THE COMPARATIVE TREATMENT EFFECT OF 50mg/kg LISINOPRIL/GLIBERCLAMIDE OR 50mg/kg METHANOL FRACTION OF SIDA ACUTA/CLEOME RUTIDOSPERMA ON HEMATOPOIETIC MODULATION OF HYPERTENSIVE/DIABETIC WISTAR RATS.

BY

OGUNSHOLA PRECIOUS RUTH

LSC2103786

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (B.Sc.) IN BIOCHEMISTRY

OCTOBER, 2025.

CERTIFICATION

This is to certify that this report was presented by Precious Ruth OGUNSHOLA, with matriculation number LSC2103786 to the department of Biochemistry, Faculty of Life Sciences, University of Benin. Under the supervision of DR. O. OLASEHINDE meets the requirement contributing to the partial fulfillment of the award of Bachelor of science (B.sc) degree in Biochemistry and is approved for its contribution to knowledge and literary presentation.

DR. O. OLASEHINDE ----------

Project Supervisor Signature & Date

DR. S.I. OJEABURU ----------

Project Coordinator Signature & Date

PROF. E.C. ONYENEKE ----------

Head of Department Signature & Date

External Examiner ----------

Signature & Date

DEDICATION

This report is dedicated to the Almighty God, who has been guiding me in every of my step and made this work a success, and to my dear parents Mr. And Mrs. Philip Shola and siblings for their endless support and encouragement and To Dr. O. OLASEHINDE who held my hands and contributed majorly to its success.

ACKNOWLEDGEMENTS

My uttermost gratitude goes to God almighty, whose strength, provision and leading has kept me. My special thanks also to my Project Supervisor DR. O. OLASEHINDE for his support and patience throughout the experiment and report.

Special thanks go to my project supervisors, Dr. Samuel I. Ojeaburu, Dr ISD. E. Osagie-Eweka and Dr. Olasehinde for their patience, guidance, and constructive criticism, which greatly improved the quality of this research work.

Special thanks also to our lab technician DR. AKPE and MR. IBEH. Special thanks to the Head of Department Prof. E.C. Onyeneke, for the privilege to improve my learning by carrying out this research work.

A special thanks goes to my parents, Mr. Philip Shola and Mrs Comfort Shola, and my siblings Success Shola, Eunice Shola and Rejoice Shola for their words of encouragement and support.

I am indebted to my amazing friends, Illevare Gift, Kevin Okhale, Irabor Mike and Favour Charles who contributed to the success of this work.

Also a very big thank you to my project colleagues for their selfless efforts to ensure the success of this project work, as well as the entire project research team. God bless you all. I appreciate the entire University of Benin for the privilege to partake in this great citadel of knowledge.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

ABSTRACT

Hypertension and diabetes are major global health problems that often occur together, worsening cardiovascular and hematological complications. Standard therapies such as Lisinopril and Glibenclamide are effective but can alter normal blood parameters during prolonged use. This study compared the effects of Lisinopril/Glibenclamide (50 mg/kg) and the methanol fraction of *Sida acuta* and *Cleome rutidosperma* (50 mg/kg) on hematopoietic modulation in hypertensive and diabetic male Wistar rats. Hypertension and diabetes were induced using L-NAME and Streptozotocin. Rats were divided into seven groups, including normal, untreated hypertensive/diabetic, and treated groups. Treatments were administered orally for five weeks. Blood samples were analyzed for red and white cell indices, hemoglobin concentration, hematocrit, and platelet parameters. Data were analyzed using ANOVA at $p \leq 0.05$. Induction of hypertension and diabetes caused elevated monocyte and granulocyte counts, indicating systemic

inflammation. Both treatments significantly reduced monocyte levels ($p < 0.001$), but only the plant extract reduced granulocyte percentage ($p = 0.003$), suggesting stronger anti-inflammatory action. Lisinopril/Glibenclamide treatment lowered hemoglobin concentration ($p = 0.016$), while the *Sida acuta*/*Cleome rutidosperma* extract maintained normal red cell values, showing hematoprotective effects. The drug combination increased platelet count and size, whereas the plant extract preserved normal platelet balance. The methanol extract of *Sida acuta* and *Cleome rutidosperma* demonstrated comparable—and in some areas superior—hematopoietic and anti-inflammatory effects to Lisinopril/Glibenclamide. Its ability to maintain erythroid and immune balance suggests potential as a natural adjunct or alternative therapy for managing hypertension–diabetes comorbidity.

Keywords: Hypertension, diabetes, *Sida acuta, Cleome rutidosperma,* Lisinopril/Glibenclamide, L-NAME and Streptozotocin

