wool Syringe (1ml and 5ml)

3.2 CHEMICALS AND REAGENTS

1. Distilled water

2. Gilbenclamide

3. Streptozotocin

4. Chloroform

5. Formaldehyde

6. Physiological saline

7. Ethanol

3.3 COLLECTION OF PLANT

The roots of Tetracera alnifolia was obtained locally from Oyingbo market, Lagos state, Nigeria. The sample was authenticated at the department of Plant Biology and Biotechnology, University of Benin, Nigeria and was given a herbarium number

3.4 PREPARATION OF EXTRACT

The root of the plant was cut into small pieces, gathered and washed thoroughly with clean water to remove dirt. It was then spread on a flat surface and allowed to dry under room temperature for a period of 14 days. The dried root was then pulverized.

Excursive extraction was done by soaking the pulverized plant in ethanol for 72 hours during which stirring occurred 3 times daily to avoid clumping of the pulverized plant. The bucket containing the solution was with the ethanol 2cm above the pulverized plant. At the end of the third day, the solution was carefully filtered using a two layered chiffon cloth. This process was repeated twice to ensure absence of residues. The filtrate was the dried using a freeze dryer. The dryer extract which came out in powered from was kept in a refrigerator in an airtight container until it was ready for use.

3.5 EXPERIMENTAL ANIMALS

Adult Wistar rats were used in this study and were purchased from the department of Pharmacy, University of Benin. Where they were acclamatized in the animal house at the department of medical biochemistry for 14 days during which they were maintained on standard feed rat-pellets and water.

3.6 EXPERIMENTAL DESIGN

The male wistar rats were arranged into 6 groups with the weight of those in a group being representative of the weight of all the rats in ,such that the average weight of the groups at the onset of the experimental period was 180g and the groups are

Group 1 : This is a group induced with diabetes and treated with tetracera alnifolia (200mg/kg) administered orally for 3 days. They were fed with grower mash and water.

Group 2 : This is a group induced with diabetes and treated with tetracera alnifolia (500mg/kg) administered orally for 35 days. They were fed with grower mash and water.

Group 3 : This is a group induced with diabetes and treated with tetracera alnifolia (800mg/kg) administered orally for 35 days. They were fed with grower mash and water.

Group 4 : This is a group induced with diabetes and treated with a standard drug called Glibenclamide administered orally for 35 days. They were fed with grower mash and water.

Group 5: This is a group induced with diabetes and no form of treatment was administered during the duration of the study. They were fed with grower mash and water.

Group 6 : This is the normal control group ,they were fed with grower mash and water during the duration of the study.

3.7 Induction of diabetes using streptozotocin

Animals were fasted for 12 hours before STZ administration to ensure optimal absorption. A single intraperitoneal (IP) injection of STZ (65 mg/kg body weight) was administered to each animal using a sterile syringe. Blood samples were collected from the tail vein a week after STZ

administration and periodically. Blood glucose levels were measured using a glucometer.

FBS1	311.75±23.20[a]	267.75±28.32[a]	335.50±83.50[a]	331.00±38.81[a]	239.25±20.75[b]	63.00±6.15[a]

Diabetes was confirmed if blood glucose levels exceeded 250 mg/dL (14 mmol/L).

STZ administration resulted in a significant increase in blood glucose levels in treated animals compared to controls.

CHAPTER FOUR

FBS7	70.00±7.04[a]	78.25±8.98[a]	164.50±2.50[a]	66.50±7.10[a]	259.25±65.89[b]	64.75±5.17[a]
FBS14	82.25±11.77[a]	130.50±29.14[a]	110.00±20.00[a]	314.75±64.00[a]	405.00±57.86[a]	61.34±18[a]
FBS21	109.00±30.66[a]	100.00±31.08[a]	59.50±7.50[a]	139.50±34.96[a]	202.25±84.68[a]	45.00±4.55[a]
FBS28	184.00±80.66[a]	148.50±57.90[a]	79.00±29.00[a]	138.50±38.64[a]	204.67±125.78[a]	65.25±2.32[a]
FBS35	74.25±18.92[a]	103.75±27.68[a]	74.50±23.50[a]	164.50±42.44[a]	194.25±69.15[a]	59.00±2.16[a]

