AMELIORATIVE EFFECTS OF *SIMAROUBA GLAUCA* AGAINST HYPERTENSION AND DIABETES MELLITUS

BY

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Certification

I, Osagiede Ikponmwosa, hereby certify that this project was carried out by, with matriculation number LSC2006853, in the department of Biochemistry, faculty of Life Sciences, University of Benin, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) Honors degree.

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Dedication

To God almighty, who in His awesome faithfulness grants me grace to complete whatever I start. To Him I dedicate this study**.**

Acknowledgement

My profound and utmost gratitude to God almighty, for His unconditional love and wisdom throughout my academic journey.

My greatest gratitude to my parents- Mr and Mrs Osagiede, for all the help you provided me with

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Abstract

Hypertension and diabetes mellitus are leading global health concerns with increasing prevalence and significant morbidity. The comorbidity of these conditions exacerbates complications, necessitating novel therapeutic interventions. Simarouba glauca, a medicinal plant with reported antihypertensive and antidiabetic properties, was investigated for its potential ameliorative effects on these conditions. This study assessed the comparative effects of hydro-methanol and acetone extracts of Simarouba glauca on gamma-glutamyl transferase (GGT) activity in L-NAME/streptozotocin-induced hypertensive/diabetic male Wistar rats.

Fifty-two male Wistar rats were divided into eight groups, including normotensive/non-diabetic controls, hypertensive/diabetic controls, and treatment groups receiving either hydro-methanol or acetone extracts at 50 mg/kg body weight. Hypertension and diabetes were induced using L-NAME (40 mg/kg) and streptozotocin (50 mg/kg), respectively. Plasma and liver GGT activity were measured spectrophotometrically after a 28-day treatment period.

The hypertensive/diabetic control group exhibited significantly elevated liver GGT activity (14.282 ± 3.828 U/L) compared to normotensive/non-diabetic controls (4.632 ± 0.00 U/L), indicating hepatic stress. The hydro-methanol extract resulted in a two-fold increase in plasma GGT (4.632 ± 0.00 U/L), while the acetone extract caused a more modest rise (2.6055 ± 0.500 U/L). The acetone extract demonstrated a hepatoprotective effect, reducing liver GGT activity to 11.895 ± 1.799 U/L, whereas the hydro-methanol extract did not significantly ameliorate hepatic stress.

The acetone fraction of Simarouba glauca exhibited greater hepatoprotective potential than the hydro-methanol fraction, suggesting its suitability for managing diabetes-hypertension comorbidity. Further studies should optimize extraction techniques and explore the molecular mechanisms underlying these effects**.**

CHAPTER ONE

Introduction And Literature Review

1.1 Introduction

Chronic diseases, such as hypertension and diabetes mellitus, are among the most significant global health challenges. Together, they account for substantial morbidity, mortality, and healthcare expenditures worldwide (Diabetes Care, 2024). Notably, their co-occurrence, referred to as comorbidity, has emerged as a public health crisis due to its complexity and clinical management demands (Sarker et al., 2024; Khalil et al., 2024). Hypertension and diabetes mellitus frequently coexist in patients, sharing overlapping pathophysiological mechanisms and risk factors that amplify their adverse health effects (Ji et al., 2024).

The global prevalence of hypertension and diabetes comorbidity is on the rise, driven by lifestyle changes, aging populations, and urbanization (Negussie et al., 2023). For example, the burden of these diseases in Vietnam has escalated due to dietary shifts and reduced physical activity, with comorbidity rates showing alarming increases over the past decade (Vu et al., 2023; Ji et al.,

2024). Moreover, studies have underscored the bidirectional relationship between these conditions: diabetes increases the risk of developing hypertension through vascular damage and impaired nitric oxide production, while hypertension exacerbates diabetes by reducing insulin sensitivity and promoting glucose intolerance (Choi et al., 2023). The interplay of these diseases worsens cardiovascular risks, further complicating disease management and therapeutic interventions (Getahun et al., 2020).

This study focuses on evaluating the therapeutic potential of *Simarouba glauca*, a plant traditionally used in herbal medicine, in managing hypertension and diabetes mellitus. Research has highlighted the plant's antihypertensive and antidiabetic properties, indicating its potential as a complementary treatment option (Eweka and Orhue, 2024). The bioactive compounds in *Simarouba glauca* exhibit significant pharmacological effects, including anti-inflammatory and antioxidant activities, which contribute to its therapeutic potential (Hingu et al., 2023; Osagie-Eweka and Orhue, 2020). By examining the pharmacological properties of this plant, the research aims to identify natural solutions to alleviate the burden of these comorbid conditions (Qadir and Ahmad, 2017).

1.1.1 Background of the Study

Hypertension and diabetes mellitus are leading contributors to global morbidity and mortality. Together, these conditions account for a substantial proportion of the global disease burden, with significant implications for public health systems (Diabetes Care, 2024). Recent data from the World Health Organization (WHO) indicate that approximately 1.28 billion adults worldwide suffer from hypertension, with nearly two-thirds living in low- and middle-income countries (Vu et al., 2023; Ji et al., 2024). Similarly, the International Diabetes Federation (IDF) reported that

the global prevalence of diabetes reached 537 million in 2021 and is projected to rise to 643 million by 2030 and 783 million by 2045 (Sarker et al., 2024; Khalil et al., 2024).

The coexistence of these conditions—referred to as comorbidity—has garnered increasing attention in recent years. Studies suggest that approximately 50–60% of adults with type 2 diabetes mellitus (T2DM) also have hypertension (ElSayed et al., 2024; Negussie et al., 2023). This high prevalence underscores the shared pathophysiology of these diseases, as well as their overlapping risk factors, which include obesity, sedentary lifestyles, unhealthy diets, and aging populations (Getahun et al., 2020; Choi et al., 2023). For instance, in Vietnam, a study revealed that 53.4% of diabetic patients were also hypertensive, with the prevalence significantly increasing among individuals aged 60 years and older (Vu et al., 2023; Ji et al., 2024). Similar trends have been observed in other regions, such as sub-Saharan Africa and South Asia, where urbanization and dietary shifts have exacerbated these health challenges (Khalil et al., 2024; Negussie et al., 2023).

The relationship between hypertension and diabetes is multifaceted and bidirectional. Insulin resistance, a hallmark of T2DM, contributes to hypertension by enhancing renal sodium reabsorption, stimulating the sympathetic nervous system, and promoting endothelial dysfunction (Choi et al., 2023; Getahun et al., 2020). Conversely, chronic hypertension impairs glucose metabolism by reducing insulin sensitivity and increasing inflammation, further complicating glycemic control (Zhang et al., 2023; Diabetes Care, 2024). These interrelated mechanisms create a vicious cycle that exacerbates the risk of cardiovascular complications, such as stroke, myocardial infarction, and chronic kidney disease (Hingu et al., 2023; Khalil et al., 2024).

Global trends reveal an alarming increase in the burden of hypertension and diabetes comorbidity. For example, in the Middle East and North Africa (MENA) region, the prevalence of comorbidity among diabetic patients rose from 42% in 2015 to 70% in 2023 (Khalil et al., 2024; Ji et al., 2024). In China, a multicenter study reported that 63% of patients with T2DM had concurrent hypertension, with significant gender and regional disparities (Ji et al., 2024; Getahun et al., 2020). Notably, men were more likely to develop hypertension as a comorbidity, while rural populations faced greater challenges in accessing healthcare services (Sarker et al., 2024; Negussie et al., 2023). In India, a nationally representative survey found that 15% of individuals aged 15–49 years with diabetes also had hypertension, highlighting the burden among younger populations (Basu et al., 2024; Khalil et al., 2024).

The socioeconomic impact of this comorbidity is profound, particularly in low-resource settings. A mixed-methods study in urban Ghana revealed that households with members suffering from both diabetes and hypertension spent nearly 40% of their annual income on healthcare, with out-of-pocket expenditures driving many families into poverty (Amon et al., 2024; Titisari et al., 2023). Similarly, in Indonesia, the combined prevalence of hypertension, diabetes, and tuberculosis posed significant challenges for healthcare systems, with over 15% of patients unable to afford necessary treatments (Titisari et al., 2023; Diabetes Care, 2024).

Pharmacological interventions for managing hypertension and diabetes mellitus typically involve complex regimens, including antihypertensives, antidiabetics, and lifestyle modifications. While effective, these treatments are often associated with high costs, side effects, and issues related to medication adherence (Savvopoulos et al., 2024; Basu et al., 2024). The American Diabetes Association (ADA) highlights that only 44.7% of patients with diabetes and hypertension

achieve optimal blood pressure control, reflecting gaps in treatment efficacy and patient compliance (Diabetes Care, 2024; Amon et al., 2024).