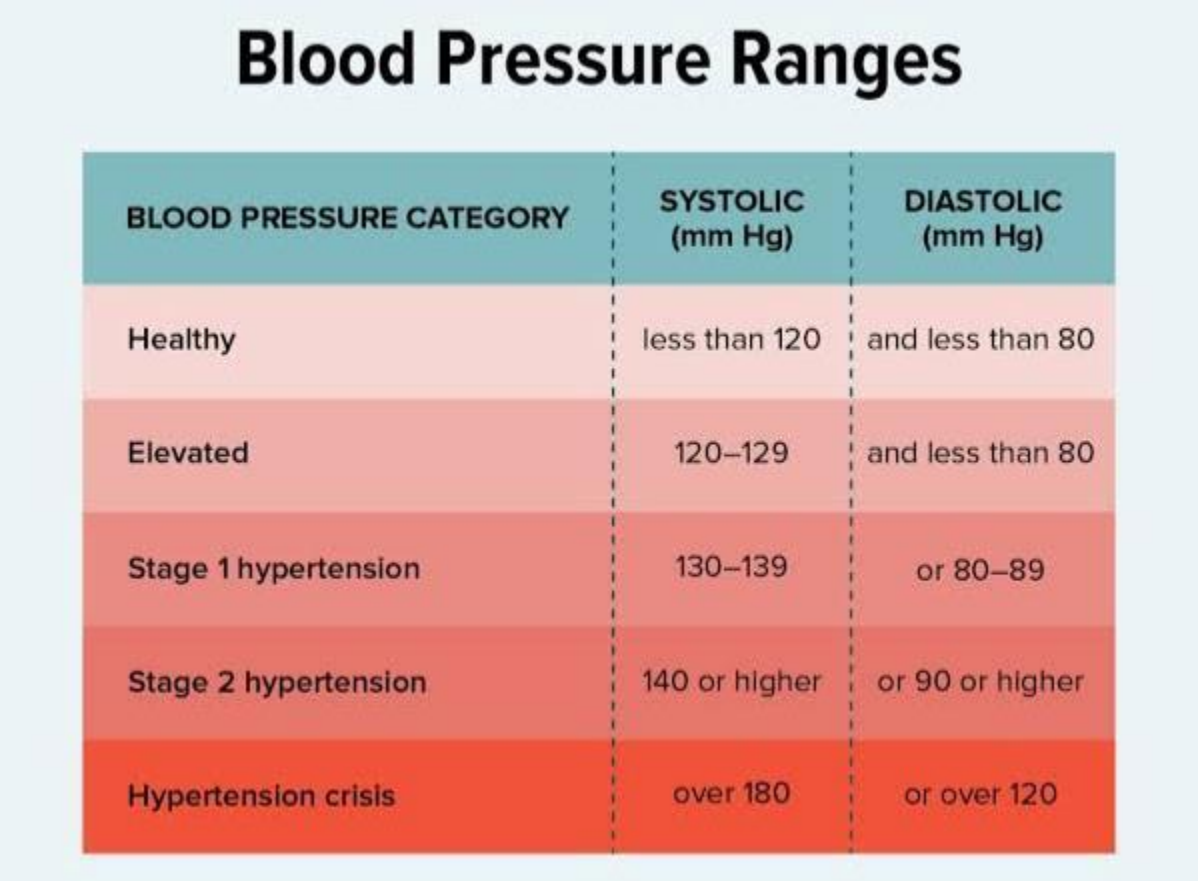
CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1.1 INTRODUCTION TO HYPERTENSION

Hypertension, commonly referred to as high blood pressure, occurs when the force of blood against the arterial walls remains consistently elevated. It is a widespread health condition affecting populations in both developed and developing nations, with its prevalence increasing with age. Blood pressure readings consist of two measurements: systolic pressure, which reflects the force exerted when the heart contracts, and diastolic pressure, which represents the pressure when the heart relaxes. Hypertension is a major health concern because it significantly increases the risk of severe complications, including myocardial infarction, stroke, heart failure, and premature death. Blood pressure levels are influenced by the heart's pumping effort and the resistance within blood vessels. The primary aim of hypertension treatment is to reduce these health risks while maintaining quality of life. Historically, hypertension was defined as a blood pressure of 140/90 mmHg or higher, but in 2017, the American College of Cardiology (ACC) and American Heart Association (AHA) updated their guidelines, lowering the threshold to 130/80 mmHg (Whelton *et al*., 2018). This revision greatly increased the number of individuals classified as hypertensive; in the United States, the prevalence rose from 31.9% to 45.6% under the new definition (Muntner *et al*., 2018). Among patients receiving treatment, 61% achieved the previous target of <140/90 mmHg, whereas only 46.6% met the new goal of <130/80 mmHg (Muntner *et al*., 2018). Antihypertensive medications that lower blood pressure and provide organ protection have been shown to significantly reduce the risks associated with hypertension. Hypertension is often called the "silent killer" because it is usually asymptomatic in its early stages while remaining the leading risk factor for cardiovascular disease, including left ventricular hypertrophy, aneurysm, congestive heart failure, chronic kidney disease (hypertensive nephropathy), hypertensive retinopathy, and stroke. The complications arise either from persistently elevated blood pressure, which leads to vascular and cardiac remodeling, or from accelerated atherosclerosis, which hypertension promotes over time. Mild to moderate primary hypertension may remain symptom-free for years. When present, common symptoms—such as headache, fatigue, dizziness, or facial flushing—are generally nonspecific. Characteristic early-morning suboccipital, pulsating headaches that improve as the day progresses have been described but are not definitive. Severe or accelerated hypertension may present with

neurological and systemic manifestations, including somnolence, confusion, visual disturbances, nausea, and vomiting.



Blood Pressure Ranges

BLOOD PRESSURE CATEGORY	SYSTOLIC (mm Hg)	DIASTOLIC (mm Hg)
Healthy	less than 120	and less than 80
Elevated	120–129	and less than 80
Stage 1 hypertension	130–139	or 80–89
Stage 2 hypertension	140 or higher	or 90 or higher
Hypertension crisis	over 180	or over 120

Figure 1.1: Blood pressure ranges (Sneha Rawat *et al.,* 2023)

