THE COMPARATIVE EFFECT OF THE ADMINISTRATION OF HYDRO-METHANOL 25MG/KG AND ACETONE-FRACTION 25MG/KG ON GAMMA-GLUTAMYL TRANSFERASE ACTIVITY OF L-NAME STREPTOZOTOCIN INDUCED HYPERTENSIVE / DIABETIC MALE WISTAR RAT.

BY

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Dedication

I dedicate this report to God Almighty for His endless grace and strength and also to my parents Mr. and Mrs. Obasogie, who has been a source of motivation to my success during the course of my academic journey.

Certification

This is to certify that this undergraduate project has been approved by the department and was carried out by **Obasogie Osamudiamen** with matriculation number **LSC2006818** from the Department of Biochemistry, Faculty of Life Science, University of Benin, Benin City, Edo State.

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Abstract

Hypertension and diabetes are associated with metabolic disturbances, including abnormal enzyme activity that can contribute to disease progression. This study evaluates the therapeutic potential of *Simarouba glauca* extracts in modulating gamma-glutamyl transferase (GGT) activity in hypertensive and diabetic rats. Both hydro-methanol and acetone fractions exhibited a significant ability to restore GGT activity in blood plasma and liver tissue, indicating their dual role in metabolic regulation and hepatoprotection. The acetone fraction demonstrated slightly superior effects, likely due to its distinct phytochemical composition, which may include a higher concentration of bioactive compounds. The ability of these extracts to normalize GGT activity suggests they possess antioxidant properties that contribute to enzyme stabilization and liver protection. These findings highlight the potential of *Simarouba glauca* as a natural therapeutic agent for managing metabolic dysfunctions associated with hypertension and diabetes. Further research is needed to elucidate the precise bioactive constituents responsible for these effects and their mechanisms of action.

Chapter One

1.0: Literature Review

Hypertension remains a leading contributor to illness and death across Africa, with Nigeria-Africa's most populous nation-playing a substantial role in this burden. In many developing nations, including Nigeria, healthcare services have traditionally emphasized infectious diseases like malaria and tuberculosis. However, in recent years, non-communicable diseases (NCDs) have emerged as a major public health concern. Hypertension, one of the most widespread NCDs globally, accounts for approximately 45% of deaths from heart disease and 51% of deaths from strokes worldwide (Calhoun *et al.,* 2008)

Among the World Health Organization's (WHO) six global regions, the African region holds the highest recorded prevalence of hypertension, affecting an estimated 46% of adults aged 25 and older, as noted in WHO's 2010 Global Status Report on Non-Communicable Diseases. In developing countries, routine medical check-ups are infrequent, meaning that the risk factors for NCDs are often not regularly monitored; Nigeria is no exception. The Executive Director of the Nigerian Heart Foundation, an NGO, has pointed out that, "the health system offers diagnosis and treatment only to those who pay for it." This financial barrier, along with limited resources, means that even when individuals undergo medical examinations, the diagnosis of chronic conditions like hypertension can be unreliable, especially at the primary healthcare level.

Research on hypertension in Nigeria extends back several decades. The first retrospective study of hypertension in Nigerian children aged 2-11 years was conducted in 1974 by Aderele and Seriki in Ibadan. Since then, various studies have explored the prevalence of hypertension in Nigerian children and adolescents, with findings ranging from 0.1% to 17.5%. Nonetheless, recent reviews of blood pressure patterns in Nigerians have primarily focused on adults or included only limited studies on children and adolescents.

Moreover, current reviews do not generally address prehypertension, leaving a gap in the understanding of its prevalence among Nigerians. Compounding this, there has yet

to be a trend analysis examining hypertension progression over time within the Nigerian population. Recognizing the connection between hypertension in childhood and high blood pressure in adulthood, it is essential to measure the number of at-risk individuals to inform resource allocation effectively, especially during times of scarcity. A detailed examination of cross-sectional studies that report on hypertension and prehypertension rates in children and adolescents in Nigeria is needed. Such analysis is critical, as Nigeria's diverse geographical, ethnic, and cultural landscape complicates the implementation of nationally representative studies, making systematic reviews essential for identifying trends across the nation.

By analyzing hypertension rates and trends comprehensively, public health stakeholders in Nigeria and other African nations can develop targeted policies and interventions to combat this silent epidemic effectively. This approach will ensure a better allocation of resources and improve early detection, ultimately aiding in the reduction of hypertension-related morbidity and mortality across Nigeria and the African continent.

1.1.0: Types of Hypertension

There are two primary hypertension types; for 95 percent of people with high blood pressure, the cause of their hypertension is unknown this is called essential, or primary, hypertension. When a cause can be found, the condition is called secondary hypertension.

1.1.1: Primary Hypertension

Primary hypertension, also known as essential hypertension, is a chronic condition where the blood pressure is consistently elevated without an identifiable secondary cause. Unlike secondary hypertension, which has a specific underlying medical cause such as kidney disease or hormonal disorders, primary hypertension is thought to be influenced by a complex interplay of genetic, environmental, and lifestyle factors. It is the most common form of hypertension, responsible for approximately 90-95% of all hypertension cases worldwide (Carretero & Oparil, 2000).

The development of primary hypertension is strongly associated with lifestyle factors, including high salt intake, physical inactivity, and excessive alcohol consumption. These habits can exacerbate the condition by causing fluid retention, vascular stiffness, and an increase in vascular resistance, leading to consistently high blood pressure. Additionally, primary hypertension is often seen in individuals with a family history of the condition, suggesting a genetic predisposition. Research indicates that specific gene variations may make certain individuals more susceptible to hypertension, especially when combined with unhealthy lifestyle practices (Harrison, 2015).

The "silent killer" aspect of primary hypertension is significant because the condition often has no noticeable symptoms, allowing it to progress undetected over years. This prolonged period of elevated blood pressure puts additional strain on the heart and blood vessels, increasing the risk of serious health issues like heart disease, stroke, and kidney failure. Early intervention, including lifestyle modification and medication when necessary, is essential in managing primary hypertension and preventing these complications (Kjeldsen, 2018).

1.1.2: Secondary Hypertension

Secondary hypertension is a type of high blood pressure caused by an underlying medical condition or specific identifiable factor. Unlike primary hypertension, which develops due to a complex interplay of genetic and lifestyle factors, secondary hypertension results directly from conditions that affect the kidneys, heart, arteries, or endocrine system. Common causes include kidney disease, hormonal disorders such as hyperthyroidism or Cushing's syndrome, and sleep apnea, all of which can alter the body’s ability to regulate blood pressure effectively (Viera and Neutze, 2010).

