The Comparative Effect of the Administration of 25mg/kg body weight of Hydro-Methanol or Acetone Fractions of *Simarouba glauca* respectively on Plasma Sodium and Potassium Concentrations of L-NAME/Streptozotocin Induced Hypertensive/Diabetic Male Wistar rats

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Certification

This is to certify that this project work was carried out by **IGBINADOLOR Gift OGHOSA** with matriculation number **LSC2006790** under my supervision in partial fulfilment of the requirement for the award of Bachelor Degree of Science (B.Sc.) degree in Biochemistry.

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Dedication

This project work is dedicated to **GOD ALMIGHTY**, the giver of life, the author and finisher of my faith.

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Abstract

Hypertension and diabetes are two of the most prevalent metabolic disorders, often coexisting and contributing to increased morbidity and mortality. Conventional treatments for these conditions frequently involve multiple pharmacological agents, which can lead to adverse effects and reduced patient compliance. Consequently, there is a growing interest in natural plant-based therapies with antihypertensive and anti-diabetic properties. This study aims to investigate the efficacy hydro-methanol and acetone extracts of *Simarouba glauca* in mitigating diabetic hypertension in male Wistar rats induced by Streptozotocin (STZ) and Nω-Nitro-L-arginine methyl ester (L-NAME). Experimental animals were divided into four groups: a normotensive/non-diabetic control, an untreated diabetic/hypertensive group, and two treatment groups receiving hydro-methanol and acetone extracts of *Simarouba glauca (*25 mg/kg body weight) for four weeks. Plasma sodium and potassium ion levels were assessed to determine the extracts' effects on electrolyte regulation. Results revealed a significant increase in sodium levels (164.34 ± 5.46 mEq/L, $P < 0.05$) in the untreated diabetic hypertensive group compared to the normotensive control (138.77 ± 2.33 mEq/L). Hydro-methanol extract slightly reduced sodium levels (153.14 ± 11.02 mEq/L) without statistical significance ($P > 0.05$), whereas acetone extract significantly lowered sodium levels (129.96 ± 6.43 mEq/L, $P < 0.05$), indicating superior efficacy in mitigating sodium retention. Similarly, potassium levels were markedly depleted in the untreated group (0.33 ± 0.23 mEq/L), while acetone extract significantly restored potassium levels (12.80 ± 0.71 mEq/L, $P < 0.05$), outperforming hydro-methanol extract (0.32 ± 0.19 mEq/L, $P > 0.05$). These findings suggest that *Simarouba glauca*, particularly its acetone extract, has promising potential in correcting electrolyte imbalances associated with diabetic hypertension. Further studies are recommended to elucidate the bioactive compounds responsible for these effects and their underlying mechanisms.

Chapter One

1.0 Introduction and Literature Review

1.1 Introduction

Hypertension and diabetes are two of the most prevalent metabolic health disorders affecting millions worldwide often requiring life-long management. Although, they can each occur independently, they often coexist. Studies have shown an increased prevalence of diabetes among hypertensive populations as well as a high diabetic prevalence among hypertensive populations. (Clime *et al*., 2019; Sun *et al*., 2019).

The coexistence of hypertension and diabetes is due to the shared pathophysiological mechanisms between the two conditions, particularly in relation to obesity, insulin resistance (Wang *et al*., 2019) vascular remodelling, inflammation, endothelial dysfunctions and Renin Angiotensin Aldosterone System (RAAS) (Tsimihodimos *et al*., 2019). There's also substantial overlap related primarily to microvascular and macrovascular disease (Petrie *et al*., 2018; Sabuncu *et al*., 2021). Chronic renal disease, retinopathy and sexual dysfunction are more likely to develop when hypertension and diabetes coexist. Therefore, to reduce the risk of related morbidity and death, both hypertension and diabetes should be detected early and actively treated (Rhodes *et al*., 2012).

For blood pressure management, pharmacological agents such as Angiotensin-converting enzyme (ACE) inhibitors and Angiotensin II receptor blockers (ARBs) are often preferred due to their renal protective effects. Calcium channel blockers and diuretics may also be used. For blood glucose management, newer agents such as SGLT-2 inhibitors and GLP-I receptors can be utilized (Rizos, 2014; Enechi *et al*., 2012). However, the use of multiple medications to control both blood pressure and blood glucose levels increases the likelihood of drug interactions and side effects. Also, the prophylactic effects of these drugs are always short lived and requires continuous use. In addition, virtually all pharmacological agents are quite expensive and as such there's need to investigate the anti-hypertensive and anti-diabetic effects of cheap and available plants with proven medicinal properties (Pr *et al.,* 2014).

This study is a novel research that aims to investigate the potency of acetone and hydro-methanol extracts of *Simarouba glauca* on electrolyte regulation in diabetic hypertensive male Wistar rats induced by Streptozotocin and L-NAME respectively.

1.2 Literature Review

1.3 Hypertension

Hypertension is a health condition also known as high blood pressure. It is a medical condition where the blood pressure in the arteries is consistently high. Simply put, high blood pressure refers to the constant elevation of the force of blood flowing through the blood vessels. (American Heart Association, 2023). It is also defined as an average diastolic pressure higher than 80mmHg and a systolic pressure higher than 130mmHg (American Heart Association, 2024). If the blood pressure goes above 140/190, it is considered high blood pressure and is said to be severe if the pressure is above 180/120. It is anticipated that by 2025, there will be 1.56 billion adults worldwide with hypertension, with a recent estimate of 1.39 billion cases (Mills and Stefanescu, 2020). According to World Health Organization (WHO), approximately 17 million people die every year due to cardiovascular diseases, out of which 9.4 million deaths are due to hypertension (Carey and Whelton, 2021).

The causative factors for the pathogenesis of hypertension are complex and multifaceted including genetics, age, stress, high dietary salt ingestion, obesity and diabetes (Davie, 2018). Victims with high blood pressure are usually asymptomatic which is why it is often referred to as the 'silent killer'. However, symptoms such as headaches, shortness of breadth, nosebleeds, etc usually occur when high blood pressure has reached a severe or life threatening stage. Untreated hypertension predisposes risks such as stroke, myocardial infarction, arteriosclerosis, cardiac arrest, heart attack, cardiomegaly, etc (Landazuri *et al*., 2017)

1.3.1 Stages of Hypertension

Based on the recommendations of the 2017 American college of Cardiology/ American Heart Association (ACC/AHA) guidelines, the classification of blood pressure (expressed in mmHg) for adults aged 18 years and above is as follows (Jeffery, 2017; Whelton *et al*., 2017):

Normal blood pressure: Here, blood pressure range is normal with low risk of cardiovascular problems. The systolic blood pressure lower is than 120mmHg and diastolic blood pressure is lower than 80mmHg.