1.1.2 INTRODUCTION TO DIABETES

Diabetes Mellitus (DM) is a chronic and progressive metabolic condition marked by consistently high blood glucose levels, a state known as hyperglycemia (Karamanou *et al*., 2016; American Diabetes Association, 2009). The global prevalence of diabetes has been increasing rapidly. In 2003, the World Health Organization (WHO) projected that by 2030, the number of adults with diabetes would double, rising from 177 million in 2000 to 370 million. In 2010, the worldwide estimated prevalence was 285 million, with predictions of an increase to 439 million by 2030. Diabetes is not a single disease but a group of related conditions, often described as “starvation in the midst of plenty”, as it occurs due to impaired insulin secretion, insulin resistance, or both (Ezeja *et al*., 2015). This disorder affects the body’s ability to produce or use insulin effectively,

leading to a range of acute and chronic complications. DM is among the most common endocrine disorders globally and poses a significant public health concern. Insulin, a hormone produced by beta cells in the pancreatic islets of Langerhans, facilitates the uptake of glucose into cells for energy use. In diabetes, beta cell dysfunction may reduce insulin secretion, or target tissues such as muscle and fat cells may become resistant to insulin, decreasing glucose absorption and metabolism. In both scenarios, blood glucose levels rise, resulting in hyperglycemia. When glucose accumulates in the bloodstream, it spills into the urine, and due to its osmotic effect, increased water loss follows, causing polyuria (frequent urination) and polydipsia (excessive thirst). The term diabetes mellitus reflects these symptoms: diabetes (Greek diabainein, "to pass through") indicates excessive urination, while mellitus (Latin, "sweetened with honey") refers to sugar in the urine. According to Janaka and Luigi (2016), hallmark symptoms of diabetes include dehydration, excessive urination, and persistent fatigue. Additional signs often observed in patients include pruritus (itching), excessive hunger, and unintended weight loss.

1.2 HISTORY OF THE PREVALENCE OF HYPERTENSION AND DIABETES

1.2.1 HISTORY OF HYPERTENSION

The recognition of hypertension is credited to Frederick Mahomed, who, in the early 1870s as a medical resident at Guy's Hospital, London, conducted blood pressure (BP) measurements in the general population. Collaborating with a watchmaker, Mahomed developed a spring-based instrument to assess radial pulse tension, effectively creating a portable adaptation of the sphygmograph that had been invented a decade earlier in France by Étienne-Jules Marey. While elevated BP was already associated with kidney disease and albuminuria, Mahomed identified a subset of individuals with high blood pressure without proteinuria, marking a pivotal discovery.

However, routine BP measurement did not become widespread until the 1890s, following Scipione Riva-Rocci's (1863–1937) invention of the inflatable BP cuff and mercury manometer. Later, Nikolai Sergeivich Korotkoff (1874–1920) introduced the use of auscultation to ensure complete arterial occlusion and accurately determine diastolic BP.

In the United States, Theodore Janeway and Harvey Cushing were instrumental in popularizing the sphygmomanometer. Early studies by Janeway and others reported that systolic BP above 140 mmHg was uncommon (0.5–1%) in adults under 65, while in those over 65, the threshold approached 160 mmHg. By 1906, insurance companies began acknowledging the increased

mortality risk associated with hypertension, a finding confirmed in a 1912 Metropolitan Life Insurance Company report. Over time, BP measurement became a standard clinical practice, and 140/90 mmHg was formally established as the diagnostic cutoff for hypertension. Subsequent research demonstrated that hypertension not only increases mortality but also predisposes patients to stroke, congestive heart failure (CHF), and chronic kidney disease (CKD).

CHF itself can reduce kidney perfusion, further worsening renal function in a condition known as cardiorenal syndrome. Over the past six to seven decades, understanding of hypertension has advanced dramatically compared to earlier years, though developing effective management strategies for this “silent killer” remains challenging. In the United States, hypertension control rates began declining around a decade ago, well before the COVID-19 pandemic, and cannot be attributed to it. Presently, only 48% of hypertensive individuals achieve a BP below 140/90 mmHg, and just 26% meet the current target of <130/80 mmHg. Key contributors to this gap include limited insurance support for extended physician consultations on asymptomatic hypertension and poor medication adherence among many patients

1.2.2 HISTORY OF DIABETES

Over the past two centuries, significant progress has been made in understanding the regulation of normal glucose metabolism. In the mid-19th century, Claude Bernard demonstrated that blood glucose levels are regulated not only through dietary carbohydrate absorption but also by the liver, which plays a pivotal role in glucose production from non-glucose precursors.

This foundational work was further advanced by subsequent researchers who identified key enzymes involved in glycogen synthesis and degradation, elucidated the influence of anterior pituitary hormones on glucose regulation and diabetes onset, and revealed the significance of reversible protein phosphorylation via protein kinases.

Furthermore, the discovery of cyclic AMP shed light on its essential role in hormonal signaling, particularly the actions of epinephrine and glucagon, both of which increase blood glucose levels and contribute to diabetic hyperglycemia (Sutherland & Rall, 1958).

A landmark moment in diabetes research occurred in 1889 when Joseph von Mering and Oskar Minkowski demonstrated that pancreatectomy in dogs resulted in fatal diabetes, thereby establishing the pancreas as a central organ in glucose regulation (von Mering & Minkowski,

1889). Building upon this finding, in 1910, Edward Albert Sharpey-Schafer proposed that diabetes was caused by the deficiency of a single pancreatic chemical, which he named insulin—derived from the Latin insula, referring to the islets of Langerhans (Sharpey-Schafer, 1910).

A decade later, in 1921, Frederick Banting and Charles Best successfully isolated insulin by reversing induced diabetes in dogs using pancreatic extracts from healthy animals. Together with James Collip and John Macleod, they purified insulin from bovine pancreases and became the first to administer it to a human patient, leading to a groundbreaking treatment that rapidly gained global acceptance (Banting *et al*., 1922).

The discovery of insulin spurred a surge of biochemical research and significant scientific breakthroughs beyond diabetes. For instance, Frederick Sanger earned the Nobel Prize in Chemistry for his pioneering work on protein sequencing, using insulin as the model protein (Sanger, 1955).