This type of hypertension is less common than primary hypertension, accounting for approximately 5-10% of hypertension cases. However, it often presents more severely and can be resistant to standard treatments. Identifying and treating the underlying cause of secondary hypertension is crucial, as blood pressure levels can return to normal or be significantly reduced once the primary condition is managed. For instance, in cases where kidney function is impaired, blood pressure regulation can be restored through medications or procedures that improve kidney health (Calhoun *et al.,* 2008).

Unlike primary hypertension, secondary hypertension often produces symptoms specific to the underlying cause. For example, in cases of adrenal gland disorders, individuals may experience symptoms such as muscle weakness, fatigue, or abnormal weight gain. Because secondary hypertension can lead to rapid health deterioration if untreated, early diagnosis and intervention are critical. Diagnostic testing, such as blood tests, urine analysis, and imaging studies, is often necessary to identify the underlying condition and determine an effective treatment approach (Pimenta and Calhoun, 2012).

1.1.3: Malignant Hypertension

Malignant hypertension is a severe and rapidly progressing form of high blood pressure that can result in life-threatening complications if not treated promptly. This condition is characterized by an extremely high blood pressure reading—typically a diastolic pressure above 120 mm Hg—accompanied by signs of organ damage, particularly affecting the eyes, kidneys, heart, and brain. Malignant hypertension is often associated with symptoms like severe headache, chest pain, shortness of breath, vision changes, and nausea, indicating the critical impact on various organs.

One of the most concerning aspects of malignant hypertension is its potential to cause irreversible damage. The high pressure can lead to swelling of the optic nerve (papilledema), kidney failure, heart failure, and even stroke. Prompt diagnosis and aggressive treatment are crucial to prevent or minimize organ damage. The condition typically requires immediate blood pressure reduction through intravenous medications and continuous monitoring in a hospital setting to stabilize blood pressure safely (Gifford, 2001).

1.1.4: Resistant Hypertension

Resistant hypertension is a type of high blood pressure that remains elevated despite the use of three or more antihypertensive medications, typically including a diuretic. This condition is particularly challenging because, even with standard treatment strategies, blood pressure levels fail to reach target goals, increasing the risk of serious cardiovascular events such as heart attack, stroke, and kidney disease. Factors contributing to resistant hypertension include lifestyle influences, like high salt intake

and obesity, as well as underlying medical issues, such as sleep apnea and chronic kidney disease. Additionally, some cases may involve medication resistance or interactions that reduce treatment effectiveness (Calhoun *et al.,* 2008).

Effective management of resistant hypertension often requires a comprehensive approach. This includes lifestyle modifications, such as dietary changes, weight loss, and physical activity, alongside optimized medication regimens that may involve adding more drugs or adjusting doses. In some cases, treatment may also include addressing secondary causes, such as treating sleep apnea or managing kidney function. With this multi-faceted approach, it is possible to bring blood pressure closer to recommended levels and reduce associated health risks.

1.2: Stages of Hypertension

Hypertension, commonly known as high blood pressure, is classified into various stages based on blood pressure measurements, which help determine the severity of the condition and guide appropriate treatment. The stages of hypertension range from normal blood pressure to hypertensive crisis, each indicating increasing levels of cardiovascular risk. Blood pressure is measured in millimeters of mercury (mm Hg) and expressed as systolic (the pressure during heartbeats) over diastolic (the pressure between beats).

1. Normal Blood Pressure: Normal blood pressure is defined as a systolic pressure below 120 mm Hg and a diastolic pressure below 80 mm Hg (Carey and Whelton, 2018). Maintaining this range is essential to reduce cardiovascular risks and prevent hypertension-related complications.

2. Elevated Blood Pressure: Elevated blood pressure, also known as prehypertension, is defined as a systolic reading between 120-129 mm Hg and a diastolic reading less than 80 mm Hg. Although not yet considered hypertensive, individuals with elevated blood pressure are at a higher risk of progressing to hypertension, especially if lifestyle changes are not made (Flack *et al.,* 2010).

3. Stage 1 Hypertension: Stage 1 hypertension occurs when systolic pressure is between 130-139 mm Hg or diastolic pressure is between 80-89 mm Hg. At this stage, lifestyle interventions such as dietary changes, increased physical activity, and stress management are recommended, and medication may be considered for those with additional risk factors, such as diabetes or cardiovascular disease.

4. Stage 2 Hypertension: Stage 2 hypertension is more severe, with a systolic pressure of 140 mm Hg or higher or a diastolic pressure of 90 mm Hg or higher. Individuals with Stage 2 hypertension typically require lifestyle interventions alongside antihypertensive medications to bring blood pressure under control and reduce health risks (Powers *et al.,* 2020).

5. Hypertensive Crisis: A hypertensive crisis is the most severe stage, with a systolic pressure above 180 mm Hg and/or a diastolic pressure above 120 mm Hg. This stage is a medical emergency that can lead to life-threatening complications like stroke, heart attack, or kidney failure if not treated immediately. Symptoms may include severe headache, shortness of breath, chest pain, and vision changes, and urgent medical intervention is needed to reduce blood pressure safely (Carey *et al.,* 2018).

1.3: Treatment of Hypertension

The treatment of hypertension is essential to reducing cardiovascular risks, preventing organ damage, and improving overall quality of life. Hypertension management typically involves a combination of lifestyle changes and, when necessary, pharmacological interventions. Lifestyle modifications are often the first line of treatment, as these can significantly lower blood pressure levels and may be sufficient for patients with mild hypertension. Recommended lifestyle adjustments include a heart-healthy diet, reducing salt intake, regular physical activity, weight management, and limiting alcohol intake.

When lifestyle changes are not enough to control blood pressure, medications are prescribed. Common classes of antihypertensive medications include diuretics, ACE inhibitors, angiotensin II receptor blockers (ARBs), calcium channel blockers, and beta-

blockers. Diuretics help the kidneys remove excess salt and water, thereby reducing blood volume and lowering blood pressure. ACE inhibitors and ARBs work by relaxing blood vessels, which helps blood flow more easily, reducing vascular resistance. Calcium channel blockers reduce blood pressure by limiting calcium entry into heart and blood vessel cells, which relaxes the vessels, while beta-blockers lower blood pressure by slowing the heart rate (Carey *et al.,* 2018).

In cases of resistant hypertension, where blood pressure remains high despite the use of three or more medications, further evaluation and combination therapy may be necessary. Patients with resistant hypertension may benefit from more intensive treatments, including adding aldosterone antagonists or modifying existing medications, alongside strict adherence to lifestyle changes (Calhoun *et al.,* 2008). The goal is to reach and maintain target blood pressure levels to reduce the risk of heart disease, stroke, and kidney damage.