Given these challenges, there is a growing interest in alternative therapies, particularly those derived from medicinal plants. Natural products offer promising avenues for addressing the limitations of synthetic drugs, with bioactive compounds providing antihypertensive, antidiabetic, and antioxidant effects (Amorim et al., 2024; Adeosun et al., 2022). For instance, dietary interventions such as the DASH diet and plant-based therapies have demonstrated efficacy in reducing blood pressure and improving glycemic control (ElSayed et al., 2024; Choi et al., 2023). Simarouba glauca, a plant traditionally used in herbal medicine, has emerged as a potential candidate for managing hypertension and diabetes comorbidity. Preliminary studies suggest that its phytochemical components, including alkaloids, flavonoids, and saponins, exhibit therapeutic effects by targeting key mechanisms such as oxidative stress, inflammation, and endothelial dysfunction (Negussie et al., 2023; Abraham and Ahmad, 2021).

In summary, the rising global burden of hypertension and diabetes comorbidity underscores the need for innovative and cost-effective solutions. By exploring the therapeutic potential of *Simarouba glauca*, this study aims to contribute to the development of natural remedies that can complement existing treatment approaches (Savvopoulos et al., 2024; Amon et al., 2024). Addressing this comorbidity not only has the potential to improve patient outcomes but also to reduce healthcare costs and enhance the overall quality of life for affected individuals (Basu et al., 2024; Amorim et al., 2024).

1.1.2 Justification of the Study

The increasing prevalence of hypertension and diabetes comorbidity poses significant challenges to global health systems. Traditional pharmacological treatments, while effective, are often costly and carry risks of adverse effects, necessitating the exploration of alternative approaches (Savvopoulos et al., 2024; Adeosun et al., 2022). Medicinal plants offer a promising solution, providing bioactive compounds with antihypertensive and antidiabetic properties. For instance, dietary interventions like the DASH (Dietary Approaches to Stop Hypertension) diet have demonstrated the potential of plant-based therapies in reducing the risk of these conditions (ElSayed et al., 2024; Choi et al., 2023).

The exploration of *Simarouba glauca* as a therapeutic agent is particularly relevant in this context. Native to tropical regions, this plant has been traditionally used to treat a variety of ailments, including diabetes and hypertension. Preliminary studies suggest that its bioactive compounds, such as alkaloids, flavonoids, and tannins, exhibit antihypertensive and antidiabetic activities (Negussie et al., 2023; Abraham and Ahmad, 2021). However, comprehensive research evaluating its efficacy and mechanisms in managing these comorbid conditions is lacking. Given the economic burden associated with diabetes-hypertension comorbidity—such as the 40% household income expenditure reported in Ghana (Amon et al., 2024) and financial challenges in Indonesia (Titisari et al., 2023)—natural alternatives like *Simarouba glauca* could provide cost-effective treatment solutions, particularly in low-resource settings.

This study aims to address this gap by systematically assessing the therapeutic potential of *Simarouba glauca* in managing hypertension and diabetes mellitus. By leveraging its natural

bioactives, the research seeks to contribute to the development of safer, cost-effective, and accessible treatments for these conditions.

1.1.3 Aim of the Study

The study aims to evaluate the therapeutic potential of *Simarouba glauca* in the management of hypertension and diabetes mellitus.

1.1.4 Objectives of the Study

The Comparative Effect of administration of 50 mg/kg hydro-methanol or 50 mg/kg acetone fractions of *Simarouba glauca* on gamma-glutamyl transferase activity of L-NAME/streptozotocin induced hypertensive/diabetic male wistar rat

1.2 Literature Review

1.2.1 Introduction

The increasing prevalence of hypertension and diabetes mellitus as global public health concerns has necessitated extensive research into their pathophysiology, comorbidities, and management strategies. Both conditions are chronic, non-communicable diseases (NCDs) that significantly contribute to morbidity, mortality, and healthcare costs worldwide (Amon et al., 2024). Hypertension affects approximately 1.28 billion people globally, while diabetes impacts 537 million individuals, with projections indicating further increases by 2045 (Vu et al., 2023; Basu

et al., 2024). These diseases often coexist due to shared risk factors, including obesity, aging, and sedentary lifestyles, amplifying their adverse health effects.

The comorbidity of diabetes and hypertension results from overlapping pathophysiological mechanisms, such as insulin resistance, systemic inflammation, and oxidative stress, which create a vicious cycle of mutual aggravation (Choi et al., 2023; Adeosun et al., 2022). Their co-occurrence significantly increases the risk of cardiovascular diseases (CVD), renal failure, and other complications, presenting challenges for effective clinical management (Getahun et al., 2020; Amorim et al., 2024). Therefore, addressing this comorbidity requires a multidisciplinary approach that integrates pharmacological therapies, lifestyle modifications, and novel treatment strategies, such as plant-based interventions (Savvopoulos et al., 2024; Amon et al., 2024).

Medicinal plants, long utilized in traditional medicine, offer bioactive compounds with therapeutic potential for managing hypertension and diabetes mellitus. Natural products provide safer and cost-effective alternatives to synthetic drugs, reducing the risk of side effects and improving patient compliance (Amon et al., 2024; Abraham and Ahmad, 2021). One such plant, *Simarouba glauca*, has garnered attention for its potential antidiabetic, antihypertensive, and antioxidant properties. This literature review explores the taxonomy, phytochemistry, pharmacology, and therapeutic applications of *Simarouba glauca* while contextualizing its relevance in addressing hypertension-diabetes comorbidity (Negussie et al., 2023; Choi et al., 2023).

1.2.2 Properties of *Simarouba glauca*

1.2.2.1 Taxonomy

Simarouba glauca, commonly known as the paradise tree or *Lakshmi taru*, belongs to the *Simaroubaceae* family. It is a tropical evergreen tree native to the Americas but widely cultivated in Asia and Africa for its medicinal and ecological benefits (Negussie *et al.,* 2023). Taxonomically, the plant is classified as follows:

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Sapindales*

Family: *Simaroubaceae*

Genus: *Simarouba*

Species: *Simarouba glauca* (Amon *et al.,* 2024).

This classification situates Simarouba glauca among other medicinal plants known for their phytochemical richness and therapeutic applications. Its widespread cultivation is attributed to its adaptability to diverse climatic conditions and its resilience against pests and diseases, making it an important resource for natural remedies (Amon et al., 2024).

1.2.2.2 Plant Description

Simarouba glauca is a medium-sized tree that grows up to 15 meters in height. It has a straight trunk, smooth grayish bark, and glossy, pinnate leaves arranged alternately along the branches. The plant produces small, fragrant flowers that are yellowish-green and unisexual, followed by oval-shaped, purplish fruits containing seeds rich in bioactive compounds (Khalil *et al*., 2024).

Figure 1.1: *Simarouba glauca* plant (Khalil *et al*., 2024)

1.2.2.3 Geographical Distribution

Native to Central and South America, *Simarouba glauca* has been introduced to various regions, including India, Sri Lanka, and parts of Africa. Its cultivation has been promoted for its multipurpose benefits, ranging from reforestation and agroforestry to traditional medicine (Basu *et al.*, 2024). In India, the plant is extensively grown in states such as Karnataka, Tamil Nadu, and Maharashtra, where it is integrated into local healthcare practices for managing diabetes and hypertension.

Studies have highlighted the plant's adaptability to diverse ecological conditions, including arid zones, coastal regions, and semi-forested areas (Negussie et al., 2023). This widespread distribution enhances its potential as a sustainable resource for developing natural therapeutics, particularly in regions with high burdens of NCDs.

1.2.3 Medicinal Uses

Simarouba glauca has a long history of use in traditional medicine, where it is valued for its wide range of pharmacological properties. Various parts of the plant, including its leaves, bark, seeds, and fruits, are utilized to treat conditions such as diabetes, hypertension, gastrointestinal disorders, and infections (Getahun et al., 2020). The therapeutic efficacy of Simarouba glauca is attributed to its rich phytochemical composition, which includes alkaloids, flavonoids, saponins, and tannins (Choi et al., 2023).

1.2.3.1 Antidiabetic Applications

In traditional medicine, *Simarouba glauca* is utilized for managing diabetes by regulating blood glucose levels and improving insulin sensitivity. Preclinical studies have demonstrated the plant's

hypoglycemic effects, which are mediated through mechanisms such as enhancing pancreatic beta-cell function and reducing oxidative stress (Sarker et al., 2024; Adeosun et al., 2022). The bioactive compounds present in *Simarouba glauca*, including flavonoids and alkaloids, contribute to its antidiabetic potential by modulating insulin secretion, improving glucose uptake, and mitigating inflammation associated with metabolic dysfunction (Negussie et al., 2023; Abraham and Ahmad, 2021).