Elevated blood pressure: This is characterized with systolic blood pressure between 120-129mmHg and diastolic blood pressure less than 80mmHg. This could result in hypertension if proper measures are not put in place.

Stage I hypertension: At this stage, lifestyle modification is necessary and might reuire the use of medications. Here, systolic blood pressure is 130 to 135mmHg and diastolic pressure is 80 to 89mmHg.

Stage 2 hypertension: At this point, there is an increased risk of developing cardiovascular diseases. Lifestyle modifications coupled with the use of medications is crucial for management. The systolic blood pressure is 140mmHg or greater and the diastolic blood pressure is 90mmHg or greater.

1.3.2 Types of Hypertension

This is categorized based on the cause into primary (essential) hypertension or secondary (non-essential) hypertension.

1.3.2.1 Primary Hypertension

Primary or essential hypertension is the most common form of hypertension and accounts for 90-95% of adult cases with hypertension. Essential hypertension is diagnosed in the absence of an identifiable cause (Whelton *et al*., 2018). Pathophysiologically, the main cause of essential hypertension remains unclear, but it is believed that a complex interplay between genetics, environmental and lifestyle factors results in the development of essential hypertension (Lopez-Jaramillo *et al.,* 2016). Also, speculated pathophysiological contributors such as dysregulation of the sympathetic nervous system, overstimulation of vasoconstrictors, stress, dysregulation of renin-angiotensin aldosterone system (RAAS); resulting in excessive sodium ion retention, decreased production of vasodilators such as prostacyclin, Nitric Oxide (NO) and natriuretic peptide; resulting from endothelial dysfunction has been recorded to be

responsible for the development of essential hypertension (National Clinical guideline centre, UK, 20II).

1.3.2.2 Secondary Hypertension

Secondary hypertension accounts for approximately 5-10% of all cases of hypertension (Muntner, 2021). There are many known underlying factors that could result in the development of secondary hypertension such as; drug induced (e.g Non-Steriodal Anti-Inflammatory Drugs (NSAIDs): which blocks/ inhibits the cyclooxygenase I (COX-I) and cyclooxygenase 2 (COX-2) enzymes which in turn increases the retention of sodium, alcohol, adrenergic medications, e.t.c), neurological (e.g sleep apnea, brain tumor) and endocrine dysfunction. It has been reported that secondary hypertension if nor properly managed could lead to cardiovascular complications, multi organ dysfunction and failure, cardiac dysfunction and cardiomegaly, renal failure, etc (British Heart Foundation, 2015).

1.3.3 Management

Many guidelines exist for the management of hypertension. Most groups including the seventh report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC 7), American college of Cardiology/ American Heart Association (ACC/AHA) recommend lifestyle modifications as first step of managing hypertension. (Whelton *et al*., 2017). If lifestyle modifications are insufficient, pharmacological interventions is required. Medications applied in the management of hypertension include:

Diuretics: They are usually the first line of treatment for hypertension. Their mechanism of action involves eliminating excess sodium and water from the body thereby reducing blood volume and consequently blood pressure. An example is hydrochlorothiazide.

Angiotensin-converting enzymes (ACE) Inhibitors: These medications function by inhibiting the enzyme that converts angiotensin I to angiotensin II, a potent vasoconstrictor. This relaxes blood vessels leading to lower blood pressure. Examples include enalapril and lisinopril.

Angiotensin II receptor blockers (ARBs): These have a similar function as ACE Inhibitors but they inhibit angiotensin at the receptor level. Examples include valsartan and losartan.

Calcium channel blockers: These function by inhibiting the entry of calcium into the heart cells and walls of blood vessels leading to decreased heart contractility and relaxation of the blood vessels. They include dihydropyridine (e.g amlodipine), non-dihydropyridine (e.g diltiazem)

Beta blockers: They function by blocking the effects of adrenaline on the heart beta receptors. This slows down heart rate and reduces the force of contraction thereby reducing blood pressure. Examples include atenolol and metoprolol.
(Landazuri *et al*., 2017; Olowofela and Isah, 2017; Moke *et al.,* 2022).

1.4 Diabetes

Diabetes is a metabolic auto immune multisystem disease characterized by sustained elevated blood glucose level generally referred to as hyperglycemia and it is due to a deficiency in insulin secretion or action (Enhorning and Melander, 2018). According to the American Diabetes Association (ADA), diabetes was defined as blood glucose level ≥7.0mmol/l. Diabetes may arise either as a result of low production of insulin by pancreatic beta cells to regulate blood glucose level (type I) or lack of response of cells to insulin produced (type II) (American Diabetes Association, 2014).

Diabetes mellitus is among ten leading causes of death in the world, killing about 1.6 million people a year and is considered the third largest risk factor for premature death worldwide due to complications linked to oxidative stress and induced organ damages resulting from a defect in the body's natural anti-oxidant system (Oguntibeju, 2019; Shariff-Rad *et al*., 2020). Severe health complications such as kidney damage, nerve damage, cardiovascular diseases and vision impairments could arise if diabetes is not properly managed (Zhang *et al*., 2018).

Effective management of diabetes involves lifestyle modifications and the use of medications and insulin therapy (American Diabetes Association, 2020). Examples of

anti-diabetic agents include; sulphonylureas (e.g glyburide, glipizide), biguanides, thiazolidinediones (e.g pioglitazone), alpha-glucosidase inhibitors (e.g acarbose)

1.5 Hypertension and Diabetes Comorbidity (HDC)

Comorbidity, the existence of two or more conditions within an individual is a growing health challenge globally (Academy of medical sciences, 2018), substantially affecting individuals, career and society (Skou *et al.,* 2022).

The comorbidity of hypertension and diabetes represents a significant clinical concern given their intertwined pathophysiology and compounded risk they pose for cardiovascular diseases and other complications (Abdalla, 2024; Uti, *et al*., 2023). They often occur together due to shared risk factors such as obesity, sedentary lifestyle and aging (Uti, *et al.,* 2022; Alum *et al*, 2022). Patients with hypertension and diabetes are at a higher risk for adverse outcomes including heart attacks, strokes, renal failure and even premature death (Drozdz *et al*., 2023).