1.4: Diabetes Mellitus

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by high blood glucose levels due to the body's inability to produce enough insulin, use insulin effectively, or both. Insulin is a hormone produced by the pancreas that helps regulate blood sugar by enabling cells to absorb glucose from the bloodstream for energy. When this process is disrupted, glucose accumulates in the blood, leading to hyperglycemia, which can damage various organs over time.

1.4.1: Causes of Diabetes Mellitus

The causes of diabetes mellitus vary depending on the type of diabetes. However, the disease generally results from a combination of genetic, environmental, and lifestyle factors that impact insulin production, insulin action, or both.

1.4.1.1: Genetic Factors:

Genetics play a significant role in the risk of developing diabetes. Family history is a known risk factor, especially in Type 2 diabetes. Certain genes influence the likelihood of diabetes by affecting insulin production, glucose metabolism, or the immune

response that leads to autoimmune destruction of insulin-producing cells in the pancreas (Olokoba, Obateru, and Olokoba, 2012). For instance, Type 1 diabetes is often linked to specific genetic markers, such as those in the HLA (human leukocyte antigen) system, which is involved in immune regulation.

1.4.1.2: Autoimmune Destruction:

In Type 1 diabetes, the immune system mistakenly attacks and destroys insulin-producing beta cells in the pancreas. This autoimmune response is thought to be triggered by genetic predisposition combined with environmental factors such as viral infections or other immune challenges. As a result, the pancreas loses its ability to produce insulin, necessitating lifelong insulin therapy (Atkinson, Eisenbarth, and Michels, 2014).

1.4.1.3: Lifestyle and Environmental Factors:

In Type 2 diabetes, lifestyle factors like poor diet, obesity, and physical inactivity are significant contributors. These factors can lead to insulin resistance, where the body's cells become less responsive to insulin, requiring the pancreas to produce more insulin to compensate. Over time, the pancreas cannot keep up with this demand, leading to elevated blood glucose levels.

1.4.2: Stages of Diabetes Mellitus

Diabetes mellitus progresses through various stages, especially in Type 2 diabetes, where individuals may experience a gradual increase in blood glucose levels over time.

1.4.2.1: Normoglycemia (Normal Blood Glucose Levels):

In this stage, blood glucose levels are within the normal range, and insulin secretion and sensitivity are balanced. No signs or symptoms of diabetes are present, and the body's glucose regulation is intact. However, individuals with certain risk factors, such as obesity or family history, may still be at risk of developing diabetes.

1.4.2.2: Pre-diabetes:

Pre-diabetes is an intermediate stage where blood glucose levels are elevated but not high enough to be classified as diabetes. In this stage, individuals often have impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), indicating that their glucose metabolism is beginning to deteriorate (Tabák *et al.,* 2012). Pre-diabetes is a warning sign and a critical intervention point, as lifestyle changes can prevent or delay the progression to Type 2 diabetes.

1.4.2.3: Diabetes:

When blood glucose levels reach a diagnostic threshold, diabetes is confirmed. In Type 1 diabetes, the onset can be sudden and severe, often manifesting in childhood or adolescence. In Type 2 diabetes, the progression is typically slower and may be asymptomatic initially, allowing blood sugar levels to rise without noticeable symptoms. As the disease progresses, symptoms such as frequent urination, excessive thirst, fatigue, and blurred vision become evident.

1.4.2.4: Complications Stage:

If blood glucose levels are not adequately managed, diabetes can lead to long-term complications. Chronic hyperglycemia damages blood vessels, nerves, and organs, leading to complications like cardiovascular disease, kidney damage (nephropathy), nerve damage (neuropathy), and eye damage (retinopathy). These complications are often severe and highlight the importance of early diagnosis, effective treatment, and long-term blood glucose control.

1.4.3: Types of Diabetes Mellitus

Diabetes mellitus is a complex and chronic metabolic disorder characterized by high blood glucose levels resulting from defects in insulin production, insulin action, or both. It encompasses several types, each with distinct causes, characteristics, and treatment approaches. The primary types of diabetes mellitus include Type 1 diabetes, Type 2 diabetes, and gestational diabetes.

1.4.3.1: Type 1 Diabetes

Type 1 diabetes, often diagnosed in childhood or early adulthood, is an autoimmune condition where the immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas. This results in little to no insulin production, necessitating external insulin administration to regulate blood glucose levels (Atkinson, Eisenbarth, and Michels, 2014). Without insulin, glucose cannot enter the cells to provide energy, leading to dangerously high blood glucose levels and potentially life-threatening complications like diabetic ketoacidosis if untreated.

1.4.3.2: Type 2 Diabetes

Type 2 diabetes is the most common form of diabetes and is typically associated with insulin resistance, where the body's cells become less responsive to insulin. Initially, the pancreas compensates by producing more insulin, but over time, it may be unable to sustain this increased output, resulting in hyperglycemia (high blood glucose levels) (Olokoba, Obateru, and Olokoba, 2012). This form of diabetes is strongly associated with lifestyle factors, including obesity, physical inactivity, and poor diet, though genetic predispositions also play a role.