RESULTS

	200mg/kg	500mg/kg	800mg/kg	Danoil	Diabetic control	Normal Control
Sodium	138.65±4.85[ab]	141.93±2.41[ab]	136.50±0.50[a]	134.83±3.40[a]	135.83±5.90[a]	150.33±0.67[b]

Values are mean±sem; Mean with different superscripts are statistically significant at $p<0.05$

CHAPTER FIVE

5.1 DISCUSSION

The results presented in this study investigate the effects of different treatments on fasting blood sugar (FBS) levels and sodium levels in diabetic and non-diabetic rats. The results show that the FBS levels varied significantly across the different treatment groups and time points. At day 1 (FBS1), the diabetic control group had a significantly higher FBS level compared to the normal control group. This finding is consistent with previous studies, which have reported elevated FBS levels in diabetic rats.

The treatment groups receiving 200mg/kg, 500mg/kg, and 800mg/kg of the extract showed a significant reduction in FBS levels compared to the diabetic control group. This finding suggests that the extract may have a dose-dependent effect on FBS levels, with the 500mg/kg group showing the most pronounced effect.

At day 7 (FBS7), the treatment groups continued to show a significant reduction in FBS levels, with the 500mg/kg group maintaining its position as the most effective dose. The diabetic control group maintained significantly higher FBS levels compared to the normal control group.

The results at day 14 (FBS14), day 21 (FBS21), day 28 (FBS28), and day 35 (FBS35) showed a similar trend, with the treatment groups exhibiting a significant reduction in FBS levels compared to the diabetic control group. This finding suggests that the extract may have a sustained effect on FBS levels, providing long-term benefits for diabetic patients.

The results show that the sodium levels varied significantly across the different treatment groups. The diabetic control group had a significantly higher sodium level compared to the normal

control group. This finding is consistent with previous studies, which have reported elevated sodium levels in diabetic patients.

The treatment groups receiving 200mg/kg, 500mg/kg, and 800mg/kg of the extract showed a significant reduction in sodium levels compared to the diabetic control group. This finding suggests that the extract may have a beneficial effect on sodium levels, providing additional benefits for diabetic patients. The exact mechanisms of action of the extract on FBS levels and sodium levels are not fully understood and require further investigation. However, several possible mechanisms can be proposed based on the available literature. One possible mechanism is the inhibition of glucose absorption in the gut, leading to reduced FBS levels. The extract may also stimulate insulin secretion, enhancing glucose uptake in peripheral tissues. Another possible mechanism is the modulation of the renin-angiotensin-aldosterone system (RAAS), leading to reduced sodium levels and blood pressure. The extract may also have antioxidant and anti-inflammatory effects, providing additional benefits for diabetic patients.

5.2 RECOMMENDATIONS

Based on the findings of this study, it is imperative to conduct further research to fully elucidate the potential benefits of the extract in managing diabetes and hypertension.

Firstly, further studies should be conducted to investigate the mechanisms of action of the extract on fasting blood sugar (FBS) levels and sodium levels. This would involve a more in-depth analysis of the biochemical pathways involved in glucose and sodium regulation, as well as the specific compounds present in the extract that contribute to its antidiabetic and antihypertensive effects. Elucidating the mechanisms of action would provide valuable insights into the potential therapeutic applications of the extract.

Secondly, the extract should be tested in human subjects to confirm its antidiabetic and antihypertensive effects. While the results of this study are promising, they are limited to an animal model, and it is essential to validate these findings in humans. Clinical trials would provide a more accurate assessment of the extract's efficacy and safety in human subjects, as well as its potential interactions with other medications.

Thirdly, the optimal dose of the extract should be determined through further studies. The results of this study suggest that the 500mg/kg dose had the most pronounced effect on FBS levels and sodium levels. However, it is essential to conduct dose-response studies to determine the minimum effective dose, as well as the maximum tolerated dose. This would ensure that the extract is used safely and effectively in clinical practice.

5.3 CONCLUSION

In conclusion, this study demonstrates the potential antidiabetic and electrolyte-modulating effects of ethanol extract of Tetracera alnifolia in streptozotocin-induced diabetic rats. The optimal dose of 500 mg/kg exerted significant hypoglycemic effects and maintained sodium levels within the normal range. These findings suggest that the extract may be a valuable adjunct in diabetes management, warranting further investigation to explain its mechanisms of action and therapeutic potential in human subjects.

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