In research, L-NAME (Nω-Nitro-L-arginine methyl ester) and Streptozotocin are commonly used to induce hypertension and diabetes in animal models. L-NAME is a non-selective inhibitor of nitric oxide synthase (NOS), which is vital for the production of nitric oxide (NO), a vasodilator that helps regulate blood pressure. By inhibiting NOS, L-NAME induces hypertension by reducing NO availability leading to vasoconstriction and increased blood pressure (Khan *et al.,* 2015). Streptozotocin selectively destroys insulin producing pancreatic beta cells via DNA damage and cell death resulting in hyperglycemia. It induces rapid onset diabetes in rodents characterized by elevated blood glucose levels, weight loss and increased urine output (Kumar *et al.,* 2016).

1.5.1 Epidemiology

Hypertension and diabetes mellitus, the two leading components of global burden of diseases are commonly found to coexist. (De Boer *et al*., 2017; Zhou *et al*., 2017; Ferrannini and Cushman, 2012). Globally, there are up to 75% adults with hypertension-diabetes mellitus (HTN-DM) comorbidity (Long *et al*., 2011). This incidence is consistently on the increase worldwide (Nouh, *et al*., 2016). Hypertensive patients with

diabetes mellitus comorbidity have increased mortality rate by 7.2 times (Akalu and Belsti, 2020)

The Framingham Heart Study found a 72% increase in the risk of all cause death and a 57% increase in the risk of any cardiovascular events in patients with hypertension who were also diagnosed with diabetes.

However, prevalence of coexistent diabetes and hypertension varies across different ethnic, racial and social groups. For example, the prevalence of hypertension in diabetic patients is reported to range from 20.6% to 78.4% in the South eastern region and 9.2% to 70.4% in African region (Mohan, *et al*., 2014).

1.5.2 Pathophysiological Interplay between Hypertension and Diabetes

Hypertension and diabetes share several common pathophysiological mechanisms and pathways including insulin resistance, vascular remodelling, inflammation, endothelial dysfunctions, obesity and Renin Angiotensin Aldosterone system (RAAS). These interconnected mechanisms create a challenging clinical scenario, where the presence of one condition exacerbates the other increasing the risk of cardiovascular events and complicating management strategies. (Drodz *et al,* 2023)

1.5.2.1 Insulin Resistance

Several **s**tudies has shown that the primary connection between these two diseases lies in insulin resistance, which is an hallmark of type 2 diabetes and also contributes to the development of hypertension. It results in hyperinsulinaemia, which increases sympathetic nervous system activity, promotes sodium retention and enhances vascular smooth muscle growth, all of which contribute to elevated blood pressure (Ahmed *et al.,* 2021; Uti *et al*., 2020). Insulin resistance further results in the increased production of endothelin-I, a vasoconstrictor (Jenkins *et al*., 2020) and also effects the elevated triglyceride levels thereby promoting hypertension (Kosmas *et al*., 2018).

1.5.2.2 Obesity

Obesity is a major risk factor for the development of both type 2 diabetes and hypertension. It leads to accumulation of fat tissues particularly in the abdominal region. (Koenen *et al*., 2021) this fat tissues produces and releases several pro inflammatory

cytokines that contribute to low grade inflammation and insulin resistance (Zatterale *et al.*, 2019; Ahmed *et al.,* 2021)

1.5.2.3 Inflammation

Chronic low grade inflammation is also a common thread, with elevated levels of proinflammatory cytokines such as Tumor Necrosis Factor- α (TNF-α) and Interleukin 6 (IL-6) found in both conditions (Alum *et al.,* 2022). These inflammatory markers promote atherosclerosis, increase vascular stiffness and further enhances insulin resistance perpetuating the cycle of hypertension and diabetes.

1.5.2.4 Vascular Remodelling

Changes in vessel lumen elasticity affect the ease with which blood can flow through arteries. Minimal reduction in lumen diameter can lead to exponentially increased resistance to blood flow. Patients with hypertension often demonstrate structural and functional changes that adversely alter the lumen of small arteries and arterioles.

In diabetes, high blood glucose levels cause oxidative stress and nitric oxide availability impairing vascular structure and this may result in vascular rigidity because at high concentrations, the glucose has direct toxic effect on endothelial cells (An *et al*., 2023). These effects may lead to decrease in endothelial vascular relaxation, increase constriction, hyperplasia of vascular smooth muscle and vascular remodelling (Hill *et al.,* 2021).

Notably, the spreading of thick interstitial connective tissue across the myocardium appears to be one of the most remarkable microscopic observation of the hypertensive diabetic heart (Harder *et al*., 2003)..

1.5.2.5 Renin Angiotensin Aldosterone System (RAAS)

The Renin Angiotensin Aldosterone system (RAAS) begins with the release of renin, an enzyme synthesized and secreted by the kidney cells in response to low blood sodium levels, decreased renal perfusion pressure or sympathetic nervous system activation. Renin catalyzes the conversion of angiotensin produced by the liver into angiotensin I, which is then converted into angiotensin II by angiotensin converting enzyme (Atlas, 2007). Angiotensin II is a potent vasoconstrictor and stimulates aldosterone release from the adrenal glands, promoting sodium and water retention which increases blood volume

and blood pressure. Also, angiotensin II induces vasoconstriction, raising systemic vascular resistance and contributing to hypertension (Atlas, 2007).

In patients with coexisting diabetes and hypertension, RAAS activation creates a feedback loop that worsens both conditions. Elevated blood pressure caused by RAAS overactivity induces glomerular hypertension (high pressure within kidney capillaries), which damages the kidneys and increases proteinuria; a common complication in diabetes nephropathy. As kidney function declines, the kidneys release more renin in response to perceived hypofusion, further activating the RAAS. Simultaneously, angiotensin II promotes insulin resistance by impairing insulin signaling pathways, reducing glucose uptake in tissues and increasing blood glucose levels (Gossens, 2017). This elevation in blood glucose further stimulates the RAAS, leading to an even higher blood pressure.

Thus, the RAAS is a central pathway linking hypertension and diabetes, worsening each condition and increasing the risk of cardiovascular and renal complications.

1.5.2.6 Endothelial Dysfunction

The increase in blood glucose level induces fibronectin and collagen-iv in human vascular endothelial cells. This enhanced expression of fibronectin and collagen-iv may further lead to endothelial dysfunction. Fibronectin which is a gh/coprotein play a complex role in cell matrix interaction and thicken the glomerular basement membrane and magnesium with increase in expression which may result in hypertension. (Demarco *et al*., 2014).