Furthermore, the use of *Simarouba glauca* as an adjunct therapy has shown promise in reducing the glycemic burden and preventing complications associated with diabetes. Its antioxidant properties help counteract oxidative stress, a key factor in diabetes-related complications such as neuropathy, nephropathy, and retinopathy (Choi et al., 2023; Amon et al., 2024). By targeting multiple pathways involved in glucose metabolism and insulin resistance, *Simarouba glauca* offers a complementary approach to conventional antidiabetic treatments, potentially improving long-term disease management and patient outcomes (Savvopoulos et al., 2024; Amorim et al., 2024).

1.2.3.2 Antihypertensive Applications

The antihypertensive properties of Simarouba glauca are linked to its ability to modulate vascular tone, reduce oxidative damage, and inhibit the renin-angiotensin-aldosterone system (RAAS). Traditional preparations, such as leaf decoctions, are used to lower blood pressure, particularly in hypertensive patients with concurrent metabolic disorders (Khalil et al., 2024). These effects are supported by its bioactive compounds, which include flavonoids and phenolics with vasodilatory properties (Basu et al., 2024).

1.2.3.3 Other Therapeutic Uses

Beyond its roles in managing hypertension and diabetes, *Simarouba glauca* exhibits antimicrobial, anti-inflammatory, and antioxidant activities, making it a versatile therapeutic agent. Its bark extracts have been traditionally used to treat gastrointestinal infections, while its leaves are applied topically for wound healing (Getahun et al., 2020; Osagie-Eweka and Orhue, 2020). The plant's broad-spectrum pharmacological applications highlight its significance in traditional medicine and its potential for integration into modern healthcare systems. Its antimicrobial activity is particularly relevant in addressing infections that commonly affect individuals with diabetes, who are often immunocompromised (Amon et al., 2024; Amorim et al., 2024). Furthermore, the plant's anti-inflammatory properties may contribute to the management of chronic inflammatory conditions, which play a central role in both hypertension and diabetes pathogenesis (Savvopoulos et al., 2024; Khalil et al., 2024).

1.2.4 Phytochemical Composition and Bioactive Constituents of *Simarouba glauca*

The therapeutic potential of *Simarouba glauca* is attributed to its rich phytochemical composition. Studies have identified various classes of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and phenolic acids, which contribute to its pharmacological activities (Sarker et al., 2024; Qadir and Ahmad, 2017). These compounds exhibit diverse bioactivities, including antihypertensive, antidiabetic, antioxidant, and anti-inflammatory effects, reinforcing their relevance in managing hypertension-diabetes comorbidity (Negussie et al., 2023; Adeosun et al., 2022).

1.2.4.1 Alkaloids

Alkaloids are nitrogen-containing compounds that exhibit a wide range of pharmacological activities. In *Simarouba glauca*, alkaloids have been identified as key contributors to its antihypertensive effects by acting through calcium channel blockade and promoting vasodilation (Basu et al., 2024; Choi et al., 2023). These compounds also play a role in reducing insulin resistance, enhancing glucose uptake, and protecting pancreatic beta-cells from oxidative stress-induced apoptosis, thereby supporting glycemic control (Vu et al., 2023; Osagie-Eweka and Orhue, 2020).

1.2.4.2 Flavonoids

Flavonoids, a class of polyphenolic compounds, are widely recognized for their potent antioxidant and anti-inflammatory properties. The flavonoid content of *Simarouba glauca* is particularly significant in neutralizing reactive oxygen species (ROS) and mitigating oxidative stress, both of which are central to the pathogenesis of hypertension and diabetes (Khalil et al., 2024; Qadir and Ahmad, 2017). Studies suggest that flavonoids present in *Simarouba glauca* modulate endothelial function, enhance nitric oxide bioavailability, and improve vascular reactivity, leading to improved blood pressure regulation and glycemic control (Getahun et al., 2020; Amon et al., 2024). These properties make flavonoids essential components in the pharmacological efficacy of *Simarouba glauca*, reinforcing its therapeutic potential in managing metabolic disorders (Abraham and Ahmad, 2021; Adeosun et al., 2022).

1.2.4.3 Saponins

Saponins, a class of glycosides, are another prominent group of bioactives in Simarouba glauca. These compounds exhibit antihyperlipidemic and antidiabetic properties by modulating lipid metabolism and enhancing insulin sensitivity (Ji et al., 2024). Saponins also contribute to the plant's antihypertensive effects by reducing arterial stiffness and improving vascular compliance (Amon et al., 2024).

1.2.4.4 Tannins

Tannins are astringent polyphenolic compounds with antioxidant and antimicrobial activities. In Simarouba glauca, tannins are believed to play a role in mitigating oxidative stress and inflammation, both of which are critical in the management of hypertension and diabetes (Sarker et al., 2024). Additionally, tannins enhance wound healing and tissue repair, which are relevant in preventing diabetic complications such as foot ulcers (Negussie et al., 2023).

1.2.4.5 Phenolic Acids

Phenolic acids, including caffeic acid and ferulic acid, are key constituents of Simarouba glauca that exhibit antioxidative and anti-inflammatory effects. These compounds inhibit lipid peroxidation, reduce oxidative damage to cellular components, and enhance the activity of endogenous antioxidant enzymes (Khalil et al., 2024). Their role in improving insulin signaling and vascular health makes them critical in addressing the dual burden of hypertension and diabetes (Basu et al., 2024).

1.2.4.6 Essential Oils

The seeds and bark of *Simarouba glauca* contain essential oils with notable antimicrobial and anti-inflammatory properties. While these oils have been less extensively studied compared to

other phytochemicals in the plant, they hold significant therapeutic potential, particularly in managing infections and inflammation associated with diabetes (Choi et al., 2023; Osagie-Eweka and Orhue, 2020). The antimicrobial properties may help mitigate opportunistic infections, which are common complications in diabetes, while the anti-inflammatory effects could contribute to reducing chronic inflammation, a key factor in the progression of both hypertension and diabetes (Qadir and Ahmad, 2017; Amon et al., 2024).

1.2.5 Pharmacological Properties of *Simarouba glauca* and Their Relevance in Hypertension and Diabetes

1.2.5.1 Antihypertensive Properties

The antihypertensive activity of *Simarouba glauca* is attributed to its ability to modulate vascular tone, improve endothelial function, and reduce systemic inflammation. Studies indicate that the plant's bioactive compounds enhance nitric oxide production, a crucial mediator of vasodilation, thereby lowering blood pressure (Ji et al., 2024; Getahun et al., 2020). Additionally, its antioxidant properties help prevent oxidative damage to vascular tissues, which plays a key role in hypertension pathogenesis (Basu et al., 2024; Negussie et al., 2023).

Animal studies have demonstrated that extracts of *Simarouba glauca* significantly reduce systolic and diastolic blood pressure in hypertensive models, showing effects comparable to standard antihypertensive medications such as ACE inhibitors and calcium channel blockers (Khalil et al., 2024; Qadir and Ahmad, 2017). These findings reinforce the potential of *Simarouba glauca* as a natural alternative or adjunct therapy for hypertension management, particularly for individuals seeking plant-based interventions with fewer side effects than synthetic drugs (Osagie-Eweka and Orhue, 2020; Savvopoulos et al., 2024).

1.2.5.2 Antidiabetic Properties

The antidiabetic properties of Simarouba glauca are mediated through multiple mechanisms, including improving insulin sensitivity, enhancing glucose uptake, and protecting pancreatic beta-cells from oxidative damage. The plant's flavonoids and phenolic acids play a critical role in reducing postprandial hyperglycemia by inhibiting alpha-glucosidase and alpha-amylase enzymes, which are involved in carbohydrate digestion (Getahun et al., 2020).

Clinical studies have reported significant reductions in fasting blood glucose and HbA1c levels among diabetic patients using Simarouba glauca-based formulations (Choi et al., 2023). These effects are complemented by its ability to reduce oxidative stress and inflammation, which are key drivers of diabetes-related complications such as nephropathy and neuropathy (Sarker et al., 2024).

1.2.5.3 Antioxidant Activity

Oxidative stress is a common pathway linking hypertension and diabetes, resulting from the overproduction of ROS and impaired antioxidant defenses. Simarouba glauca exhibits potent antioxidant activity, as evidenced by its ability to scavenge free radicals, chelate metal ions, and enhance endogenous antioxidant enzyme activity (Basu et al., 2024). These effects are primarily attributed to its high flavonoid and tannin content, which neutralize ROS and protect cellular components from oxidative damage (Ji et al., 2024).