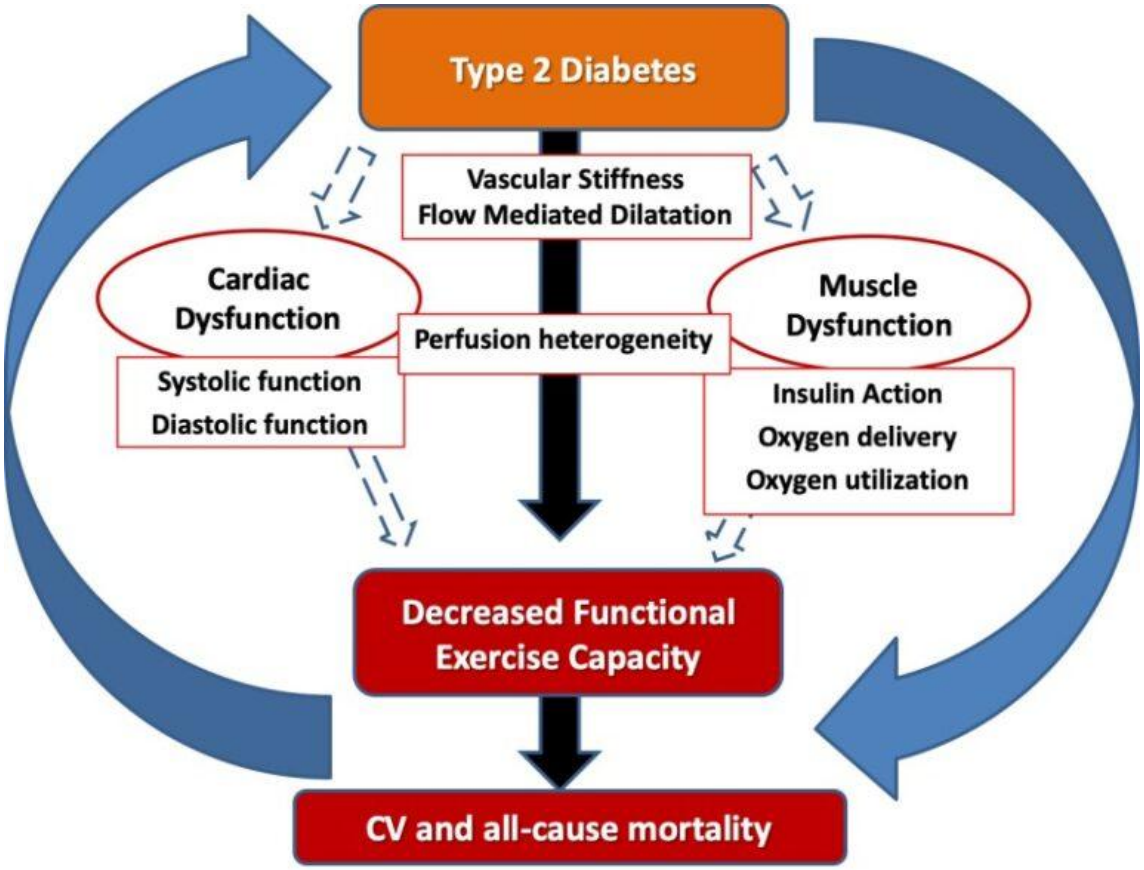

Fig. 1.1 How exercise help manage type 2 diabetes
Hellwing and Hellwing (2025)

1.4.3.3: Gestational Diabetes

Gestational diabetes occurs in pregnant women who previously did not have diabetes but develop high blood glucose levels during pregnancy. It typically appears in the second trimester and can pose risks to both the mother and the baby, including higher chances of birth complications and an increased risk of developing Type 2 diabetes later in life (Kim, 2010). Although gestational diabetes usually resolves after childbirth, careful monitoring and management are essential during pregnancy.

1.4.4: Treatment of Diabetes Mellitus

The treatment of diabetes mellitus varies depending on the type and severity of the condition. While all types require blood glucose monitoring, the therapeutic strategies differ significantly.

1.4.4.1: Insulin Therapy for Type 1 Diabetes

Since Type 1 diabetes results from an absolute deficiency of insulin, lifelong insulin therapy is required. Insulin can be administered through injections or an insulin pump, and treatment is tailored to maintain blood glucose within a target range. Insulin types vary in their onset, peak, and duration, including rapid-acting, short-acting, intermediate-acting, and long-acting insulin. A combination of these may be used to mimic natural insulin secretion and ensure stable blood glucose levels (Atkinson, Eisenbarth, and Michels, 2014). In addition, individuals with Type 1 diabetes often use continuous glucose monitoring (CGM) devices to track glucose levels in real time, allowing for precise insulin dose adjustments.

1.4.4.2: Lifestyle Changes and Medication for Type 2 Diabetes

The cornerstone of Type 2 diabetes treatment is lifestyle modification, which includes dietary changes, regular physical activity, weight management, and smoking cessation. Dietary changes focus on reducing carbohydrate intake, choosing low glycemic index foods, and incorporating fiber-rich fruits and vegetables.

For many patients, lifestyle changes alone may not suffice, and medication is often needed. Oral medications are common in Type 2 diabetes treatment, including metformin, which lowers blood glucose by reducing liver glucose production and improving insulin sensitivity in peripheral tissues (Olokoba, Obateru, and Olokoba, 2012). Other classes of drugs include sulfonylureas, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists. Some patients may eventually require insulin therapy if their condition progresses and oral medications are no longer effective.

1.4.4.3: Gestational Diabetes Management

Gestational diabetes treatment primarily focuses on dietary modifications and regular physical activity to manage blood glucose levels during pregnancy. A diet high in fiber, low in simple sugars, and balanced in carbohydrates, proteins, and fats is recommended. Regular physical activity can improve insulin sensitivity and help maintain a healthy weight during pregnancy (Kim, 2010). In cases where lifestyle modifications are insufficient to control blood glucose, insulin therapy is preferred over oral

hypoglycemic agents due to safety concerns for the fetus. Blood glucose is closely monitored throughout pregnancy, as poorly controlled gestational diabetes can lead to complications during delivery.

Gestational diabetes typically resolves after childbirth, but women who experience it are encouraged to continue a healthy lifestyle, as they have an elevated risk of developing Type 2 diabetes in the future. Regular follow-up glucose testing post-pregnancy is also recommended.

1.5: Comorbid Hypertension and Diabetes

Hypertension and diabetes are two prevalent chronic conditions that frequently occur together, forming a dangerous combination that significantly increases the risk of cardiovascular disease, kidney disease, and other complications. This comorbidity is especially common in individuals with Type 2 diabetes, where high blood pressure and elevated blood glucose levels can create a cascade of adverse health effects. Managing both conditions simultaneously presents unique challenges, but effective control is crucial for reducing long-term health risks and improving quality of life (Cheung and Li, 2012).

1.5.1: Relationship between Hypertension and Diabetes

The co-occurrence of hypertension and diabetes is not coincidental; rather, these conditions share overlapping mechanisms and risk factors. Insulin resistance, a hallmark of Type 2 diabetes often leads to changes in blood vessel function, sodium retention, and increased sympathetic nervous system activity, which collectively contribute to elevated blood pressure. Obesity, physical inactivity, and poor dietary habits are common risk factors that further increase the likelihood of both conditions appearing together (Ferrannini and Cushman, 2012).

When diabetes and hypertension occur together, they exacerbate each other's effects. High blood glucose levels can lead to damage in blood vessel walls, making them more susceptible to hypertension-induced stress. In turn, elevated blood pressure can worsen insulin resistance and accelerate diabetes-related complications. Together, these

conditions significantly raise the risk of cardiovascular diseases such as heart attack and stroke, and can also accelerate kidney damage, leading to chronic kidney disease, a common outcome for individuals with both diabetes and hypertension.

1.5.2: Complications of Comorbid Hypertension and Diabetes

The combination of hypertension and diabetes markedly increases the risk of several severe health complications, especially those affecting the cardiovascular and renal systems:

1.5.2.1: Cardiovascular Disease

Individuals with both hypertension and diabetes have a much higher risk of developing cardiovascular disease compared to those with only one of these conditions. Chronic high blood pressure and hyperglycemia damage the blood vessels and can lead to atherosclerosis, which is the buildup of plaque in the arteries. This narrowing of the arteries restricts blood flow, which increases the likelihood of a heart attack or stroke (Cheung and Li, 2012).