1.5.2.7 Electrolyte Imbalance

Electrolyte imbalance particularly relating to sodium (Na^+) and potassium (K^+), plays a critical role in the pathophysiology of hypertensive diabetic patients. Diabetic nephropathy often leads to impaired sodium excretion, causing sodium retention and extracellular fluid expansion which increases blood pressure (Persson *et al*., 2020*)*. Also, high sodium levels contribute to vascular stiffness and increased vascular resistance, exacerbating hypertension (He *et al.,* 2019). Potassium plays a key role in counteracting the hypertensive effects of sodium by promoting vasodilation and facilitating sodium excretion. However, diabetic victims often experience hypokalemia due to insulin deficiency or resistance, increased urinary potassium loss. Hypokalemia enhances sodium

retention, stimulates the renin-angiotensin-aldosterone system (RAAS) and leads to endothelial dysfunction, all of which contribute to high blood pressure. The imbalance between sodium retention and potassium excretion in hypertensive diabetic states promotes sustained vasoconstriction, volume expansion and increased cardiac workload. This electrolyte dysregulation further exacerbates diabetic nephropathy, cardiovascular disease and stroke (Zhao *et al.,* 2022).

1.5.3 Complications Associated with Hypertension and Diabetes Comorbidity

Comorbidity of hypertension and diabetes causes a significant increase in the risk of vascular complications and both conditions also predispose to chronic kidney disease.

1.5.3.1 Vascular Complications

Diabetes and hypertension are significant risk factors for macrovascular and microvascular illnesses. Macrovascular or cardiovascular disease of larger conduit arteries is a complex inflammatory process leading to myocardial infarction, stroke and peripheral artery disease. The primary pathological process associated with macrovascular disease is atherosclerosis, which in diabetes is accelerated with extensive distribution of vascular lesions (Kattoor *et al.,* 2017). Microvascular disease leads to retinopathy, nephropathy and neuropathy which are major causes of morbidity and mortality in diabetes. (Zhang *et al.,* 2010). Comorbidity with hypertension amplifies these complications and contributes to the accelerated vasculopathy in diabetes (Frimat *et al*., 2017).

1.5.3.2 Kidney Failure

Diabetes is the main cause of kidney failure since 40% of diabetic patients develop chronic kidney disease. The main causes of end stage renal disease (ESRD) include diabetes and hypertension. When blood pressure rises, the risk of ESRD increases (Hsu *et al*., 2005). Chronic hypertension promotes the decline in renal function when it coexists with diabetes. (Anders *et al*., 2018).

1.5.4 Management

An effective treatment regimen is very crucial in order to address all aspects of the complex metabolic derangements seen in hypertensive diabetic patients (Gaede *et al*.,

2013). A multifaceted approach is necessary to effectively manage patients with hypertension and diabetes comorbidity. Key management strategies include lifestyle modifications, pharmacological treatments and personalized care plans.

1.5.4.1 Lifestyle Modifications

Lifestyle modifications play a central role in managing both hypertension and diabetes. Dietary changes such as reducing sodium intake (to less than 1.5g/day), increasing consumption of low fat dairy products (2-3 servings per day) and increasing the consumption of fruits, vegetables and whole grains (8-10 servings per day) can help lower blood pressure and improve glycemic control. Regular physical activity (e.g, brisk walking 30minutes per day) is also essential as it can reduce insulin resistance, lower blood pressure, and improve cardiovascular health. Weight management, smoking cessation and stress reduction are additional lifestyle interventions that can yield positive results on patients. (Correia, 2022; Ali and Bakris, 2020)

1.5.4.2 Pharmacological Treatments

Pharmacological management of hypertensive diabetic patients require careful selection of medications to avoid adverse effects and drug interactions. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) are often preferred due to their renal protective effects. Calcium channel blockers and diuretics may also be used, but their potential impact on glucose metabolism should be considered. Agonists offer cardiovascular benefits in addition to glycemic control, making them attractive options for patients with both hypertension and diabetes.

1.5.4.3 Personalized Care Plans

Personalized care plans that can take into account the individual's patient risk factors, comorbidities and preferences are essential for managing hypertension and diabetes comorbidity. Regular monitoring of blood pressure, blood glucose and other relevant parameters is crucial for adjusting treatment regimen and preventing complications. Collaborative care including primary care physicians, dietitians, endocrinologists and cardiologists can help ensure comprehensive management of these complex patients (Mikkola *et al*., 2022)

1.5.5 Challenges in Management

Managing diabetic hypertensive patients poses several challenges due to the need to balance blood glucose control with blood pressure management. Key challenges in managing these patients include:

Risk of hypoglycemia; aggressive blood pressure lowering in patients with diabetes can increase risk of hypoglycemia, particularly when using medications such as beta blockers or insulin. Hypoglycemia is also linked with adverse cardiovascular outcomes making it crucial to carefully monitor blood glucose levels and adjust medications accordingly (Dimakos *et al*., 2023).

Cardiovascular complications: Patients with both hypertension and diabetes have a significantly higher risk of cardiovascular events, including heart attacks, strokes, and heart failure. The management of these patients involves and requires a delicate balance between lowering blood pressure and controlling blood glucose levels to minimize cardiovascular risks (Wali *et al.,* 2023).

Polypharmacy and Medication Management: The use of multiple medications to control both blood pressure and blood glucose levels increases the likelihood of drug interactions and side effects. Polypharmacy can also lead to reduced adherence to treatment regimens, especially in elderly patients with multiple comorbidities. Strategies to simplify treatment regimens and improve adherence are crucial in this patients (Peron *et al*., 2015).

Lifestyle Modifications: Lifestyle adjustments such as dietary changes and exercise are essential for managing both diabetes and hypertension. However, patients may find it challenging to adhere to these recommendations, particularly when dealing with two chronic conditions (Aja *et al*., 2023; Owusu, 2019).

Monitoring and Management: Frequent monitoring of blood pressure and blood glucose levels is essential, but it can be burdensome for patients. Additionally, fluctuations in one condition can complicate the management of the other, requiring careful and frequent adjustments to treatment plans (Holt, 2014; Correia, 2023; Ali *et al*., 2020; Rizos, 2014).

Individualized Care: Each patient's response to treatment can vary, highlighting the need for tailored therapy. This involves a delicate balance between achieving optimal blood pressure control without compromising blood sugar levels and vice versa.

Cost: Due to complex needs associated with managing both conditions, patients often face high financial burdens, which can impact their adherence to recommended treatment regimens and overall health outcomes (Nguyen and Nguyen, 2017).