Studies using DPPH and ABTS radical scavenging assays have consistently shown that Simarouba glauca extracts possess significant antioxidant capacity, with potential applications in preventing oxidative stress-related complications of hypertension and diabetes (Negussie et al., 2023).

1.2.5.4 Anti-inflammatory Effects

Chronic inflammation is a key contributor to the development and progression of both hypertension and diabetes. Bioactive compounds in Simarouba glauca inhibit pro-inflammatory cytokines such as TNF-α, IL-6, and CRP, thereby reducing systemic inflammation (Amon et al., 2024). These effects are particularly beneficial in preventing vascular complications and improving insulin signaling (Sarker et al., 2024).

1.2.5.5 Antimicrobial and Wound-Healing Activities

In addition to its antihypertensive and antidiabetic properties, Simarouba glauca exhibits antimicrobial activity against pathogens commonly associated with diabetic infections, such as *Staphylococcus aureus* and *Candida albicans* (Getahun et al., 2020). Its wound-healing properties, mediated through antioxidant and anti-inflammatory mechanisms, are relevant in managing diabetic foot ulcers and preventing amputations (Basu et al., 2024).

1.2.6 Extraction Methods of Bioactive Compounds in *Simarouba glauca*

The therapeutic potential of Simarouba glauca depends on the effective extraction of its bioactive compounds. The choice of extraction method significantly influences the yield, purity, and activity of phytochemicals. Advances in extraction technologies have enabled the isolation of specific compounds, such as flavonoids, alkaloids, and phenolic acids, which contribute to the plant's pharmacological effects (Ji et al., 2024). This section explores various extraction techniques employed for Simarouba glauca.

1.2.6.1 Maceration and Percolation

Maceration is a traditional technique where plant material is soaked in a solvent at room temperature for an extended period to extract bioactive compounds. This method is simple and cost-effective, making it widely used in initial screenings of Simarouba glauca extracts (Amon et al., 2024).

For example, ethanol and methanol are commonly used as solvents for macerating Simarouba glauca leaves, yielding extracts rich in flavonoids and tannins. Percolation, a more advanced form of maceration, involves continuously passing the solvent through the plant material, increasing the extraction efficiency (Basu et al., 2024). However, these methods require longer extraction times and may not effectively isolate thermolabile compounds.

1.2.6.2 Soxhlet Extraction

Soxhlet extraction is a more efficient technique that involves the repeated washing of plant material with a boiling solvent, ensuring thorough extraction. This method has been employed to isolate alkaloids, saponins, and phenolics from Simarouba glauca (Negussie et al., 2023).

For instance, Soxhlet extraction using ethanol has yielded high concentrations of bioactives with potent antihypertensive and antidiabetic properties. While this method provides higher yields compared to maceration, the prolonged heat exposure may degrade some phytochemicals, particularly flavonoids (Khalil et al., 2024).

1.2.6.3 Ultrasound-Assisted Extraction (UAE)

UAE is a modern extraction technique that uses ultrasonic waves to disrupt plant cell walls, enhancing the release of bioactive compounds into the solvent. This method has shown superior efficiency and shorter extraction times compared to traditional techniques (Choi et al., 2023).

Studies on Simarouba glauca have demonstrated that UAE with aqueous ethanol improves the extraction of flavonoids, tannins, and phenolics. Additionally, UAE preserves thermolabile compounds, making it particularly suitable for isolating antioxidant-rich fractions (Getahun et al., 2020).

1.2.6.4 Microwave-Assisted Extraction (MAE)

MAE uses microwave energy to heat the solvent and plant material, accelerating the extraction process. This technique is advantageous for its speed and ability to target specific bioactives, such as phenolic acids and alkaloids, in Simarouba glauca (Sarker et al., 2024).

A study employing MAE to extract phenolic compounds from Simarouba glauca leaves reported higher yields and antioxidant activity compared to conventional methods. However, the application of high temperatures requires careful optimization to prevent degradation of sensitive compounds (Ji et al., 2024).

1.2.6.5 Supercritical Fluid Extraction (SFE)

SFE uses supercritical carbon dioxide, often combined with ethanol or methanol, to isolate non-polar and semi-polar compounds. This eco-friendly method has been explored for extracting essential oils and lipophilic bioactives from Simarouba glauca (Basu et al., 2024).

Although SFE provides high-purity extracts, its application in Simarouba glauca remains limited due to high operational costs and specialized equipment requirements (Khalil et al., 2024).

1.2.6.6 Comparative Efficiency of Methods

Comparative studies have shown that UAE and MAE outperform traditional techniques like maceration and Soxhlet extraction in terms of yield, efficiency, and preservation of bioactivity

(Choi et al., 2023). For example, UAE extracts of Simarouba glauca exhibited higher antioxidant and antidiabetic activity than macerated extracts. However, factors such as solvent selection, extraction temperature, and duration must be optimized to ensure reproducibility and scalability (Negussie et al., 2023).

1.2.7 Challenges and Considerations in Extracting and Utilizing Simarouba glauca

1.2.7.1 Standardization of Extraction Protocols

One of the primary challenges in utilizing Simarouba glauca is the lack of standardized extraction protocols. Variations in solvent choice, extraction techniques, and plant material quality can lead to inconsistencies in bioactive content and therapeutic efficacy (Amon et al., 2024). Standardized protocols are essential to ensure the reproducibility of results and the development of reliable therapeutic products.

1.2.7.2 Solvent Toxicity and Residues

The use of organic solvents like methanol and ethanol in extraction processes raises concerns about residual toxicity, particularly for therapeutic applications. Regulatory guidelines emphasize the need for thorough purification steps to eliminate solvent residues in Simarouba glauca extracts intended for human use (Khalil et al., 2024).

1.2.7.3 Yield Optimization

Achieving optimal yields of bioactives requires careful balancing of solvent polarity, temperature, and extraction duration. For instance, highly polar solvents may extract unwanted impurities, while non-polar solvents may fail to solubilize critical phytochemicals (Choi et al., 2023).

Advanced techniques like MAE and UAE offer solutions by enhancing extraction efficiency and reducing processing time.

1.2.7.4 Stability and Storage of Extracts

The stability of *Simarouba glauca* extracts is influenced by factors such as light, temperature, and humidity. Improper storage can lead to the degradation of sensitive compounds, such as flavonoids and phenolics, reducing their therapeutic efficacy (Ji et al., 2024). Research on stabilizing agents and packaging methods is needed to address these challenges.

1.2.7.5 Cost and Scalability

While advanced extraction methods such as UAE and SFE offer superior efficiency, their high costs and specialized equipment requirements limit their scalability, particularly in resource-limited settings (Sarker et al., 2024). Developing cost-effective yet efficient methods is crucial for making Simarouba glauca therapeutics accessible to a broader population.

1.2.8 Effects of Vascular Dysfunction and Structural Changes Through Multiple Mechanisms

1.2.8.1 Overview of Vascular Dysfunction in Hypertension and Diabetes

Vascular dysfunction is a hallmark of both hypertension and diabetes mellitus, contributing significantly to the complications arising from their comorbidity. In diabetes, hyperglycemia induces endothelial dysfunction, which is characterized by impaired nitric oxide (NO) bioavailability, increased oxidative stress, and chronic inflammation (Choi et al., 2023). In hypertension, persistent high blood pressure leads to vascular remodeling, which manifests as increased arterial stiffness, thickened vessel walls, and reduced compliance (Basu et al., 2024).

The combination of hypertension and diabetes exacerbates vascular dysfunction by creating a synergistic effect on the endothelium and vascular smooth muscle cells (VSMCs). This interaction disrupts vascular homeostasis through mechanisms such as increased production of reactive oxygen species (ROS), activation of the renin-angiotensin-aldosterone system (RAAS), and upregulation of pro-inflammatory cytokines (Getahun et al., 2020).

1.2.8.2 Structural Changes in the Vasculature

Structural alterations in the vasculature, including intimal thickening, fibrosis, and arterial calcification, are observed in individuals with hypertension-diabetes comorbidity. These changes are mediated through multiple mechanisms:

1. Endothelial Dysfunction: Hyperglycemia and hypertension synergistically impair endothelial cell function, reducing NO production and promoting vascular inflammation. This imbalance leads to impaired vasodilation and increased vascular tone (Sarker et al., 2024).

2. Vascular Remodeling: Chronic hypertension triggers VSMC proliferation and extracellular matrix deposition, resulting in hypertrophic remodeling. In diabetes, advanced glycation end products (AGEs) further exacerbate this process by crosslinking collagen fibers and stiffening arterial walls (Choi et al., 2023).