Despite these monumental advances, improvements in individual patient outcomes have not translated into equivalent success at a public health level. Diabetes prevalence continues to rise worldwide, reflecting the scale of this chronic disease as a global health challenge. In the United States, the number of diagnosed diabetes cases increased from 5.6 million to 20.9 million, representing a rise from 2.5% to 6.9% of the population, with nearly 27% of adults over 65 years now affected (Centers for Disease Control and Prevention, 2014).

Projections indicate that 1 in 3 U.S. adults may develop diabetes by 2050 if current trends persist (CDC, 2014). Furthermore, the American Diabetes Association estimated that the economic burden of diagnosed diabetes in the U.S. was $174 billion in 2007, highlighting the strain on healthcare systems (ADA, 2008).

Globally, the International Diabetes Federation (IDF) reported in 2013 that the prevalence of diabetes among adults was 8.3% (382 million cases), with 198 million men and 184 million women affected, predominantly between 40 and 59 years of age. The IDF predicts that by 2035, diabetes cases will exceed 592 million, reaching a 10.1% global prevalence (IDF, 2013).

1.3. TYPES OF HYPERTENSION AND DIABETES

1.3.1 TYPES OF HYPERTENSION

1.3.1.1 Essential (Primary) Hypertension:

Essential hypertension accounts for over 90% of hypertension cases and is diagnosed when no clear underlying cause can be identified. Although its precise etiology remains unknown, several risk factors have been strongly associated with its development. These include obesity, diabetes mellitus, a diet high in salt and fat, excessive alcohol intake, and cigarette smoking

1.3.1.2 Secondary Hypertension:

Secondary hypertension represents fewer than 10% of all hypertension cases and is characterized by elevated blood pressure caused by a specific, identifiable underlying condition. Clinically, it is suspected in patients presenting with markedly elevated BP and rapidly progressive end-organ damage, such as grade 3 or 4 hypertensive retinopathy, renal impairment with proteinuria, or hypertensive encephalopathy. Without appropriate management, this condition can be fatal within a few months.

1.3.1.3 Malignant or Accelerated Hypertension:

Malignant (or accelerated) hypertension is a severe clinical syndrome that can develop as a complication of hypertension from any cause. It is characterized by rapidly progressive microvascular injury, including fibrinoid necrosis of arteriolar walls and intravascular thrombosis, leading to significant tissue ischemia and organ dysfunction.

1.3.2 TYPES OF DIABETES

1.3.2.1 TYPE 1 DIABETES MELLITUS (Autoimmune type 1 diabetes)

Type 1 diabetes mellitus (T1DM) accounts for approximately 5–10% of all diagnosed cases of diabetes and is primarily caused by the destruction of pancreatic β-cells (American Diabetes Association, 2014). It is the predominant form of diabetes in children and adolescents, representing 80–90% of cases in these age groups.

According to the International Diabetes Federation (IDF), in 2013, there were an estimated 497,100 children under the age of 15 living with type 1 diabetes worldwide, with approximately 78,900 new diagnoses reported annually. The pathogenesis of T1DM is largely autoimmune, involving a T-cell–mediated inflammatory response (insulitis) and a humoral B-cell response that collectively lead to β-cell destruction.

The hallmark of T1DM is the presence of autoantibodies targeting pancreatic islet cells, which can often be detected months to years prior to disease onset. These autoantibodies include:

- Islet cell autoantibodies (ICA)
- Insulin autoantibodies (IAA)
- Autoantibodies to glutamic acid decarboxylase (GAD65)
- Autoantibodies to protein tyrosine phosphatase (IA-2, IA-2β)
- Autoantibodies to zinc transporter protein (ZnT8A).

Type 1 diabetes exhibits a strong genetic predisposition, with susceptibility linked to HLA-DR and HLA-DQ alleles, some of which are predisposing while others are protective. This autoimmune-mediated form of diabetes is characterized by a near-complete absence of insulin secretion and occurs most frequently in children and adolescents. In addition to genetic factors, numerous environmental triggers have been implicated in T1DM development. Viral infections such as congenital rubella, enterovirus, rotavirus, herpes virus, cytomegalovirus, endogenous retrovirus, and Ljungan virus have been proposed as potential contributors.

Type 1 diabetes often has a sudden onset and presents with classic hyperglycemic symptoms, including polydipsia, polyuria, polyphagia, enuresis, extreme fatigue, sudden weight loss, slow wound healing, recurrent infections, blurred vision, and severe dehydration. In children, it can rapidly progress to diabetic ketoacidosis (DKA), making early detection critical. Symptoms are typically more severe in children than in adults.

Patients with autoimmune T1DM are also predisposed to other autoimmune disorders, including Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac disease, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

1.3.2.2 TYPE 2 DIABETES MELLITUS

In patients with type 2 diabetes mellitus (T2DM), insulin resistance in target tissues such as skeletal muscle, adipose tissue, and the liver creates an increased demand for insulin production to maintain glucose homeostasis. However, this heightened insulin demand cannot be met effectively due to functional defects in pancreatic β-cells. Over time, β-cell function continues to deteriorate, and insulin secretion progressively declines, leading to worsening hyperglycemia. In

some individuals, this decline may result in a transition from insulin independence to insulin dependence.

Despite this progressive β-cell failure, most individuals with T2DM do not become fully insulin-dependent, as insulin production generally persists, and absolute insulin depletion is rare. This feature distinguishes T2DM from type 1 diabetes mellitus (T1DM), where autoimmune β-cell destruction results in absolute insulin deficiency. Furthermore, ketoacidosis is uncommon in T2DM, and there is no autoimmune-mediated destruction of β-cells in most cases resembling T2DM also exhibit autoantibodies, such as islet cell antibodies or anti-GAD65 antibodies. This group is classified as having latent autoimmune diabetes in adults (LADA), a hybrid form of diabetes with features of both T1DM and T2DM.