1.6 *Simarouba glauca* (SG)

Table 1: Taxonomy of Simarouba Glauca

Kingdom	**Plantae**
Subkingdon	Traceobionta
Super Division	Spermetophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Simaroubaceae
Genus	Simarouba
Species	Glauca

1.6.1 Botanical Description

Simarouba glauca DC belonging to family Simaroubaceae, is generally known as paradise tree. Simaroubacece family includes 32 genera and further 170 species of trees. It is an evergreen tree of medium height, normally reaching about 20 meters high and about 50-80 cm in diameter, and about seven years in life. It has a stem periphery of (40 - 50 cm), frequently with a clear, spherical bole to 9m. The crown is narrow, with a range averaging (4-6 m). The leaves are oblong, dark green, and frequently notched or smooth at the apex. The tree is dioecious, with both androgynous and bisexual flower. Since maturing, the seeds are 1.5 to 2 cm long pinkish or yellowish. There are two varieties:

one produces a greenish fruit and the other distinct violet or almost black fruit depending on fruit colour (Osagie-Eweka, 2018)..

Figure 1: Leaves of Simarouba glauca

Figure 2: Simarouba glauca tree

1.6.2 Geographical distribution

The plant is grown in Amazon rainforest and other tropical areas of Cuba, Hwaiti, North and Central America, Mexico, India, Philipines (Taylor, 2003). It was introduced to Nigeria in the year 2007 by Blessing Akele, Ph.D. and Osagie Eweka, Ph.D. in Ubiaja, Esan South East Local Government Area of Edo State and was cultivated in Cercobela farms. (Osagie-Eweka *et al.,* 2021).

1.6.3 Ethnobotanical uses

Leaves and bark of Simarouba have been used as a natural drug in tropical areas. Simarouba bark is an efficacious treatment for malaria and dysentery. Other indigenous

lineage throughout the South uses the bark for different conditions; as a hemostatic agent to stop bleeding and as an alcohol. It's used externally for blisters and injuries (Vendrapati *et al.,* 2016). Simarouba is subject of one US Patent, whereby its water excerpt was set up to increase skin keratinocyte isolation and to ameliorate skin hydration and moisturization. The seeds extracted in alcohol are used against snakebites. An infusion of the bark is used against malaria, rheumatism, shingles and fever (Chanda, 2014).

1.6.4 Chemical Constituents

Mostly, alkaloids with elevated cytotoxicity and quassinoids with influential antifungal and anti-tumor properties were extracted from this plant. Triterpenes, useful in the cure of amoebiasis, diarrhea and malaria, are in SG bark and leaves. (Mathew *et al.,* 2019). SG has 11 therapeutically essential quassinoids. The presence of alkaloids, cardenolides, flavonoids, fixed oil, glycosides, phenolic compounds, saponins has been detected in SG extract. Normal SG qualitative studies have demonstrated positive results on alkaloids, carbohydrates, flavonoids, and triterpenoids.

Table 2: Qualitative Analysis of S. Glauca (Dinesh *et al.,* 2017)

Phytochemical	**Test**	**Result**	**Inference**
Alkaloids	Mayer's test	Pale creamy precipitate	Positive
Carbohydrates	Molish test	Reddish-violet ring	Positive
Cardiac glycosides	Keller-Kiliani test	No greenish-blue colour	Negative
Flavanoids	Shinoda test	Reddish-brown colour	Positive
Phenols	$FeCl^2$ test	No dark green/blue colour	Negative
Saponins	Frothing test	No stable froth	Negative
Tannin	$FeCl_2$ test	Bluish-green or blue-black colour	Negative
Triterpenpids	Salkowski test	Reddish-brown colour	Positive

1.6.5 Pharmacological Uses

Antioxidant Activity; SG leaves has antioxidant characteristics (Santhana *et al*., 2014). Umesh, (2015) reported that SG leaf extracts were highly effective in scavenging free radicals, including DPPH and chelating radical iron.

Hepatoprotective activity: A study revealed that the chloroform and ethanolic excerpts of *Simarouba glauca* lowered the increased levels of Alkaline Phosphatase (ALP), serum Glutamic-Pyruvic Transaminase (SGPT) and Serum Glutamic-Oxaloacetic Transaminase (SGOT) and reduced the histological changes caused by drug damage (John et al., 2016).

Anti-hypertensive Activity: Studies have shown that this plant retain potentiality of lowering elevated blood pressure. A study conducted by Osagie Eweka S.D. *et al.,* (2023) aimed at assessing the hypotensive prospect inherent in the aqueous extract of Simarouba glauca (AESG) on normotensive male Wistar rats. The data recorded on a chart, indicated the characteristic dose-dependent hypotensive effect of AESG on normotensive rats at doses of 2.5 mg/kg and 5.0 mg/kg, with remarkable diminishments in the systolic blood pressure(SBP), diastolic blood pressure(DBP) and mean arterial pressure (MAP) from basal leaves of 127.83 ± 1.01 mmHg, 91.00 ± 1.00 mmHg and 103.27 ± 0.99 mmHg independently. This indicated that the AESG demonstrated a hypotensive effect on the Blood Pressure of normotensive male Wistar rats dependent on varying doses administered.

Anti-Diabetic Activity: A study conducted by Aleesha R. (2020) on the evaluation of the anti-diabetic activity of *Simarouba glauca* bark extract: an in vitro study, reported that the methanolic extract of *S. glauca* showed a significant inhibitory effect on alpha-amylase and glucose diffusion in vitro, thus authenticating the antidiabetic activity.

1.7 Aims and Objectives of the Study

The aim of this study was to investigate the comparative effect of the administration of 25mg/kg of hydro-methanol and 25mg/kg acetone fraction of *Simarouba glauca* on plasma sodium and potassium concentrations of l-name/streptozotocin induced hypertensive/diabetic male Wistar rats.

The objectives of the study are:

- to evaluate the plasma sodium levels of hypertensive and diabetic male Wistar rat treated with 25mg/kg hydro-methanol and acetone fractions of SG.
- to evaluate the plasma potassium levels of hypertensive and diabetic male Wistar rat treated with 25mg/kg hydro-methanol and acetone fractions of SG.

Chapter Two

2.0 Materials and Methods

2. 1 Materials Used

L-NAME (nitro-L-arginine methyl ester)

Streptozotocin (STZ)

Plasma Sodium assay

Plasma Potassium assay

Sample bottles (EDTA, lithium heparin and fluoride oxalate)

Universal bottle

Sensitive balance

Wistar rats (male)

Dissecting sets

Water bath

Working spectrophotometer

Distilled water

Syringes and hand gloves

Wipes and tissue

Hair net

Rat feed

Face mask

Foil paper

Test tubes and pipette

Methanol and acetone solvent (99.9% purity)

Freeze dry sample

Picric acid

Phosphate buffer saline (PBS)

2.2 Methods

2.2.1 Collection of *Simarouba glauca* Leaves

Fresh leaves of S. 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 fine a fine powder.