1.5.2.2: Kidney Disease

The kidneys are particularly vulnerable to damage from the combined effects of hypertension and diabetes. High blood pressure can damage the tiny blood vessels in the kidneys, reducing their ability to filter blood effectively. In individuals with diabetes, high blood sugar levels also stress the kidneys, leading to diabetic nephropathy. Together, these conditions can cause significant and often irreversible kidney damage, resulting in chronic kidney disease or end-stage renal disease, which may require dialysis or kidney transplantation (Ferrannini and Cushman, 2012).

1.5.2.3: Retinopathy and Neuropathy

The combined effects of hypertension and diabetes can also affect the eyes and nerves. Diabetic retinopathy, where high blood glucose levels damage the blood vessels in the retina, is worsened by hypertension. This can lead to blindness if not managed carefully. Neuropathy, or nerve damage, is also accelerated when both conditions are present,

potentially resulting in loss of sensation, particularly in the limbs, and increasing the risk of foot ulcers and infections.

1.5.3: Management of Comorbid Hypertension and Diabetes

The management of comorbid hypertension and diabetes requires a comprehensive and integrated approach, often involving lifestyle modifications, pharmacotherapy, and regular monitoring.

1.5.3.1: Lifestyle Modifications

Dietary changes, regular exercise, weight management, and smoking cessation are key components of managing both diabetes and hypertension. A diet low in sodium, refined sugars, and saturated fats can help manage both blood glucose and blood pressure levels. Physical activity improves insulin sensitivity and reduces blood pressure, while weight loss can decrease the burden on the cardiovascular system and enhance metabolic control (Cheung and Li, 2012).

1.5.3.2: Pharmacological Treatment

Medications are often necessary to control both hypertension and diabetes. Common medications for managing blood pressure in patients with diabetes include ACE inhibitors or angiotensin II receptor blockers (ARBs), which help protect the kidneys as well as lower blood pressure. For glucose control, medications such as metformin or GLP-1 receptor agonists are commonly used, with careful consideration to avoid hypoglycemia. Additionally, patients with both conditions may require lipid-lowering drugs, as high cholesterol is another common issue in these individuals (Ferrannini and Cushman, 2012).

1.5.3.3: Regular Monitoring and Target Setting

Monitoring blood pressure and blood glucose levels frequently is crucial for managing these comorbid conditions. Generally, individuals with both hypertension and diabetes are advised to aim for a blood pressure target of below 130/80 mmHg, though targets may vary depending on individual health profiles and existing complications. Regular

visits to healthcare providers allow for adjustments to treatment regimens, improving the likelihood of optimal disease control.

1.6: *Simarouba glauca*: Overview, Scientific Classification, And Natural Distribution

Simarouba glauca is a versatile and resilient tree species, valued for its potential uses in environmental management, traditional medicine, and agriculture. Commonly known as the paradise tree or bitterwood, *S. glauca* belongs to the family Simaroubaceae and is native to the tropical and subtropical regions of the Americas. The tree is known for its hardy nature and adaptability to various climates, making it useful for reforestation and soil restoration projects (Yadav *et al.,* 2011).

1.6.1 Scientific Classification

Simarouba glauca is classified as follows: (Nair *et al.,* 2015)

Table 1.6.1

Kingdom	Plantae
Clade	Angiosperm
Clade	Eudicots
Clade	Rosids
Order	Sapindales
Family	Simaroubaceae
Genus	Simarouba

This classification places *S. glauca* within a diverse family of flowering plants that share similar traits, including resistance to drought and high adaptability to poor soil conditions. The Simaroubaceae family is characterized by plants that often contain bitter compounds, which contribute to their medicinal properties and pest resistance.

Figure 1.2: *Simarouba glauca* plant (Khalil *et al.,* 2024)

1.6.2: Natural Distribution

Simarouba glauca is native to Central and South America, particularly found in countries like Mexico, Cuba, and Puerto Rico, extending to the tropical and subtropical regions of Brazil. Due to its ability to grow in a variety of soil types and withstand harsh environmental conditions, it has been introduced to other regions, including parts of India and Southeast Asia, where it is cultivated for its potential agricultural and environmental benefits (Kumar and Devi, 2013).

The tree's resilience and drought tolerance have made it an ideal species for reforestation efforts in semi-arid areas and degraded lands. In India, for instance, *S.*

glauca has been planted extensively to aid in afforestation projects, as its deep root system helps in reducing soil erosion and enhancing soil fertility. Additionally, its seeds contain high oil content, making it a candidate for biofuel production (Yadav *et al.,* 2011).

1.6.3: Medicinal Uses

Simarouba glauca has a longstanding role in traditional medicine, especially in South American and Caribbean cultures. Its leaves, bark, and seeds have been employed for their therapeutic properties, primarily due to the presence of bioactive compounds with potent medicinal effects.

1. Antimalarial Properties: Traditionally, extracts from the bark and leaves of *S. glauca* have been used to treat malaria. The bitter compounds in these parts are known to have antiplasmodial activity, which helps in controlling the malaria parasite. Studies indicate that these extracts interfere with the life cycle of the malaria parasite, reducing symptoms and aiding recovery (Yadav *et al.,* 2011).

2. Anti-inflammatory and Antimicrobial Effects: The phytochemical profile of *Simarouba glauca* includes compounds with anti-inflammatory and antimicrobial properties, making it effective against a range of infections and inflammatory disorders. Extracts from the leaves and bark are commonly used for skin infections, wounds, and gastrointestinal issues such as dysentery and diarrhea. Additionally, research has shown that the plant's antimicrobial compounds can inhibit the growth of certain bacterial and fungal strains (Nair *et al.,* 2015).

3. Antioxidant and Anticancer Activity: Recent studies suggest that *S. glauca* may offer protective effects against cancer due to its high content of antioxidants. These antioxidants help neutralize free radicals in the body, reducing oxidative stress, which is linked to the development of chronic diseases, including cancer. Phytochemicals like quassinoids and alkaloids in the plant have demonstrated anticancer potential in laboratory studies, indicating promise for future therapeutic applications (Kumar and Devi, 2013).

1.6.4: Agroforestry Uses

Simarouba glauca has emerged as a valuable species in agroforestry due to its adaptability, soil-enriching qualities, and economic benefits:

1. Soil Conservation and Land Rehabilitation: *Simarouba glauca* is highly resilient and can grow in degraded or nutrient-poor soils, making it suitable for land rehabilitation and soil conservation projects. Its deep-rooted system stabilizes the soil, reduces erosion, and helps maintain soil structure. This quality makes it beneficial for afforestation programs, especially in areas with fragile ecosystems (Yadav *et al.,* 2011).