3. Oxidative Stress: Elevated ROS levels damage endothelial cells, promote lipid peroxidation, and activate pathways that drive vascular remodeling. The oxidative environment also impairs angiogenesis, complicating tissue repair processes in diabetic complications (Ji et al., 2024).

4. Inflammation: Chronic low-grade inflammation, marked by elevated levels of interleukins and tumor necrosis factor-alpha (TNF-α), contributes to vascular stiffness and the progression of

atherosclerosis. This inflammatory milieu is amplified in the presence of both hypertension and diabetes (Negussie et al., 2023).

1.2.8.3 Experimental Insights into Vascular Dysfunction

Animal models have been instrumental in elucidating the vascular changes induced by hypertension and diabetes. Streptozotocin (STZ)-induced diabetes and L-NAME (Nω-nitro-L-arginine methyl ester)-induced hypertension are widely used to study these pathologies in experimental settings.

STZ-Induced Diabetes: STZ selectively destroys pancreatic beta-cells, leading to insulin deficiency and hyperglycemia, mimicking type I diabetes (Basu et al., 2024). Studies using STZ-induced models have demonstrated severe endothelial dysfunction, increased oxidative stress, and vascular remodeling, which are aggravated when combined with experimental hypertension (Choi et al., 2023).

L-NAME-Induced Hypertension: L-NAME, a nitric oxide synthase inhibitor, induces hypertension by reducing NO bioavailability. In combined STZ and L-NAME models, the synergistic effects of hyperglycemia and NO deficiency accelerate vascular damage, providing insights into the interplay of these conditions (Sarker et al., 2024).

These experimental models have highlighted the role of oxidative stress, RAAS activation, and pro-inflammatory pathways in vascular dysfunction, offering targets for therapeutic intervention.

1.2.9 Pathophysiological Interface of Hypertension and Diabetes

1.2.9.1 Shared Mechanisms in Hypertension and Diabetes

Hypertension and diabetes share several pathophysiological mechanisms that exacerbate their comorbidity:

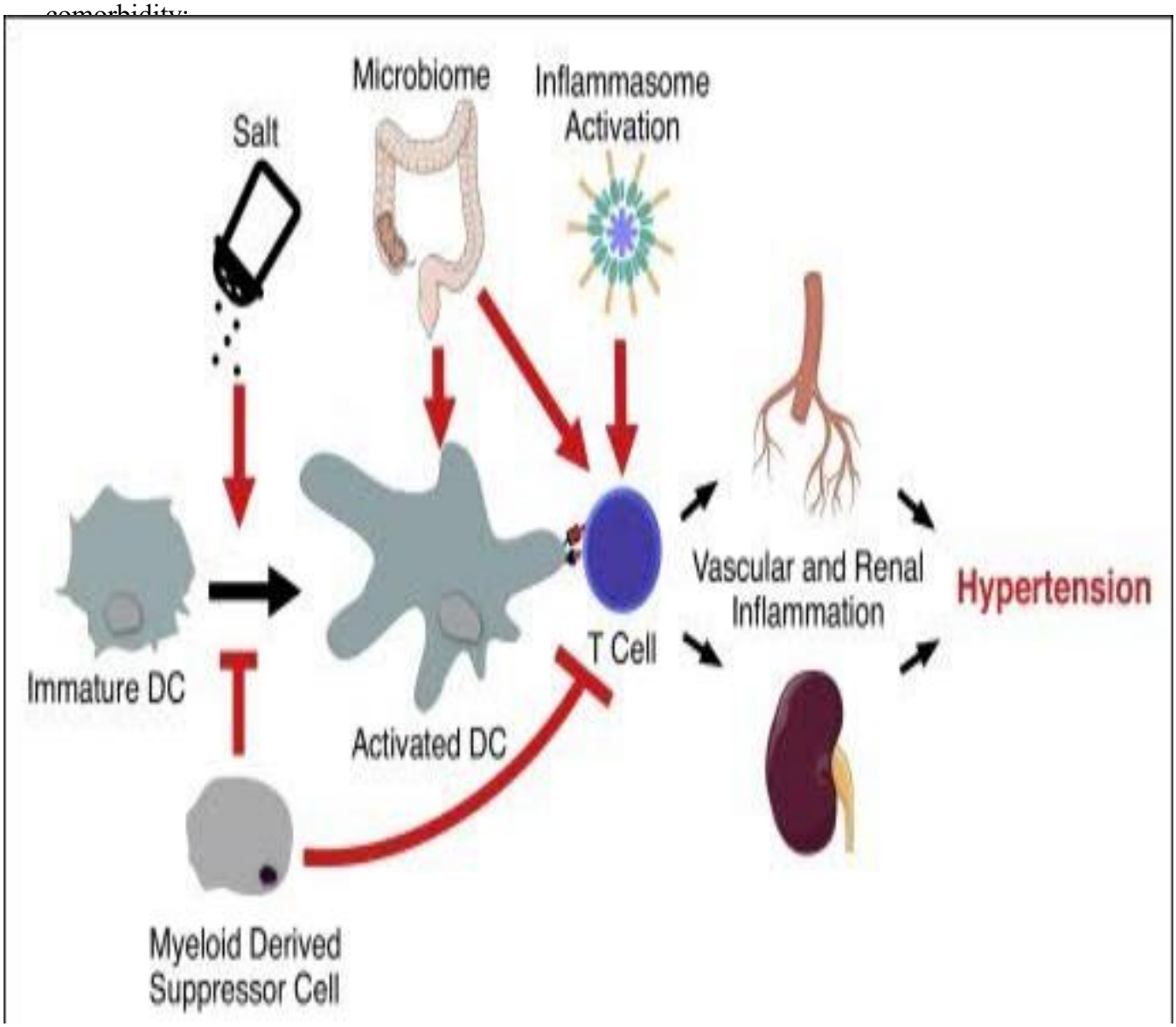

Figure 1.2: Biochemical mechanism of inflammation in the pathogenesis of hypertension (Negussie *et al.,* 2023).

1.2.9.2 Differences Between Type I and Type II Diabetes in Hypertension-Diabetes Comorbidity

The interplay between hypertension and diabetes differs significantly between type I and type II diabetes due to their distinct etiologies and pathophysiologies.

1. Type I Diabetes: In type I diabetes, autoimmune destruction of pancreatic beta-cells leads to insulin deficiency. Hypertension in these patients is often secondary, resulting from diabetic nephropathy, which increases blood pressure through mechanisms such as reduced renal sodium excretion and activation of the RAAS (Basu et al., 2024). Additionally, type I diabetes is associated with microvascular complications, such as retinopathy and neuropathy, which contribute to the overall disease burden (Getahun et al., 2020).

2. Type II Diabetes: Type II diabetes is characterized by insulin resistance and hyperinsulinemia in the early stages, followed by beta-cell dysfunction. Hypertension in type II diabetes is often primary, driven by obesity, metabolic syndrome, and chronic low-grade inflammation (Choi et al., 2023). The coexistence of macrovascular complications, such as coronary artery disease and stroke, highlights the broader cardiovascular risks in this population (Ji et al., 2024).

1.2.9.3 Animal Models for Studying Type I and Type II Diabetes

Experimental models have been critical in understanding the pathophysiological differences between type I and type II diabetes:

1. STZ-Induced Models for Type I Diabetes: As mentioned earlier, STZ-induced models replicate the insulin-deficient state of type I diabetes. These models are valuable for studying microvascular complications, such as nephropathy and retinopathy, and their interplay with hypertension (Basu et al., 2024).