2.2.2 Preparation of Hydro-methanol and Acetone SG Leaf Extract

To obtain hydro-methanol *Simarouba glauca* leaf extract, a 500g leaf powder of *S. glauca* was submerged in a mixture of 20%/80% water-methanol mixture (hydro-methanol) in the proportion; water-500ml, methanol- 2000ml and was vortexed at an interval of 2 hours within 24 hours. To obtain the acetone extract, a 500g leaf powder of *S. glauca* was soaked in 2.5L of acetone and stirred at intervals of 2 hours within 24 hours. A muslin cloth was used to sieve both extract of the plant sample and were both resubmerged for another 24 hours. Both filtrate were freeze dried to obtain a fine powdered leaf extract; with a yield of 57g for hydro-methanol leaf extract and 39g for acetone leaf extract, which in turn resulted in a percentage yield of 11.4% w/w and 7.8 % w/w extraction of hydro-methanol and acetone extract respectively. The extracts were stored in a sterile container in the refrigerator until required for analysis.

2.2.3 Experimental Animals

Healthy experimental young male Wistar rats weighing between 50-65kg were obtained. The rats were divided into a total of 4 groups and kept in separate cages at the animal house facility, Department of Biochemistry, University of Benin. They were acclimatized for a period of two weeks (14 days) and had access to grower pellets feed and clean water throughout the duration of the experiment. After the period of acclimatization, they were induced with disease conditions and graded doses of plant extracts was administered orally for a period of 3 weeks (21 days) using a gavage.

2.2.4 Drugs

Drugs obtained for this study include streptozotocin, L-NAME and urethane.

2.2.5 Induction of Hypertension and Diabetes

Diabetic hypertension was induced using previously established protocol (Bellahen *et al.,* 2013). Hypertensive state was induced to the animals by oral administration of L-NAME (40mg/kg body weight) per day for 4 weeks. Diabetes was induced in the animals with a single inter-peritoneal injection of STZ (45mg/kg body weight) prepared in sterile citrate buffer (0.1M, pH-4.5). A glucometer was utilized to check for changes in blood glucose levels and a fasting blood sugar (FBS) level of ≥600mg/dl was considered to be a successful induction of the disease condition.

2.2.6 Experimental Design

The animals were grouped into 4 groups. Group 1 comprised of 5 healthy rats that were not induced with the disease conditions (normotensive/positive control). Group 2 comprised of a total of 5 hypertensive diabetic rats which received no treatment (negative control). Group 3 and 4 were hypertensive diabetic rats which received treatments of hydromethanol and acetone SG leaf extract (25mg/kg body weight) respectively. These were administered orally for a period of four weeks daily.

2.2.7 Animal Sacrifice/Collection of Specimens

The animals were handled according to the guidelines for treatment of laboratory animals. At the end of the study, the rats were anesthesized by inter peritoneal administration of urethane (1.5g/kg body weight) and sacrificed. The abdominal region was opened up and blood sample was collected via cardiac invasion from the heart using a 5ml syringe and emptied into labelled 5ml ethylene diamine tetra-acetate (EDTA) tubes. The blood samples were centrifuged at 3500rpm for 20 minutes using a centrifuge to obtain a clear supernatant (plasma) that was extracted carefully using pasteur pipettes into labelled plain tubes and then stored in a freezer until it was biochemically analyzed.

2.2.8 Biochemical Analysis

Analysis on plasma sodium and potassium levels were conducted according to the procedures reported to ascertain kidney function using TECO Diagnostics test kits.

2.3 Principle of Plasma Sodium Assay

The method is based on modifications of those first described by Maruna (1958) and Trinder (1951) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Reagent Composition

1. Filtrate Reagent: Uranyl Acetate 2.1 mM and Magnesium Acetate 20 mM in ethyl alcohol.
2. Acid Reagent: A diluted acetic acid.
3. Sodium Colour Reagent: Potassium Fenrocyanide, non-reactive stabilizers, and fillers.
4. Sodium Standard: Sodium Chloride solution: 150 mEq/L of sodium.

Procedure

Filtrate Preparation:

1. Label test tubes: blank, standard and samples (plasma).
2. Pipette 1.0 ml of Filtrate Reagent to all tubes.
3. Add 50 µl of sample to all tubes and distilled water to the blank.
4. Shake all tubes vigorously and mix continuously for 3 minutes.
5. Centrifuge tubes at high speed (1,500G) for 10 minutes and test the supernatant fluids as described below, taking care not to disturb the protein precipitate.

Colour Development

1. Label test tubes corresponding to the above Filtrate tubes.
2. Pipette 1.0 ml Acid Reagent to all tubes.
3. Add 50 µl of Supernatant to respective tubes and mix.
4. Add 50 µl of Colour Reagent to all tubes and mix.
5. Zero spectrophotometer with distilled water at 550 nm.
6. Read and record absorbance of all tubes.

Calculations

Abs. = Absorbance

S = Sample

STD = Standard

(Abs. of Blank – Abs. of S) ×Conc. of STD (mEq/L) = Conc. of S (mEq/L)(Abs. of Blank – Abs. of STD)

2.4 Principle of Plasma Potassium Assay

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspenrion (Maruna, 1958). The turbidity of which is proportional in potassium concentration in the range of 2–7 mEq/L.

Reagent Contents

1. Potassium Reagent Sodium Tetraphenylboron 2.1 mM, preservatives and thickening agents.
2. Potassium Standard: Equivalent to 4 mEq/L

Procedure

1. Label test tubes: standard, control, samples. A blank is necessary
2. Pipette 1.0mL of Potassium Reagent to all tubes.
3. Add 0.01 mL (10µl) of samples to represent tubes. Mix and let sit at room temperature for 3 minutes.
4. After 3 minutes, set the wavelength of spectrophotometer to 500 nm, zero spectrophotometer with reagent blank. Read and record the absorbance of all tubes,

Calculations

Abs. = Absorbance

STD = Standard

Abs. of unknown x Conc. Of Std (mEq/L) = Potassium Conc. (mEq/L)
Abs. of STD

2.5 Statistical Analysis

Data obtained from the study are expressed as mean and standard deviation (mean ± SD) and analyzed by one way analysis of variance (ANOVA). P-values < 0.05 were taken as significant and not significant at P-values > 0.05. Data was presented in tables.

Chapter Three

3.0 Results

This chapter presents the statistical comparison of plasma sodium (mEq/L) and plasma potassium (mEq/L) concentrations across experimental groups; Normotensive/Non-Diabetic (N/ND), Hypertensive/Diabetic (H/D), Hydro-Methanol Fraction (HM 25mg/kg body weight) and Acetone Fraction (A 25mg/kg body weight).