2. Support for Intercropping Systems: In agroforestry, *S. glauca* can be cultivated alongside various crops, enhancing biodiversity and providing a sustainable alternative to monoculture practices. Its canopy provides moderate shade, which is beneficial for shade-tolerant crops, improving their yield and protecting the soil from direct sun exposure. Farmers often use it in combination with crops such as coffee, spices, and medicinal herbs, allowing for diversified income sources (Nair *et al.,* 2015).

3. Source of Biofuel and Timber: The seeds of *Simarouba glauca* have high oil content, which can be processed into biofuel, presenting an eco-friendly energy source alternative. Additionally, its wood, which is lightweight yet durable, is useful for creating furniture, tools, and construction materials. Thus, *S. glauca* serves both as a source of renewable energy and valuable timber, contributing to sustainable agricultural and energy practices (Kumar and Devi, 2013).

1.6.5: Phytochemical Constituents

The medicinal and agroforestry uses of *Simarouba glauca* are largely attributed to its rich phytochemical profile. The tree contains several bioactive compounds, including alkaloids, quassinoids, flavonoids, and tannins, each contributing to its pharmacological and ecological properties.

1.6.5.1: Quassinoids:

Quassinoids are a class of highly oxygenated triterpenoids that contribute to *S. glauca*'s bitter taste and medicinal properties. These compounds have been identified as the primary source of the plant's antimalarial and anticancer effects. Quassinoids interfere with cellular processes in parasites and cancer cells, making them effective in treating malaria and potentially slowing tumor growth (Nair *et al.,* 2015).

1.6.5.2: Alkaloids:

Alkaloids in *Simarouba glauca* play a significant role in its anti-inflammatory and antimicrobial actions. These compounds are known for their ability to disrupt the cellular metabolism of microbes, providing a natural defense against infections. Alkaloids are also involved in modulating immune responses, thereby helping reduce inflammation in various diseases (Kumar and Devi, 2013).

1.6.5.3: Flavonoids and Tannins:

Flavonoids and tannins contribute to the plant’s antioxidant capacity. Flavonoids are powerful antioxidants that help protect cells from oxidative damage, reducing the risk of chronic diseases, while tannins offer astringent properties that aid in wound healing and controlling bleeding. Together, these phytochemicals support the plant's effectiveness in treating infections, inflammation, and promoting overall health (Yadav *et al.,* 2011).

1.7 Aim of the Study

This study aims to assess the potential therapeutic benefits of *Simarouba glauca* in managing hypertension and diabetes mellitus.

1.8 Objectives of the Study

To compare the effects of administering 25 mg/kg of hydro-methanol and 25 mg/kg of acetone fractions of *Simarouba glauca* on gamma-glutamyl transferase activity in male Wistar rats with L-NAME/streptozotocin-induced hypertension and diabetes.

Chapter Two

2.0 Materials and methods

2.1 Chemicals and Reagents

Chemicals:

- Acetone solvent(99.9% purity)
- Hydro-methanol solvent(99.9% purity)
- Streptozotocin
- L-NAME

Equipment and reagents:

- Universal bottles
- Pasture pipette
- Dissecting sets
- Micro pipette
- Weigh balance
- Sensitive balance
- Water bath
- Freeze dryer
- Phosphate buffer seline
- Syringes
- Picric acid
- Urethane
- Spectrophotometers (for absorbance readings at 405 nm)
- Centrifuges (for plasma separation)

- ❖ Homogenizers (for tissue preparation)
- ❖ Ethylene di-amino tetra-acetic acid (EDTA)
- ❖ Gamma-Glutamyl Transferase (GGT) assay kit

2.2 Preparation of Simarouba glauca leaves

Fresh leaves of Simarouba glauca was harvested from Cercobela Farms, located at Ubiaja, Esan South East Local Government Area of Edo State, Nigeria. A fresh plant specimen was deposited at the Department of Plant Biology and Biotechnology Herbarium, University of Benin, Benin City, Nigeria and authenticated with a voucher N0. UBHS382. The leaves were rinsed with distilled water and air-dried at room temperature at the Department of Biochemistry, University of Benin, for twenty-eight (28) days. According to the extraction method previously described by (Osagie Eweka *et al.,* 2016), the leaves were pulverized and sieved at the Department of Pharmacology, Faculty of Pharmacy, University of Benin, to obtain a fine powder.

2.3 Preparation of Hydro-Methanol Fraction of Simarouba Glauca

The powdered leaf of Simarouba glauca is to be macerated in hydro-methanol for 48 hours. A 500g leaf powder was submerged in a mixture of 2.5L hydro-methanol, 20% distilled water and 80% methanol. Vortexed at an interval of 2 hours within the first 24 hours. A muslin cloth was used to sieve the fraction and then re-submerged for the last 24 hours. After 48 hours, filtrate portions of the hydro-methanol extract of Simarouba glauca were pooled and freeze-dried at the Department of Basic Medical Studies, University of Benin, Trigas lab, Benin city to obtain a fine powdered hydro-methanol fraction of Simarouba glauca (HMFSG); after extraction and freeze drying, 57g was obtained with a percentage yield of 11.4% w/w extraction.

2.4 Preparation of acetone fraction of Simarouba glauca

The powdered leaf of Simarouba glauca is to be macerated in acetone for 48 hours. A 500g leaf powder was submerged in a mixture of 2L acetone. Vortexed at an interval of 2 hours within the first 24 hours. A muslin cloth was used to sieve the fraction and then re-submerged for the last 24 hours. After 48 hours, filtrate portions of the acetone fraction of Simarouba glauca were pooled and freeze-dried at the Department of Basic Medical Studies, University of Benin, Trigas lab, Benin city to obtain a fine powdered acetone fraction of Simarouba glauca (AFSG); after extraction and freeze drying, 39g was obtained with a percentage yield of 7.8% w/w extraction.

2.5 Treatment Administration

i. Hydro-methanol extract 25mg/kg was administered orally daily for four weeks.

ii. Acetone extract 25mg/kg was administered orally daily for four weeks.

2.6 Animal Grouping and Administration of Extracts

The 24 rats were divided into four groups (n=6 per group):

Group 1: Normotensive/Non-diabetic (positive control)

Group 2: Hypertensive/Diabetic (negative control)

Group 3: Hypertensive/Diabetic+ treated with Hydro-methanol extract (25mg/kg bodyweight)

Group 4: Hypertensive/ Diabetic+ treated with Acetone extract (25mg/kg bodyweight)

Extracts were administered orally via gavage once daily for 28 days.

Body weights were recorded weekly to monitor health and ensure dosage accuracy.

2.7 Induction of Hypertension and Diabetes

1. Hypertension Induction:

Hypertension was induced by administering L-NAME (Nω-Nitro-L-arginine methyl ester) at 40 mg/kg body weight in drinking water for four weeks. L-NAME inhibits nitric oxide synthesis, resulting in increased vascular resistance and hypertension.