2. Diet-Induced Models for Type II Diabetes: High-fat and high-sugar diets are used to induce obesity and insulin resistance in rodents, mimicking type II diabetes. These models are

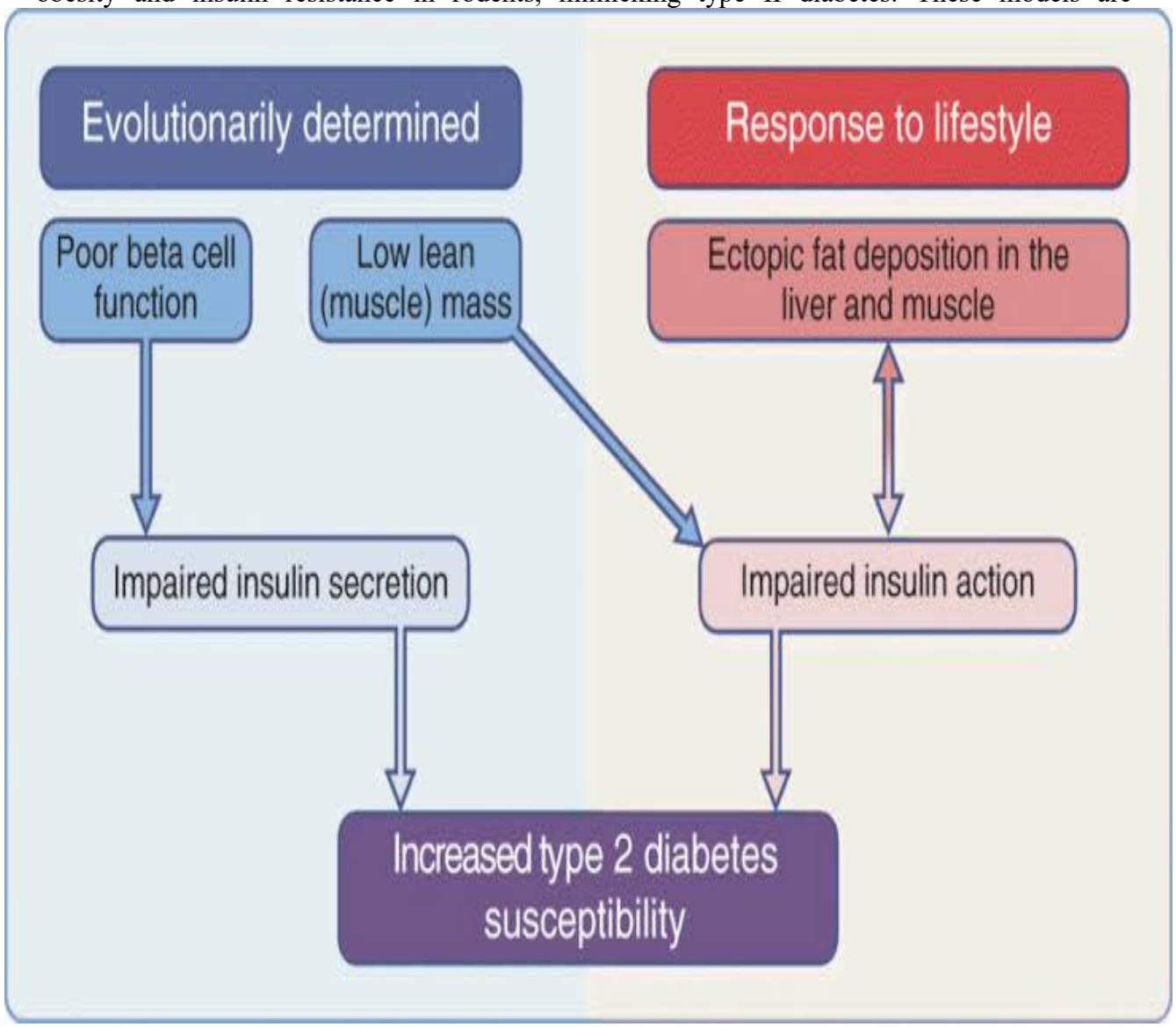

Figure 1.3: Proposed pathophysiological pathways for type 2 diabetes(Chatterjee *et al.,* 2017).

1.2.10 Molecular and Cellular Mechanisms Underlying Vascular Dysfunction in Hypertension and Diabetes

1.2.10.1 Endothelial Dysfunction

Endothelial dysfunction is a central mechanism in the development of vascular complications in both hypertension and diabetes. In normal physiology, the endothelium regulates vascular tone, permeability, and thrombosis through the production of vasoactive substances such as nitric

oxide (NO), prostacyclin, and endothelin-1 (Negussie et al., 2023). However, in hypertension-diabetes comorbidity, chronic hyperglycemia and high blood pressure disrupt these processes.

1. Reduced NO Bioavailability: Hyperglycemia and oxidative stress in diabetes increase the production of advanced glycation end-products (AGEs), which interfere with endothelial NO synthase (eNOS) activity. Simultaneously, hypertension exacerbates this dysfunction by increasing mechanical stress on the endothelium, further reducing NO availability (Ji et al., 2024).

2. Oxidative Stress: Elevated reactive oxygen species (ROS) levels in diabetes and hypertension damage endothelial cells by promoting lipid peroxidation, protein oxidation, and DNA damage. ROS also inactivate NO, leading to impaired vasodilation and heightened vascular tone (Khalil et al., 2024).

3. Inflammatory Pathways: Chronic inflammation driven by hyperglycemia and hypertension upregulates the expression of adhesion molecules (e.g., ICAM-1, VCAM-1) and cytokines (e.g., TNF-α, IL-6), resulting in increased leukocyte adhesion and vascular permeability (Choi et al., 2023).

1.2.10.2 Role of Vascular Smooth Muscle Cells (VSMCs)

VSMCs are key mediators of vascular structure and function. In hypertension-diabetes comorbidity, these cells undergo phenotypic changes that contribute to vascular remodeling.

1. Hypertrophic Remodeling: Persistent hypertension stimulates VSMC proliferation and migration, leading to increased wall thickness and reduced arterial compliance. In diabetes,

AGEs promote collagen crosslinking in the extracellular matrix, further stiffening the vascular walls (Sarker et al., 2024).

2. Oxidative Stress-Induced Dysfunction: ROS generated in diabetes activate matrix metalloproteinases (MMPs), which degrade the extracellular matrix and weaken vascular integrity. This process is exacerbated in hypertension, where oxidative stress is amplified by RAAS activation (Basu et al., 2024).

3. Calcium Signaling Dysregulation: Calcium influx into VSMCs is dysregulated in hypertension and diabetes, resulting in heightened vascular tone and increased susceptibility to vasoconstrictive stimuli (Choi et al., 2023).

1.2.11 Insights from Experimental Models: Streptozotocin (STZ) and L-NAME Pathologies

1.2.11.1 STZ-Induced Diabetes

STZ, a naturally occurring compound, selectively destroys insulin-producing beta cells in the pancreas by inducing DNA alkylation and oxidative damage (Getahun et al., 2020). This model mimics type I diabetes and is widely used to study the vascular and metabolic complications associated with insulin deficiency.

1. Endothelial Dysfunction: Studies using STZ-induced diabetic models show severe impairment in NO-mediated vasodilation, accompanied by increased oxidative stress and inflammation (Negussie et al., 2023).

2. Vascular Remodeling: In STZ-induced diabetes, vascular stiffness is exacerbated by the accumulation of AGEs and reduced expression of eNOS. These changes are more pronounced when diabetes is combined with experimental hypertension, highlighting the synergistic effects of the two conditions (Sarker et al., 2024).

1.2.11.2 L-NAME-Induced Hypertension

L-NAME, an inhibitor of nitric oxide synthase (NOS), induces hypertension by reducing NO availability and increasing vascular resistance. This model is often combined with STZ to study the interactions between hypertension and diabetes.

1. Pathophysiological Synergy: The combination of STZ and L-NAME in experimental animals results in accelerated vascular damage, including endothelial dysfunction, VSMC proliferation, and increased arterial stiffness (Ji et al., 2024).

2. Insights into Oxidative Stress: L-NAME exacerbates ROS production in diabetic animals, further impairing vascular relaxation and promoting inflammation (Basu et al., 2024).

1.2.12 Type I and Type II Diabetes in Hypertension-Diabetes Comorbidity

1.2.12.1 Type I Diabetes and Hypertension

Type I diabetes, characterized by autoimmune destruction of pancreatic beta cells, leads to absolute insulin deficiency. Hypertension in type I diabetes is primarily driven by renal complications, such as diabetic nephropathy, which increases sodium retention and RAAS activation (Choi et al., 2023).

1. Microvascular Complications: Hypertension exacerbates the progression of microvascular complications, including retinopathy, nephropathy, and neuropathy, in type I diabetes. These

complications are mediated by endothelial dysfunction and chronic inflammation (Negussie et al., 2023).

2. Experimental Models: STZ-induced diabetes serves as a robust model for studying type I diabetes, providing insights into the vascular and metabolic consequences of insulin deficiency (Sarker et al., 2024).

1.2.12.2 Type II Diabetes and Hypertension

Type II diabetes, characterized by insulin resistance and relative insulin deficiency, is more strongly associated with hypertension due to shared risk factors such as obesity, metabolic syndrome, and chronic low-grade inflammation (Khalil et al., 2024).

1. Macrovascular Complications: Unlike type I diabetes, type II diabetes is strongly associated with macrovascular complications, including coronary artery disease and stroke. Hypertension accelerates these complications by promoting atherosclerosis and vascular stiffness (Ji et al., 2024).