1.3.3 RELATIONSHIP BETWEEN LONG TERM HYPERTENSION AND DIABETES.

There is considerable pathophysiological overlap between diabetes mellitus (DM) and hypertension (HTN), as both conditions share multiple etiological factors and mechanistic pathways (Bernard *et al*., 2012). This interrelationship has been well documented in clinical and molecular studies, with several key contributors identified:

1.3.3.1 Obesity:

Obesity plays a central role in the development of both diabetes and hypertension. It is widely regarded as the consequence of a dysregulated hypothalamic feeding center, an imbalance between caloric intake and energy expenditure, and genetic predispositions. Individuals with obesity have a significantly elevated risk of developing type 2 diabetes mellitus (T2DM) and hypertension. Given its strong association with both conditions, it is unsurprising that diabetes, obesity, and hypertension share susceptibility genes, further reinforcing their interconnected nature (Bernard *et al*., 2012).

1.3.3.2. Inflammation and Oxidative Stress:

Chronic low-grade inflammation is a hallmark of both diabetes and hypertension, and even periodontitis has been identified as a latent risk factor for metabolic and cardiovascular diseases, including T2DM, HTN, and metabolic syndrome. In many respects, both diabetes and hypertension can be conceptualized as chronic inflammatory disorders. Elevated levels of inflammatory biomarkers such as C-reactive protein (CRP) are consistently observed in patients

with diabetes, hypertension, and metabolic syndrome, and they also predict future disease development. Moreover, oxidative stress has been highlighted through gene regulatory network analyses as a central molecular mechanism in the pathogenesis of these conditions, serving as a shared mechanistic cascade linking inflammation, vascular dysfunction, and metabolic abnormalities

1.3.3.3. Insulin Resistance:

Insulin resistance, defined as a diminished cellular response to normal insulin levels in skeletal muscle, adipose tissue, liver, and cardiovascular tissue, is another key factor linking hypertension and diabetes. This condition is associated with impaired insulin signaling, pro-inflammatory states, and reduced fibrinolytic activity. The majority of patients with T2DM are insulin resistant, and approximately 50% of individuals with essential hypertension also exhibit this abnormality. Thus, insulin resistance represents a critical mechanistic bridge between hypertension and diabetes, explaining their frequent coexistence.

1.4 INTRODUCTION TO HYPERTENSION AND DIABETES UNDER CO-MORBIDITY DISORDER.

Comorbidity refers to the coexistence of two or more chronic diseases or conditions within the same individual, and it significantly increases the global burden of non-communicable diseases (NCDs). Among the most clinically relevant comorbidities, hypertension is highly prevalent in individuals with diabetes mellitus (DM) and is a leading contributor to cardiovascular morbidity, disability, and premature mortality (American Diabetes Association [ADA], 2022). This association is particularly concerning because hypertension accelerates the development of both macrovascular and microvascular complications in diabetes, thereby compounding disease severity and treatment complexity (Cheung *et al*., 2015).

Pharmacological management of hypertension in diabetic patients often prioritizes angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) as cost-effective first-line therapies, provided no contraindications exist. When additional blood pressure (BP) control is required, calcium channel blockers or thiazide-type diuretics serve as effective second-line agents. Notably, hypertension is twice as common in patients with diabetes as in those without the condition (Cheung *et al*., 2015).

This increased prevalence is thought to reflect, at least in part, the vascular and renal consequences of insulin resistance, a hallmark of type 2 diabetes mellitus (T2DM). Interestingly, emerging evidence indicates that the relationship between hypertension and diabetes is bidirectional. While diabetes increases the risk of hypertension, glucose metabolism abnormalities are also more frequently observed in hypertensive patients, suggesting shared pathogenic mechanisms (Ferrannini & Cushman, 2012). Clinical trials consistently demonstrate that BP reduction leads to substantial decreases in cardiovascular and microvascular morbidity and mortality; however, optimal BP control remains challenging, as a large proportion of patients with diabetes continue to have uncontrolled hypertension despite treatment (Cheung *et al*., 2015). The pathophysiology of hypertension in diabetes involves complex interactions between the autonomic nervous system, immune dysregulation, and overactivation of the renin-angiotensin-aldosterone system (RAAS), compounded by environmental influences such as physical inactivity and excess caloric intake (Sowers *et al*., 2001; Montezano & Touyz, 2012). These factors promote adiposity, which worsens insulin resistance and triggers a cascade of oxidative stress, vascular inflammation, and endothelial dysfunction, largely mediated by reduced nitric oxide (NO) bioavailability (Muniyappa & Sowers, 2013). Such vascular alterations lead to arterial stiffness and persistent BP elevation, heightening the risk of cardiovascular disease (CVD). Additionally, patients with diabetes often experience renal impairment and cardiac comorbidities, which further limit their tolerance to intensive antihypertensive regimens (Cheung *et al*., 2015).

1.4.1 CHALLENGES ASSOCIATED WITH CO-EXISTENCE OF HYPERTENSION AND DIABETES

1.4.1.1 Stroke Risk:

Persistent elevation of blood pressure weakens cerebral blood vessels, making them highly susceptible to structural damage and significantly increasing the risk of stroke. Stroke is a leading cause of cognitive impairment, physical disability, and multiple comorbidities, placing a substantial burden on patients and healthcare systems. Notably, hypertension is the most significant modifiable risk factor for stroke, emphasizing the importance of early diagnosis and effective BP control in stroke prevention.

1.4.1.2. Kidney Damage:

Hypertension exerts profound effects on the kidneys by damaging their delicate glomerular filtration units, ultimately leading to chronic kidney disease (CKD). If left untreated, CKD can progress to end-stage renal disease (ESRD), necessitating long-term hemodialysis or renal transplantation. This progression not only compromises quality of life but also imposes substantial economic strain on global healthcare systems.