Table 3: Plasma Sodium concentration across Treatment Groups

Group	Plasma Sodium (mEq/L)
N/ND	138.77 ± 2.33^{a}
H/D	164.35 ± 5.46^{b}
HM, 25 mg/kg	153.14 ± 11.02^{b}
A, 25mg/kg	129.96 ± 6.43^{c}

Values are expressed as mean ± standard deviation (SD)
Data with different alphabetical superscript are significantly different at $p \leq 0.05$ when compared hypertensive/diabetic group. Whereas data with similar alphabetical superscript are not significantly different.

Table 3 above presents the prophylactic effect of hydro-methanol and acetone leaf extract of *Simarouba glauca* on plasma Sodium ion levels of L-NAME and Streptozotocin induced hypertensive and diabetic male Wistar rats

The Hypertensive/Diabetic group (164.35 ± 5.46) had the highest value compared to Normotensive/Non-Diabetic (138.77 ± 2.33) and is significantly different at $P<0.05$. There's a non-significant ($P>0.05$) decrease in Hydro-methanol extract 25mg/kg (153.14 ± 11.02) when compared to Hypertensive/Diabetic group (164.35 ± 5.46). Acetone extract 25mg/kg (129.96 ± 6.43) significantly ($P<0.05$) decreased when compared to Hypertensive/Diabetic group (164.35 ± 5.46). A significant ($P<0.05$) increase was observed when Hydro-methanol extract 25mg/kg (153.14 ± 11.02) was compared with acetone extract 25mg/kg (129.96 ± 6.43).

Table 4. Plasma Potassium Concentrations across Treatment Groups.

Group	Plasma Potassium (mEq/L)
N/ND	11.66 ± 0.36^{a}
H/D	0.33 ± 0.23^{b}
HM, 25 mg/kg	0.32 ± 0.19^{b}
A, 25mg/kg	12.80 ± 0.71^{c}

Values are expressed as mean ± standard deviation (SD)
Data with different alphabetical superscript are significantly different at $p \leq 0.05$ when compared hypertensive/diabetic group. Whereas data with similar alphabetical superscript are not significantly different.

Table 4 above illustrates the prophylactic effect of hydro-methanol and acetone leaf extract of *Simarouba glauca* on plasma Potassium ion levels of L-NAME and Streptozotocin induced hypertensive and diabetic male Wistar rats.

The Hypertensive/diabetic (0.33 ± 0.23) when compared to Normotensive (11.66 ± 0.36) showed a significant ($P<0.05$) decrease. Hydro-methanol extract 25mg/kg (0.32 ± 0.19) showed a non-significant ($P>0.05$) decrease when compared to Hypertensive/diabetic (0.33 ± 0.23). There's a significant ($P<0.05$) increase in Acetone extract 25mg/kg (12.80 ± 0.71) when compared to Hypertensive/diabetic (0.33 ± 0.23). A significant ($P<0.05$) decrease was observed when hydro-methanol extract 25mg/kg (0.32 ± 0.19) was compared with acetone extract 25mg/kg (12.80 ± 0.71).

Chapter Four

4.0 Discussion and Conclusion

4.1 Discussion

The regulation of plasma sodium ion concentration is a critical aspect of electrolyte homeostasis, particularly in pathological conditions such as hypertension and diabetes. Table 3 highlights the differential effects of hydro-methanol and acetone leaf extracts of Simarouba glauca on plasma sodium ion levels across the experimental groups.

The normotensive group exhibited sodium levels of 138.77 ± 2.33 mEq/L, reflecting normal physiological conditions. In contrast, the hypertensive/diabetic group demonstrated a significant increase (164.35 ± 5.46 mEq/L, $P < 0.05$) in plasma sodium ion levels, indicative of disrupted electrolyte balance in hypertensive and diabetic states. This aligns with Osagie-Eweka *et al* (2018), who reported that hypertensive and diabetic conditions exacerbate sodium retention through mechanisms such as impaired renal sodium excretion and increased reabsorption in the distal nephron.

The administration of hydro-methanol extract at 25 mg/kg resulted in a slight reduction in sodium levels (153.14 ± 11.02 mEq/L) compared to the hypertensive/diabetic group. However, this decrease was not statistically significant ($P > 0.05$), suggesting that hydro-methanol extract may partially mitigate sodium retention but lacks robust efficacy at this dosage. Conversely, the acetone extract at 25 mg/kg exhibited a pronounced and significant reduction (129.96 ± 6.43 mEq/L, $P < 0.05$) in sodium levels compared to the hypertensive/diabetic group, demonstrating its potential to restore electrolyte balance effectively.

The significant difference between the hydro-methanol extract and the acetone extract groups highlights the superior efficacy of the latter in reducing plasma sodium levels. These findings corroborate previous studies, such as Patil and Gaikwad (2020), which identified specific bioactive compounds in *Simarouba glauca* with potent effects on electrolyte regulation.

The role of potassium in maintaining cellular function and cardiovascular health is well-documented, and its dysregulation is a hallmark of hypertensive and diabetic conditions.

Table 4 presents the effects of hydro-methanol and acetone extracts on plasma potassium levels across the experimental groups.

The normotensive group displayed potassium levels of 11.66 ± 0.36 mEq/L, reflecting a balanced electrolyte state. The hypertensive/diabetic group exhibited a substantial decline in potassium levels (0.33 ± 0.23 mEq/L), although this reduction was not statistically significant ($P > 0.05$) compared to the normotensive group. This finding aligns with previous research by Osagie-Eweka *et al* (2023), which highlighted the interplay between hypertension, diabetes, and potassium depletion, often mediated by renal tubular dysfunction.

Hydro-methanol extract administration resulted in a slight, non-significant decrease in potassium levels (0.32 ± 0.19 mEq/L) compared to the hypertensive/diabetic group. While this indicates a modest effect on potassium regulation, it suggests that hydro-methanol extract alone may not be sufficient to correct significant potassium imbalances. On the other hand, the acetone extract at 25 mg/kg induced a significant increase (12.80 ± 0.71 mEq/L, $P < 0.05$) compared to the hypertensive/diabetic group, demonstrating its capacity to restore potassium levels effectively.

The significant reduction in potassium levels observed in the hypertensive/diabetic group is consistent with findings by Moke *et al.* (2023), which identified the deleterious effects of combined hypertension and diabetes on electrolyte homeostasis. The pronounced efficacy of the acetone extract in restoring potassium levels may be attributed to its higher flavonoid and phenolic content, which enhance its antioxidant and nephroprotective properties (Patil and Gaikwad, 2020).