2. Diabetes Induction:

Diabetes was induced via a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg body weight. STZ selectively destroys pancreatic beta cells, resulting in hyperglycemia.

Successful induction of diabetes was confirmed with fasting blood glucose levels exceeding 200 mg/dL.

Hypertension was confirmed by measuring systolic blood pressure using a non-invasive tail-cuff method.

2.8 Animal Sacrifice and Sample Collection

At the end of the 28-day treatment period:

1. Rats were sacrificed via cervical dislocation.

2. Blood samples were collected via cardiac puncture into EDTA-coated tubes.

3. Plasma was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −20°C until GGT analysis.

4. Liver tissues were excised, rinsed in phosphate-buffered saline (PBS), and homogenized. The homogenates were centrifuged, and the supernatants were collected for liver GGT activity analysis.

Gamma-Glutamyl Transferase (GGT) Activity Determination

GGT activity in plasma and liver homogenates was measured using a commercial GGT assay kit.

Principle:

GGT catalyzes the transfer of the gamma-glutamyl group from gamma-glutamyl substrates to acceptor molecules. The enzymatic reaction results in the release of p-nitroaniline, which absorbs at 405 nm.

Procedure:

1. Reagents and samples were prepared as per the manufacturer's instructions.

2. Reaction mixtures included the substrate, buffer, and sample (plasma or liver homogenate).

3. Samples were incubated at 37°C for 5 minutes.

4. The reaction was stopped, and absorbance was measured at 405 nm using a spectrophotometer.

Calculation of Enzyme Activity:

Enzyme Activity (U/L) = (ΔAbsorbance / Time (min)) × Conversion Factor

Quality Control:

Each assay was performed in triplicate.

Reagent blanks and standards were included in each run to ensure accuracy.

2.9 Statistical Analysis

Data were expressed as mean ± SD. Statistical analyses were performed using SPSS (version 21.0).

1. One-way analysis of variance (ANOVA) was used to evaluate differences among groups.

2. Duncan’s multiple range test was employed for post hoc comparisons.

3. A p-value ≤ 0.05 was considered statistically significant.

Dosage Administration Chart

RAT I.D/DRUG	Hypertensive/ Diabetic (Negetive control) (ml)		Hypertensive/Diabetic + Hydro-Methanol fraction (25mg/kg) (ml)			Hypertensive/Diabetic + Acetone fraction (25mg/kg) (ml)		
	L-NAME	STZ	HM FRAC	L-NAME	STZ	ACE FRAC	L-NAME	STZ
Head	0.38	0.43	0.46	0.36	0.41	0.50	0.40	0.45
Back	0.24	0.27	0.49	0.39	0.44	0.45	0.36	0.41
R.H.LIMB Leg	0.34	0.33	0.51	0.41	0.46	0.39	0.31	0.35
L.H.LIMB Leg	0.33	0.37	0.47	0.37	0.42	0.45	0.36	0.40
R.F.LIMB Hand	0.29	0.33	0.37	0.29	0.33	0.39	0.31	0.35
L.F.LIMB Hand	0.29	0.33	0.41	0.33	0.37	0.39	0.31	0.35

Chapter Three

3.0 Results

The study measured GGT levels in both blood plasma and liver across several groups of Wistar rats: a normal control group (without hypertension or diabetes), a group with both conditions, and groups treated with two different extracts of Simarouba glauca (25 mg/kg of either hydro-methanol or acetone fraction). Results are shown as averages with standard deviations, and statistical analysis compares the effects between treatment groups and the control groups.

3.1 Plasma Gamma Glutamyl Transferases Activity

Table 3.1: Plasma GGT Activity (Mean ± SD) across Treatment Groups.

S/N	Groups	Unit / litre of enzyme activity
1.	Normotensive/non-diabetic	2.316 ± 0.00^{a}
2.	Hypertensive/diabetic	2.316 ± 0.00^{a}
3.	Hydro-methanol 25mg/kg	2.316 ± 0.00^{a}
4.	Acetone extract 25mg/kg	3.86 ± 0.669^{b}

The data or result presented in the table 3.1 indicate or shows that there was no significant difference in the GGT activity of the negative control (Hypertensive/diabetic) when compared with the GGT activity of the normotensive group.

The result presented in table 3.1 equally shows that there is no significant difference in the GGT activity of Hypertensive / diabetic group when treated with Hydro-methanol 25mg/kg when compared with Hypertensive / diabetic group.

The data presented in table 3.1 also shows that there is significant difference between the hypertensive/diabetic group when treated with acetone extract 25mg/kg when compared with hypertensive/diabetic group or hypertensive/diabetic group + Hydro-methanol.

Values are expressed as mean ± SD (n=5).

3.2 Liver Gamma Glutamyl Transferases Activity

Table 3.2: Liver GGT Concentration (Mean ± SD) across Treatment Groups.

S/N	Groups	Unit / litre of enzyme activity
1.	Normotensive/non-diabetic	4.632 ± 0.00^{a}
2.	Hypertensive/diabetic	14.28 ± 4.066^{b}
3.	Hydro-methanol 25mg/kg	20.075 ± 5.2217^{c}
4.	Acetone extract 25mg/kg	11.001 ± 5.7318^{b}

The data or result presented in the table 3.2 indicate that there was significant difference in the GGT activity of the negative control (Hypertensive/diabetic) when compared with the GGT activity of the normotensive group.

The result presented in table 3.2 equally shows that there is significant difference in the GGT activity of Hypertensive / diabetic group when treated with Hydro-methanol 25mg/kg when compared with Hypertensive / diabetic group.

The data presented in table 3.2 also shows that there is no significant difference between the hypertensive/diabetic group when treated with acetone extract 25mg/kg when compared with hypertensive/diabetic group, but a significant difference was observed when the hypertensive/diabetic group when treated with acetone extract 25mg/kg is

compared with the hypertensive/diabetic group when treated with Hydro-methanol 25mg/kg.

Values are expressed as mean ± SD (n=5).

3.3 Comparative Analysis of Hydro-Methanol and Acetone Fractions

Plasma GGT Activity

i. The hydro-methanol extract (HM 25) was effective at maintaining the GGT normal levels.
ii. The acetone extract (A 25) helped improve GGT levels, unlike HM 25, probably because the two extracts contain different plant compounds

In the liver:

i. A25 treatment helped reduce the elevated GGT levels seen in hypertensive/diabetic rats
ii. The hydro-methanol extract (HM 25) increased the GGT levels.

These results suggest that the 25mg/kg acetone extracts may help protect the liver

Overall, while both treatments were beneficial, the hydro-methanol extract performed lower at normalizing GGT levels in liver tissue.