1.4.1.3. Metabolic Syndrome:

Hypertension frequently coexists with other metabolic disturbances, including central obesity, hyperglycemia, and dyslipidemia, collectively referred to as metabolic syndrome. This clustering of risk factors significantly heightens the likelihood of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD), underscoring the interrelated nature of metabolic and vascular pathologies.

1.4.1.4. Diabetic Complications:

The coexistence of hypertension and diabetes substantially amplifies the risk of microvascular complications, such as diabetic nephropathy, retinopathy, and neuropathy. Elevated BP accelerates vascular injury in diabetic patients, further complicating disease management and prognosis. The combined burden of hypertension and diabetes thus represents a major contributor to long-term morbidity and mortality

1.4.2 UNDERLINED CONDITIONS ASSOCIATED WITH CO-EXISTENCE OF HYPERTENSION AND DIABETES

1.4.2.1. Premature Vascular Aging:

Alterations in arterial lumen elasticity significantly influence blood flow, as even minimal reductions in lumen diameter can exponentially increase vascular resistance. Individuals with hypertension frequently exhibit structural and functional vascular changes, particularly in the small arteries and arterioles, that disrupt lumen integrity. In patients with diabetes, hypertension-driven vascular remodeling, low-grade inflammation, fibrosis, and arterial stiffening are common maladaptive responses to persistently elevated blood pressure.

Consequently, diabetic patients experience accelerated vascular aging, characterized by impaired endothelial-mediated vasodilation, heightened vascular smooth muscle contraction, and progressive arterial stiffness. These maladaptive changes not only facilitate the development of hypertension but also intensify its deleterious impact on vascular structure and function.

1.4.2.2. Autonomic Nervous System Dysregulation:

The autonomic nervous system (ANS) is central to blood pressure regulation, with both sympathetic and parasympathetic branches playing critical roles. Sympathetic overactivation elevates blood pressure by increasing heart rate, myocardial contractility, peripheral vascular resistance, and renal fluid retention, while reduced parasympathetic outflow exacerbates this imbalance, creating a state of sympathetic dominance (Naha, 2021).

Dysregulation of these pathways is frequently associated with central obesity, insulin resistance, and sleep disturbances, often resulting in resistant hypertension and concurrent renin-angiotensin-aldosterone system (RAAS) activation. Furthermore, heightened sympathetic activity contributes to insulin resistance and elevates the risk of type 2 diabetes mellitus (T2DM). Conversely, autonomic dysfunction seen in diabetes further exacerbates hypertension, creating a reciprocal pathogenic cycle. Evidence of this relationship is supported by studies showing that renal denervation, which interrupts central sympathetic output, improves insulin sensitivity, glycemic control, and blood pressure regulation.

1.4.2.3. Renal Dysfunction:

Hypertension is well-established as a major risk factor for chronic kidney disease (CKD) in diabetic populations; however, diabetic nephropathy also plays a crucial role in the pathogenesis of hypertension. This bidirectional relationship is especially evident in type 1 diabetes mellitus (T1DM) patients without pre-existing hypertension, where microalbuminuria is often observed before hypertension onset. As CKD progresses, the prevalence of hypertension increases, reaching nearly 90% in T1DM patients with end-stage renal disease.

Mechanistic explanations include volume expansion due to enhanced renal sodium retention, endothelial dysfunction leading to peripheral vasoconstriction, hyperactivation of RAAS, endothelin-1 upregulation, and reduced nitric oxide bioavailability. These pathophysiological

processes highlight the interdependence between renal impairment and hypertension in diabetes progression.

1.5 TREATMENT OF HYPERTENSION AND DIABETES

Managing hypertension in individuals with diabetes presents notable difficulties, especially when they exhibit resistant hypertension—defined as persistently elevated blood pressure (BP > 140/90 mm Hg) despite optimal lifestyle changes and the combined use of a diuretic plus two different classes of antihypertensive medications at proper doses.

1.5.1. Lifestyle Modifications

Lifestyle interventions may suffice as sole therapy when BP is below 140/90 mm Hg. However, for systolic BP (SBP) ≥ 140 mm Hg or diastolic BP (DBP) ≥ 90 mm Hg, combining lifestyle changes with pharmacologic treatment is advised. Although modest in effect, lifestyle adjustments can reduce BP by approximately 5–10 mm Hg. Recommended modifications include:

Limiting sodium intake to under 1.5 g/day

Consuming 8–10 servings of fruits and vegetables each day

Including 2–3 servings of low-fat dairy daily

Engaging in regular aerobic physical activity (e.g., brisk walking for 30 minutes per day)

Reducing excess body weight

Avoiding excessive alcohol—no more than two standard drinks per day for men (≈ 30 ml ethanol) and one for women (≈ 15 ml ethanol)

1.5.2. Angiotensin-Converting Enzyme (ACE) Inhibitors

ACE inhibitors prevent the conversion of angiotensin I to angiotensin II, thereby reducing peripheral vascular resistance and lowering BP. Notably, they selectively dilate efferent arterioles in the kidneys, leading to reduced intraglomerular pressure. This property makes them particularly effective in slowing the progression of proteinuria, offering a renal-protective advantage over many other antihypertensive classes.

1.5.3. Diuretics

Diuretics initially lower BP by promoting renal sodium excretion and reducing plasma volume. Over time, although volume may normalize, the BP-lowering effect persists due to a reduction in peripheral vascular resistance. Thiazide diuretics, such as hydrochlorothiazide (HCTZ) and chlorthalidone, are effective in patients with mild to moderate hypertension and an estimated glomerular filtration rate (eGFR) above 50 ml/min/1.73 m^2.