The observed differences in the effects of hydro-methanol and acetone extracts can be attributed to their distinct phytochemical compositions. According to Osagie-Eweka et al. (2018), the hydro-methanol extract contains a moderate concentration of bioactive compounds such as saponins, tannins, and alkaloids, which exhibit diuretic and anti-inflammatory properties. However, the acetone extract possesses higher levels of flavonoids and polyphenols, conferring superior antioxidant and electrolyte-modulating effects.

The significant reduction in sodium and increase in potassium levels observed in the acetone extract group may also be linked to its ability to modulate renal function. Studies have shown that flavonoids can enhance the activity of Na+/K+-ATPase, a critical enzyme in maintaining electrolyte gradients (Patil and Gaikwad, 2020). This mechanism may explain the pronounced effects of the acetone extract in restoring electrolyte balance in hypertensive and diabetic rats.

The findings of this study underscore the potential of *Simarouba glauca* extracts as complementary therapies for managing electrolyte imbalances in hypertension-diabetes comorbidity. The acetone extract, in particular, demonstrates significant efficacy in normalizing plasma sodium and potassium levels, suggesting its utility in mitigating the cardiovascular and renal complications associated with these conditions.

Further research is warranted to explore the molecular pathways underlying the observed effects, as well as to evaluate the long-term safety and efficacy of *Simarouba glauca* extracts in clinical settings. Additionally, the differential effects of hydro-methanol and acetone extracts highlight the importance of selecting the appropriate extraction method to optimize therapeutic outcomes.

4.2 Recommendations

1. Dose Optimization: Future studies should investigate the dose-dependent effects of *Simarouba glauca* extracts to identify the most effective therapeutic dose.
2. Mechanistic Studies: Detailed investigations into the molecular pathways modulated by the extracts will provide insights into their mode of action.
3. Clinical Trials: Translational studies are needed to evaluate the efficacy of *Simarouba glauca* extracts in human populations with hypertension-diabetes comorbidity.
4. Combination Therapies: Exploring the synergistic effects of *Simarouba glauca* extracts with standard antihypertensive and antidiabetic drugs may enhance therapeutic efficacy.

4.3 Conclusion

This study demonstrated the differential effects of hydro-methanol and acetone extracts of *Simarouba glauca* on plasma sodium and potassium ion levels in hypertensive and diabetic male Wistar rats. The acetone extract exhibited superior efficacy, highlighting its potential as a therapeutic agent for managing electrolyte imbalances in hypertension-diabetes comorbidity. These findings contribute to the growing body of evidence supporting the pharmacological applications of *Simarouba glauca* and underscore the need for further research to elucidate its therapeutic potential**.**

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Appendix

Appendix 1: Plasma Potassium at 500nm

S/N	OD VALUE	CAL. VLAUE (mEq/L)	GROUP
1	1.314	11.865	
2	1.246	11.251	Normotensive/
3	1.315	11.874	Non-diabetic
X ± SD		11.66 ± 0.36	
1	0.065	0.587	
2	0.018	0.163	Hypertensive/
3	0.027	0.244	Diabetic
X ± SD		0.33 ± 0.23	
1	0.012	0.108	
2	0.054	0.488	
3	0.043	0.389	HM 25mg/kg
X ± SD		0.32 ± 0.19	
1	1.368	12.352	
2	1.509	13.625	ACE 25mg/kg
3	1.377	12.433	
X ± SD		12.80 ± 0.91	

Appendix 2: Plasma Sodium at 550nm

S/N	OD VALUE	CAL. VLAUE (mEq/L)	GROUP
1	0.463	138.781	
2	0.436	142.089	Normotensive/
3	0.470	137. 109	Non-diabetic
4	0.470	137.109	
X ± SD		138.77 ± 2.33	
1	0.240	170.801	
2	0.309	160.693	Hypertensive/
3	0.299	162.158	Diabetic
X ± SD		164.35 ± 5.46	
1	0.295	162.744	
2	0.400	147.363	
3	0.300	162.012	HM 25mg/kg
4	0.447	140.478	
X ± SD		153.14 ± 11.03	
1	0.569	124.100	
2	0.534	128.947	ACE 25mg/kg
3	0.477	136.842	
X ± SD		129.96 ± 6.43	

Appendix 3: Plasma Sodium concentration across Treatment Groups

GROUP	PLASMA SODIUM (mEq/L)
N/ND	138.77 ± 2.33^{a}
H/D	164.35 ± 5.46^{b}
HM, 25 mg/kg	153.14 ± 11.02^{b}
A, 25mg/kg	129.96 ± 6.43^{c}

Values are expressed as mean ± standard deviation (SD)
Data with different alphabetical superscript are significantly different at $p \leq 0.05$ when compared hypertensive/diabetic group. Whereas data with similar alphabetical superscript are not significantly different

Appendix 4. Plasma Potassium Concentrations across Treatment Groups.

GROUP	PLASMA POTASSIUM (mEq/L)
N/ND	11.66 ± 0.36^{a}
H/D	0.33 ± 0.23^{b}
HM, 25 mg/kg	0.32 ± 0.19^{b}
A, 25mg/kg	12.80 ± 0.71^{c}

Values are expressed as mean ± standard deviation (SD)
Data with different alphabetical superscript are significantly different at $p \leq 0.05$ when compared hypertensive/diabetic group. Whereas data with similar alphabetical superscript are not significantly different.

Appendix 5: Dosage Administration Chart

Rat LD/Drug	Hypertensive/ Diabetic (Negative control) (ml)		Hypertensive/Diabetic + Hydro-Methanol fraction (25mg/kg) (ml)			Hypertensive/Diabetic + Acetone fraction (25mg/kg) (ml)		
	L-Name	STZ	HMFRAC	L-Name	STZ	ACEFRAC	L-Name	STZ
Head	0.38	0.34	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

Appendix 6: Urethane Dosage Administration Chart

	N/ND		H/D		HM 25		A25	
	Dose (g)	Dose (ml)	Dose (g)	Dose (ml)	Dose (g)	Dose (ml)	Dose (g)	Dose (ml)
H	0.218	2.18	0.155	1.55	0.143	1.43	0.174	1.74
G	0.237	2.37	0.116	1.16				
R_H			0.126	1.26	0.134	1.34	0.195	1.95
L_H	0.288	2.28			0.120	1.20	0.137	1.37
R_F	0.305	3.05	0.129	1.19	0.146	1.46		
L_F	0.161	1.61	0.135	1.35	0.126	1.26		