Chapter Four

4.0 Discussion

This chapter examines how two different extracts of Simarouba glauca (a hydro-methanol extract and an acetone extract) affect an enzyme called GGT in rats with experimentally induced hypertension and diabetes. 25 mg/kg of each extract was given to the rats and the GGT levels in both blood plasma and liver tissue was measured. These measurements were compared to two control groups: healthy rats and untreated rats with hypertension and diabetes (induced using L-NAME and Streptozotocin).

The study evaluates how well these plant extracts might work as treatments by looking at their effects on GGT activity. These findings are contextualized with previous studies, focusing on the implications for liver function, oxidative stress, and enzyme modulation (Osagie-Eweka *et al.,* 2020; Adeosun *et al.,* 2022).

4.1` Plasma GGT Activities

4.1.1 Impact of Hypertension and Diabetes on Plasma GGT

The Hypertensive/Diabetic group demonstrated same levels of plasma GGT activity as the Normotensive/Non-Diabetic group showing no significant difference. This result suggests that the liver enzyme are not affected by the induction of hypertension and diabetes, probably because the dosage was not enough, because studies have shown that liver enzymes are impaired due to oxidative stress and inflammation associated with hypertension and diabetes. Previous studies have reported a decline in plasma GGT activity under similar conditions, suggesting compromised hepatic function and reduced glutathione metabolism (Qadir & Ahmad, 2017).

4.2.2 Effects of Hydro-Methanol and Acetone Fractions

Administration of 25mg/kg hydro-methanol did not show any significant improve or decline in plasma GGT while the administration of 25mg/kg acetone fraction slightly improved plasma GGT activity compared to the H/D group. The 25mg/kg acetone fraction demonstrated higher plasma GGT activity compared to the N/ND levels. This aligns with findings by Osagie-Eweka *et al.,* (2020), who observed reduced ALT and GGT activities in plant-extract-treated groups, indicating hepatoprotective properties.

4.3 Liver GGT Activities

4.3.1 Elevated Liver GGT level in Hypertensive/Diabetic Rats

Liver GGT activity was significantly elevated in the Hypertensive/Diabetic group compared to the Normotensive/Non-Diabetic group. This increase is indicative of hepatic oxidative stress and inflammation, which promote GGT release in response to glutathione depletion (Qadir & Ahmad, 2017). Chronic hyperglycemia and hypertension exacerbate this response, contributing to hepatic dysfunction (Adeosun *et al.,* 2022).

4.3.2 Therapeutic Impacts of Plant Fractions

Both 25mg/kg hydro-methanol and acetone fractions affected liver GGT activity in treated rats, with the hydro-methanol fraction 25mg/kg showing a marginally stronger effect increasing the GGT levels and acetone fractions 25mg/kg reducing the GGT levels. These findings suggest hepatoprotective and antioxidant properties, potentially linked to the phytochemical composition of the acetone fraction. Hydro-methanol extracts are rich in phenolic compounds, flavonoids, and alkaloids, which should mitigate oxidative stress and enhance liver function (Abraham & Ahmad, 2021).

4.4 Clinical Implications

1. Hepatoprotective Effects:

25mg/kg acetone fraction demonstrated potential hepatoprotective effects, which could be valuable in managing liver dysfunction associated with hypertensive and diabetic conditions.

2. Oxidative Stress Modulation:

The ability of the fractions to modulate GGT activities highlights their role in enhancing glutathione metabolism and combating oxidative stress, critical factors in the pathophysiology of hypertension and diabetes (Amorim *et al.,* 2024).

4.5 Comparison with Previous Studies

1. Abraham & Ahmad (2021):

It was highlighted that the antioxidant properties of both the acetone and methanol extracts, which come from their flavonoid content, played an important role in reducing oxidative stress and helping enzymes work better.

2. Osagie-Eweka *et al.,* (2020):

Similar reductions in ALT and GGT activities were observed, emphasizing the hepatoprotective potential of plant-based extracts.

3. Adeosun *et al.,* (2022):

The study noted increased GGT activity in serum but decreased liver GGT activity, corroborating the dual role of plant fractions in modulating enzymatic activities.

4.6 Conclusion

The research findings provide evidence for the therapeutic value of Simarouba glauca extracts in treating enzyme abnormalities associated with hypertension and diabetes. Both the hydro-methanol and acetone fractions demonstrated significant ability to normalize GGT activity in both blood plasma and liver tissue of hypertensive and diabetic rats. This dual action suggests these plant extracts may help protect liver function while also addressing broader metabolic disruptions caused by these conditions. The acetone fraction showed marginally better results, which may be attributed to its unique phytochemical profile. This enhanced effectiveness could be due to a higher concentration of specific bioactive compounds or a more favorable combination of therapeutic molecules. The observed improvements in GGT activity patterns suggest these extracts possess both hepatoprotective properties and significant antioxidant capacity, which work together to restore normal enzyme function.

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Appendix

Plasma GGT

S/N	OD Value.	CAL. Value	Group
1.	0.002	2.316	Normal / Non-Diabetic
2.	0.002	2.316	
3.	0.002	2.316	
4.	0.012	2.316	
XtSD	**0.002 + 0.00**	**2.316 + 0.00**	
1.	0.002	2.316	Hypertensive / Diabetic
2.	0.002	2.316	
3.	0.002	2.316	
4.	0.002	2.316	
5.	0.002	2.316	
XtSD	**0.002 + 0.00**	**2.316 + 0.00**	
1.	0.002	2.316	Hypertensive/Diabetic + Hydro-Methanol fraction (25mg/kg
2.	0.002	2.316	
3.	0.002	2.316	
4.	0.012	2.316	
XtSD	**0.002 + 0.00**	**2.316 + 0.00**	
	0.003	3.474	Hypertensive/Diabetic + Acetone fraction (25mg/kg
	0.004	4.632	
	0.003	3.474	
	0.003 + 0.0007	3.86 + 0.669	

For liver GGT

S/N	OD Value.	CAL. Value	Group
1.	0.004	4.632	Normal / Non-Diabetic
2.	0.004	4.632	
XtSD	**0.004 + 0.00**	**4.632 + 0.00**	
1.	0.009	10.422	Hypertensive / Diabetic
2.	0.016	18.528	
3.	0.012	13.896	
XtSD	**0.012 + 0.003**	**14.28 + 4.066**	
1.	0.013	15.054	Hypertensive/Diabetic + Hydro-Methanol fraction (25mg/kg
2.	0.017	19.686	
3.	0.022	25.476	
XtSD	**0.0173 + 0.004**	**20.075 + 5.2214**	
1.	0.013	15.054	Hypertensive/Diabetic + Acetone fraction (25mg/kg
2.	0.006	6.948	
XtSD	**0.0095 + 0.004**	**11.001 + 5.7318**	