In patients with eGFR below 30 ml/min/1.73 m^2, loop diuretics or a loop-thiazide combination are more effective (Ajibola *et al.,* 2018). Adverse effects can include hypokalemia, hyperglycemia, and hyperuricemia, which can often be mitigated by combining diuretics with an ACE inhibitor, ARB, potassium-sparing diuretic, or aldosterone antagonist.

1.5.4. Calcium Channel Blockers (CCBs):

CCBs are divided into two subclasses:

Dihydropyridines (DHPs) (e.g., amlodipine, felodipine, isradipine, nicardipine, nifedipine), which primarily reduce BP through peripheral vasodilation without notably affecting cardiac conduction or contractility.

Non-DHPs (NDHPs) (e.g., verapamil, diltiazem), which lower BP modestly but are also influential on cardiac rate and electrical conduction, making them particularly useful in treating arrhythmias.

1.6 LITERATURE REVIEW

1.6.1 EFFICACY OF MEDICINAL PLANTS IN TREATMENT OF HYPERTENSION AND DIABETES

Medicinal plants have played a central role in human health for centuries, valued for both their nutritional and therapeutic properties. For thousands of years, natural sources have been harnessed for medicinal purposes, and numerous modern pharmaceuticals have been derived from these natural compounds. Historically, plant isolations were often guided by their traditional therapeutic applications. For instance, within Indian traditional medicine, diuretics and sedatives have long been employed in the treatment of blood disorders, bile-related illnesses, liver conditions, and neurological ailments.

In Nigeria, a wide variety of medicinal plants is traditionally used to manage hypertension and other chronic diseases. However, most studies examining the mechanisms of action of these plants' phytochemical constituents on hypertension have been conducted outside Nigeria.

Due to variations in plant composition—influenced by factors such as species diversity, soil composition, fertilizer use, and environmental conditions—scholars have emphasized the need for locally conducted research. Such studies would strengthen the scientific basis for the therapeutic efficacy of these plants in managing hypertension and other diseases, while providing region-specific data to validate traditional practices.

Medicinal plants have been a vital resource in managing diabetes, offering natural therapeutic options for this chronic condition. According to the World Health Organization, about 80% of the global population relies on herbal medicines for primary healthcare, especially in areas with limited access to conventional treatments. These plants have anti-diabetic properties that help restore pancreatic function, enhance insulin secretion, or inhibit glucose absorption, thereby regulating blood sugar levels.

Plant-derived antioxidants can also help prevent or mitigate diabetes-related complications, such as oxidative stress. The World Health Organization recognizes the significance of hypoglycemic agents derived from plants used in traditional medicine. These natural compounds offer a complementary or alternative approach to conventional diabetes treatments, highlighting the importance of integrating traditional knowledge with modern healthcare practices.

By combining traditional knowledge with modern healthcare, medicinal plants can play a crucial role in improving patient outcomes and managing diabetes more effectively. This approach emphasizes the potential of natural therapies in addressing the complexities of diabetes care.

1.7 DISEASES AND CONDITIONS ASSOCIATED WITH *SIDA ACUTA*, *CLEOME RUTIDOSPERMA* AND *HUNTERIA UMBELLATA*.

1.7.1.*Sida acuta* Burm. f (Malvaceae):

one of the herbaceous indigenous plants used for many application to treat specific health issues. *Sida acuta* is recognized as astringent, tonic, and beneficial in urinary disorders.

Figure 1.2 : *Sida acuta* plant (University of Benin Anatomy back gate)
Voucher number: UBH- S454

Botanical Name: *Sida acuta*. English Name: Common Wireweed, Broomweed, Fanpetals Yoruba Name: Ìsẹ̀tù (or Ishẹtu) Igbo Name: Mkpuru-ala Hausa Name: Karkashin-kwarya Edo (Benin) Name: Erhinvbon-ofo or Amu-egbe (though Erhinvbon-ofo seems more common for this specific plant). The *Sida acuta* species is widely recognized for its adaptogenic and immunomodulatory properties, making it valuable as a general nutritive tonic that is traditionally believed to promote longevity. Its roots have been historically utilized in the treatment of tuberculosis, cardiovascular conditions, respiratory ailments, and trauma-related disorders (Ramesh Babu H.N., et al 2024). Scientific studies have further demonstrated that *Sida acuta* extracts possess antifungal activity against a range of pathogens, including Candida albicans, Aspergillus flavus, Aspergillus niger, Sorghum smut (Sporisorium sorghi), lead smut, and long smut (Tolyposporium ehrenbergii). Consequently, *Sida acuta* is considered a promising antimicrobial and antifungal agent with significant potential in regulating pathogenic fungal growth in commercially valuable crops, such as Sorghum species. These findings highlight the

importance of isolating bioactive phytochemicals from medicinal plants to maximize their therapeutic applications.

1.7.2 Cleome rutidosperma DC:

commonly referred to as the “fringed spider flower” or “purple cleome,” is a medicinally significant species from the Cleomaceae family. In traditional Ayurvedic medicine, different parts of this plant—including its leaves, roots, and seeds—have been employed for managing pain, muscular spasms, and dermatological conditions. In African ethnomedicine, the leaf sap of C. rutidosperma is widely used to relieve symptoms of deafness and earaches, while its leaf extracts have been applied as a treatment for convulsions. These traditional applications demonstrate its multifaceted therapeutic potential and warrant further phytochemical and pharmacological investigation.

Figure 1.3: *Cleome rutidosperma* plant (University of Benin football field).
Voucher Number: UBH-C148.