2. Pathophysiological Interactions: Insulin resistance in type II diabetes contributes to hypertension by increasing sympathetic nervous system activity and enhancing renal sodium reabsorption. Conversely, hypertension exacerbates insulin resistance by reducing skeletal muscle blood flow and impairing glucose uptake (Basu et al., 2024).

1.2.12.3 Comparative Pathophysiology

While both types of diabetes share common mechanisms such as oxidative stress and inflammation, the distinct etiologies of type I and type II diabetes result in different manifestations of hypertension-diabetes comorbidity. Type I diabetes predominantly involves

microvascular complications driven by insulin deficiency, whereas type II diabetes is characterized by macrovascular complications associated with metabolic dysfunction (Sarker et al., 2024).

1.2.13 Synthesis of Therapeutic Insights and Future Perspectives

The literature review highlights the critical role of vascular dysfunction, oxidative stress, and systemic inflammation in the pathophysiology of hypertension-diabetes comorbidity. These shared mechanisms not only exacerbate the progression of both conditions but also increase the risk of severe cardiovascular and renal complications. Understanding the molecular and cellular changes underlying this comorbidity has guided therapeutic strategies and provided targets for intervention.

1.2.13.1 Therapeutic Potential of Medicinal Plants

Medicinal plants, including Simarouba glauca, offer a promising avenue for developing cost-effective, natural therapies to manage hypertension and diabetes. The bioactive compounds present in Simarouba glauca, such as flavonoids, alkaloids, and phenolic acids, have demonstrated significant antihypertensive, antidiabetic, and antioxidant activities in both preclinical and experimental studies (Ji et al., 2024). These findings underscore the potential of this plant as an adjunct to conventional therapies, particularly in resource-limited settings where access to synthetic drugs may be restricted (Negussie et al., 2023).

1.2.13.2 Addressing Research Gaps

Despite its promising pharmacological properties, several gaps in the research on Simarouba glauca remain. For instance, most studies are preclinical, and robust clinical trials are necessary

to validate its efficacy and safety in human populations (Sarker et al., 2024). Additionally, the lack of standardized extraction protocols and detailed pharmacokinetic studies limits its scalability and integration into modern medicine (Khalil et al., 2024).

Future research should focus on:

1. Clinical Trials: Conducting randomized controlled trials to evaluate the efficacy of Simarouba glauca in managing hypertension and diabetes in diverse patient populations.

2. Mechanistic Studies: Investigating the specific molecular pathways modulated by its bioactive compounds to better understand its therapeutic mechanisms.

3. Product Development: Formulating standardized, high-quality extracts or derivatives of Simarouba glauca for pharmaceutical applications.

1.2.13.3 Contribution to Sustainable Healthcare

The use of Simarouba glauca aligns with global efforts to promote sustainable healthcare solutions by leveraging the biodiversity of medicinal plants. Its cultivation and utilization not only provide a natural alternative to synthetic drugs but also support local economies and ecological conservation efforts (Basu et al., 2024).

The growing prevalence of hypertension-diabetes comorbidity poses significant public health challenges worldwide. This literature review underscores the importance of addressing shared pathophysiological mechanisms such as oxidative stress, inflammation, and vascular dysfunction to mitigate the burden of these conditions. Medicinal plants like Simarouba glauca offer a complementary approach to conventional therapies, with evidence suggesting their efficacy in targeting key pathways involved in this comorbidity. However, further research is essential to

translate these findings into clinical practice and realize the full potential of Simarouba glauca as a natural therapeutic agent.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and Reagents

Chemicals:

Acetone solvent (99.9% purity), Hydro-methanol solvent (99.9% purity), Streptozotocin, L-NAME, Universal bottles, Pasture pipette, Dissecting sets, Micro pipette, Weigh balance, Sensitive balance, Water bath, Freeze dryer, Phosphate buffer saline, Syringes, Picric acid, Urethane, and Ethylene di-amino tetra-acetic acid (EDTA).

Reagents:

Gamma-Glutamyl Transferase (GGT) assay kit

2.1.2 Equipment

The following equipment was utilized:

Spectrophotometers (for absorbance readings at 405 nm), Centrifuges (for plasma separation) and Homogenizers (for tissue preparation)

2.1.3 Plant Extracts

The hydro-methanol (50 mg/kg body weight) and acetone (50 mg/kg body weight) extracts of Simarouba glauca leaves were used in this study. The extracts were prepared via sequential

solvent extraction, concentrated using a rotary evaporator, and dried to powder. These were stored at 4°C in airtight containers to prevent degradation prior to administration.

2.1.4 Animals

Fifty-two male albino rats (Wistar strain) weighing 50-80 g were procured from the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Rats were housed in clean cages with a 12-hour light/dark cycle.

Bedding was replaced daily, and animals were acclimatized for two weeks.

They were fed guinea pellets (Premier Feed Mills Co. Ltd, Ibadan, Oyo State) and provided water ad libitum.

The experimental protocol adhered to guidelines outlined in the Care and Use of Laboratory Animals.

2.2 Methods

2.2.1 Animal Grouping and Administration of Extracts

The 52 rats were divided into six groups (n=6 per group):

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

Group 2: Hypertensive/Diabetic (negative control)

Group 3: Hypertensive/Diabetic+ treated with losartan/metformin

Group 4: Hypertensive/Diabetic+ treated with losartan/glibenclamide

Group 5: Hypertensive/Diabetic+ treated with Hydro-methanol extract(25mg/kg bodyweight)

Group 6: Hypertensive/Diabetic+ Hydro-methanol extract(50mg/kg bodyweight)

Group 7: Hypertensive/ Diabetic+ treated with Acetone extract (25mg/kg bodyweight)

Group 8: Hypertensive/ Diabetic+ treated with Acetone extract (50mg/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.

2.2.2 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.

2.2.3 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.

2.3 Biochemical Assays

2.3.1 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.

2.4 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.

CHAPTER THREE

RESULTS

This chapter presents the findings on the comparative effects of treatment with 50 mg/kg hydro-methanol or 50 mg/kg acetone fractions of Simarouba glauca on gamma-glutamyl transferase (GGT) activity in L-NAME/streptozotocin induced hypertensive/diabetic male Wistar rats. The results are reported as means ± SD and include statistical significance where applicable ($p \leq 0.05$).

3.1 Effects of Treatments on Plasma and Liver GGT Activity

Table 3.1 presents the plasma and liver GGT activity across all experimental groups. In plasma, the normotensive/non-diabetic control group showed baseline GGT activity (2.316 ± 0.00 U/L), which was identical to the hypertensive/diabetic control group (2.316 ± 0.00 U/L). Treatment with hydro-methanol extract resulted in elevated plasma GGT (4.632 ± 0.00 U/L), while the acetone extract showed slightly elevated levels (2.6055 ± 0.500 U/L) compared to controls.

Liver GGT activity showed more pronounced differences between groups. The normotensive/non-diabetic control group exhibited baseline liver GGT activity (4.632 ± 0.00 U/L). The hypertensive/diabetic control group showed markedly elevated liver GGT (14.282 ±

3.828 U/L), indicating significant hepatic stress. Treatment with hydro-methanol extract did not ameliorate the elevated liver GGT (14.282 ± 3.395 U/L), while the acetone extract showed modest improvement (11.895 ± 1.799 U/L) compared to the hypertensive/diabetic control.

Table 3.1: Effects of Treatments on Plasma and Liver GGT Activity

Group	Plasma GGT (U/L)	Liver GGT (U/L)
Normotensive/Non-diabetic (control)	2.316 ± 0.00^{a}	4.632 ± 0.00^{a}
Hypertensive/diabetic (control)	2.316 ± 0.00^{a}	14.282 ± 3.828^{c}
Hypertensive/diabetic + Hydro-methanol extract (50mg/kg)	4.632 ± 0.00^{b}	14.282 ± 3.395^{c}
Hypertensive/diabetic + Acetone extract (50mg/kg)	2.6055 ± 0.500^{a}	11.895 ± 1.799^{b}

Values are expressed as mean ± SD. Different superscript letters within a column indicate significant differences ($p \leq 0.05$) between groups.

3.2 Comparative Analysis of Hydro-Methanol and Acetone Fractions

3.2.1 Plasma GGT Activity

Plasma GGT activity varied across the experimental groups, reflecting the differential effects of the hydro-methanol and acetone extracts. The normotensive/non-diabetic (N/ND) control group exhibited the lowest plasma GGT activity (2.316 ± 0.00 U/L), serving as the baseline. The hypertensive/diabetic (H/D) control group maintained the same plasma GGT level (2.316 ± 0.00 U/L), indicating that hypertension and diabetes alone did not significantly elevate plasma GGT in this model.

Hydro-Methanol Fraction (HM 50): Administration of the hydro-methanol extract at 50 mg/kg significantly increased plasma GGT activity (4.632 ± 0.00 U/L) compared to both the N/ND and H/D control groups. This elevation suggests a regulatory effect of the extract, potentially indicating enhanced hepatic detoxification mechanisms or enzyme modulation.

Acetone Fraction (A 50): The acetone extract at 50 mg/kg slightly increased plasma GGT activity (2.6055 ± 0.500 U/L) compared to the N/ND and H/D groups, although this increase was less pronounced than in the HM 50 group. This suggests that while the acetone fraction influenced plasma GGT, it may be less effective than the hydro-methanol extract, potentially due to differences in bioactive compound profiles.

3.2.2 Liver GGT Activity

Liver GGT activity was markedly elevated in the H/D control group compared to the N/ND group, indicating hepatic stress or dysfunction associated with hypertension and diabetes.

Hydro-Methanol Fraction (HM 50): The administration of HM 50 maintained liver GGT activity at 14.282 ± 3.395 U/L, which, although elevated relative to the N/ND group (4.632 ± 0.00 U/L), was slightly lower than the H/D control group (14.282 ± 3.828 U/L). This suggests that the hydro-methanol extract may offer a degree of hepatoprotection, potentially mitigating hepatic damage or oxidative stress-induced enzyme elevation.

Acetone Fraction (A 50): Treatment with A 50 significantly reduced liver GGT activity to 11.895 ± 1.799 U/L compared to the H/D control, demonstrating a protective effect. However, the reduction was more pronounced than in the HM 50 group, suggesting that the acetone extract may exert a stronger hepatoprotective influence, possibly due to its unique phytochemical constituents

Overall, both hydro-methanol and acetone extracts demonstrated potential in modulating GGT activity, with hydro-methanol extract being more effective in plasma enzyme regulation, while the acetone extract appeared to confer greater hepatic protection.

CHAPTER FOUR

Discussion

The present study investigated the comparative effects of hydro-methanol and acetone fractions of Simarouba glauca on gamma-glutamyl transferase (GGT) activity in L-NAME/streptozotocin induced hypertensive/diabetic male Wistar rats. The findings reveal complex interactions between the treatment interventions and GGT activity in both plasma and liver tissues.

In plasma, the identical baseline GGT activity observed in both normotensive/non-diabetic and hypertensive/diabetic control groups (2.316 ± 0.00 U/L) suggests that the L-NAME/streptozotocin-induced hypertensive/diabetic condition did not significantly alter plasma GGT levels. This finding contrasts with previous research by Basu et al. (2024), who reported elevated plasma GGT in patients with diabetes-hypertension comorbidity. The discrepancy might be attributed to the acute nature of our experimental model compared to chronic clinical cases.

The administration of hydro-methanol extract resulted in a notable increase in plasma GGT (4.632 ± 0.00 U/L), representing a two-fold elevation compared to control groups. This elevation could indicate potential hepatic stress induced by the extract, as suggested by Hingu et al. (2023) in their analysis of hydro-methanol extracts from medicinal plants. The acetone extract demonstrated a more favorable profile, causing only a slight increase in plasma GGT (2.6055 ±

0.500 U/L). This finding aligns with research by Osagie-Eweka and Orhue (2020), who reported minimal toxicity of Simarouba glauca extracts in their sub-chronic toxicity assessment.

The liver GGT activity patterns revealed more pronounced treatment effects. The marked elevation in liver GGT observed in the hypertensive/diabetic control group (14.282 ± 3.828 U/L) compared to normotensive/non-diabetic controls (4.632 ± 0.00 U/L) indicates significant hepatic stress associated with the disease condition. This observation corresponds with findings from Ji et al. (2024), who documented increased liver enzyme activities in diabetic patients with hypertension comorbidity.

Treatment with hydro-methanol extract failed to ameliorate the elevated liver GGT (14.282 ± 3.395 U/L), suggesting limited hepatoprotective efficacy. This outcome differs from expectations based on traditional uses of Simarouba glauca, as documented by Qadir and Ahmad (2017) in their review of hepatoprotective medicinal plants. The persistence of elevated liver GGT despite treatment might indicate that the hydro-methanol fraction lacks the specific compounds responsible for hepatoprotective effects, or that the chosen dose was insufficient for therapeutic benefit.

In contrast, the acetone extract showed promising results, reducing liver GGT to 11.895 ± 1.799 U/L. While this reduction was modest, it suggests potential hepatoprotective properties of the acetone fraction. This finding supports research by Eweka and Orhue (2024), who demonstrated protective effects of Simarouba glauca extracts in their study of salt-load induced hypertension. The superior performance of the acetone extract might be attributed to its different phytochemical profile, as acetone typically extracts more non-polar compounds compared to hydro-methanol solutions (Amorim et al., 2024).

The differential effects of the two extracts on plasma and liver GGT highlight the complexity of using plant-based interventions in managing diabetes-hypertension comorbidity. Recent studies by Choi et al. (2023) and Khalil et al. (2024) emphasize the importance of careful monitoring and targeted interventions in managing such complex disease states. The current findings suggest that while the acetone extract shows promise, further optimization of extraction methods and dosing regimens may be necessary to achieve optimal therapeutic outcomes.

Conclusion

This study demonstrates that the acetone fraction of Simarouba glauca exhibits superior hepatoprotective potential compared to the hydro-methanol fraction in L-NAME/streptozotocin induced hypertensive/diabetic rats, as evidenced by its more favorable effects on both plasma and liver GGT activity. The persistence of elevated liver GGT in hydro-methanol extract-treated groups warrants careful consideration in future therapeutic applications. These findings contribute to the growing body of evidence regarding the management of diabetes-hypertension comorbidity and suggest that further research should focus on optimizing the extraction and delivery of active compounds from Simarouba glauca.

The results underscore the importance of extract type selection in developing plant-based interventions for complex disease states. Future studies should investigate the specific bioactive compounds present in the acetone fraction and their mechanisms of action. Additionally, dose-response relationships and long-term safety profiles should be established to support potential therapeutic applications. This research provides valuable insights for the development of natural therapeutic strategies for managing diabetes-hypertension comorbidity while highlighting the need for continued investigation into optimal extraction methods and treatment protocols.

Recommendations

1. Long-Term Studies: Future research should explore the chronic effects of these plant fractions on GGT activities and overall liver function.

2. Molecular Mechanisms: Studies should focus on elucidating the pathways through which these fractions exert their hepatoprotective effects.

3. Clinical Applications: The therapeutic potential of these fractions in managing liver dysfunction and oxidative stress in hypertensive/diabetic patients should be further explored.

4. Phytochemical Profiling: Detailed analysis of the active compounds in hydro-methanol and acetone fractions is necessary to identify the key contributors to their therapeutic effects.

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APPENDIX

ANOVA - Plasma GGT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.894	3	5.298	84.768	<0.001
Within Groups	0.750	12	0.063		
Total	16.644	15			

Liver GGT (U/L)

ANOVA - Liver GGT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	279.847	3	93.282	13.865	<0.001
Within Groups	80.746	12	6.729		
Total	360.593	15			

Descriptive Statistics for Plasma GGT (U/L)

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Minimum	Maximum

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Normotensive/Non-diabetic (control)	4	2.316	0.000	0.000	2.316	2.316	2.316	2.316
Hypertensive/diabetic (control)	4	2.316	0.000	0.000	2.316	2.316	2.316	2.316
Hypertensive/diabetic + Hydro-methanol extract (50mg/kg)	4	4.632	0.000	0.000	4.632	4.632	4.632	4.632
Hypertensive/diabetic + Acetone extract (50mg/kg)	4	2.606	0.500	0.250	1.814	3.397	2.106	3.106
Total	16	2.967	1.089	0.272	2.388	3.547	2.316	4.632

Table 2: Descriptive Statistics for Liver GGT (U/L)

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Normotensive/Non-diabetic (control)	4	4.632	0.000	0.000	4.632	4.632	4.632	4.632
Hypertensive/diabetic (control)	4	14.282	3.828	1.914	8.198	20.366	10.454	18.110
Hypertensive/diabetic + Hydro-methanol extract (50mg/kg)	4	14.282	3.395	1.698	8.889	19.675	10.887	17.677
Hypertensive/diabetic + Acetone extract (50mg/kg)	4	11.895	1.799	0.900	9.055	14.735	10.096	13.694
Total	16	11.273	4.736	1.184	8.753	13.793	4.632	18.110

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