Botanical Name: Cleome rutidosperma English Name: Fringed Spider Flower or Purple Cleome Yoruba Name: Àjéfò Igbo Name: Nkpatu-ata Hausa Name: Gidan-kifi Benin (Edo State) Name: Ebe-ebo (also sometimes called Ebe ewure)

1.7.2.1 Antioxidant Activity:

Extensive research has demonstrated that *Cleome rutidosperma* possesses significant antioxidant, anti-inflammatory, antinociceptive, anti-tumor, and antimicrobial properties. These therapeutic effects are attributed to the plant's diverse bioactive compounds, including phenolics, alkaloids, tannins, terpenoids, and saponins. Studies have shown that ethanol extracts of the aerial parts of C. rutidosperma, along with fractions prepared using diethyl ether, petroleum ether, ethyl acetate, and butanol, exhibit potent antioxidant activity in a dose-dependent manner. Notably, these activities were comparable to reference antioxidants such as ascorbic acid and α-tocopherol.

Further analysis suggested that flavonoids, tannins, and terpenoids may be primarily responsible for these antioxidant effects (Trang, 2023). Additional studies have confirmed that both ethanol and aqueous extracts of C. rutidosperma effectively scavenge free radicals, including ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), nitric oxide, and hydroxyl radicals, demonstrating antioxidant potential equivalent to that of ascorbic acid (Trang, 2023).

1.7.2.2 Anti-inflammatory Activity:

In vitro studies have revealed that 70% ethanol extract of C. rutidosperma demonstrates an inhibitory effect on bovine albumin denaturation (IC50 = 201.58 μg/mL), although this activity was notably weaker than that of diclofenac (IC50 = 31.32 μg/mL) (Trang, 2023). This anti-inflammatory potential has been linked to several phenolic compounds, such as ferulic acid, sinapic acid, ellagic acid, myricetin, and quercetin.

Furthermore, the plant has shown anti-neuroinflammatory properties, as ethanol extracts inhibited nitric oxide (NO) release and iNOS (inducible nitric oxide synthase) expression in LPS-stimulated BV2 microglial cell lines. These findings highlight the therapeutic promise of C. rutidosperma in the regulation of inflammatory pathways (Trang, 2023).

1.7.2.3 Anticancer Activity:

Methanol extracts of C. rutidosperma leaves have been evaluated for cytotoxic activity against HepG2 liver cancer cells, revealing a dose-dependent inhibitory effect. At a concentration of 100 µg/mL, the extract demonstrated notable cytotoxic potential, suggesting its possible role as a natural anticancer agent (Trang, 2023).

1.7.2.4 Antidiabetic Activity:

Studies have also established that C. rutidosperma exhibits antidiabetic properties. In vitro assays demonstrated that its extract exerts α-amylase inhibitory activity comparable to that of diclofenac (Trang, 2023). In vivo investigations in streptozotocin-induced diabetic rats indicated that aqueous leaf extracts enhanced hepatic glucokinase activity and inhibited glucose-6-phosphatase, thereby reducing gluconeogenesis. Further studies in alloxan-induced diabetic rats showed that methanol leaf extracts (200 mg/kg) produced superior glycemic control compared to glibenclamide, supporting the plant's hypoglycemic potential (Trang, 2023).

1.8 AIMS AND OBJECTIVES

This study aims to evaluate the pharmacological effectiveness of three medicinal plants—Cleome rutidosperma, Sida acuta, and Hunteria umbellata—in managing hypertension and diabetes, with a particular focus on their potential to protect the pancreas in male Wistar rats. With the increasing prevalence of these metabolic conditions worldwide, especially in resource-limited settings like Nigeria, this research seeks to provide scientific support for the traditional use of these plants. By examining their effects on blood pressure and blood glucose regulation, the study intends to offer valuable insights into their therapeutic potential, safety, and possible role in modern healthcare.

1.8.1 OBJECTIVES OF THE RESEARCH

1. To assess the antihypertensive properties of Cleome rutidosperma, Sida acuta, and Hunteria umbellata extracts in male Wistar rats, with a focus on their capacity to influence blood pressure levels and related cardiovascular metrics.

2. To evaluate the antidiabetic effects of these plant extracts by analyzing their impact on blood glucose control, insulin sensitivity, and lipid metabolism in rats with experimentally induced diabetes.
3. To explore the pancreatic-protective properties of the chosen plant extracts, emphasizing histological examinations of pancreatic tissues to ascertain whether these plants can prevent or alleviate β-cell injury and dysfunction.
4. To compare the effectiveness of the plant extracts from the three species, identifying both similarities and differences in their pharmacological effects.
5. To enhance scientific understanding by substantiating the traditional applications of these plants in the treatment of metabolic disorders, thus bridging the divide between ethnomedicine and contemporary pharmacology.

Through these aims, the research is anticipated to underscore not only the therapeutic potential of Cleome rutidosperma, Sida acuta, and Hunteria umbellata but also the necessity for additional investigations into their bioactive compounds, mechanisms of action, and clinical relevance.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Animals

Adult male Wistar rats were fed with pellets, given water, and were acclimatized with a restrainer daily (for two weeks). With the cages always clean in the morning before feeding the rats at the animal house of the Department of Biochemistry, University of Benin.

2.2 Drugs

L-NAME (Nitro-L arginine methyl ester) and streptozotocin (STZ) were the drugs used in the induction of hypertension and diabetes respectively. While Lisinopril and Glibenclamide were used for treatment.

2.3 MATERIALS USED

Materials were made used of such as;

Universal bottles, Glucometer, Restrainer, 1mL, 2mL & 5mL syringes, Plasma sodium, Plasma calcium, Sample bottles, Sensitive balance, Dissecting set, Spectrophotometer, Methanol, Picric acid, Foil paper, Chicken Feed (Happy Chicken Feed Grower Pellets), Test tubes, Distilled and sterile water, Sodium citrate, Formal saline, Paper towel, Pastor's pipette, Micropipettes and EDTA bottles.

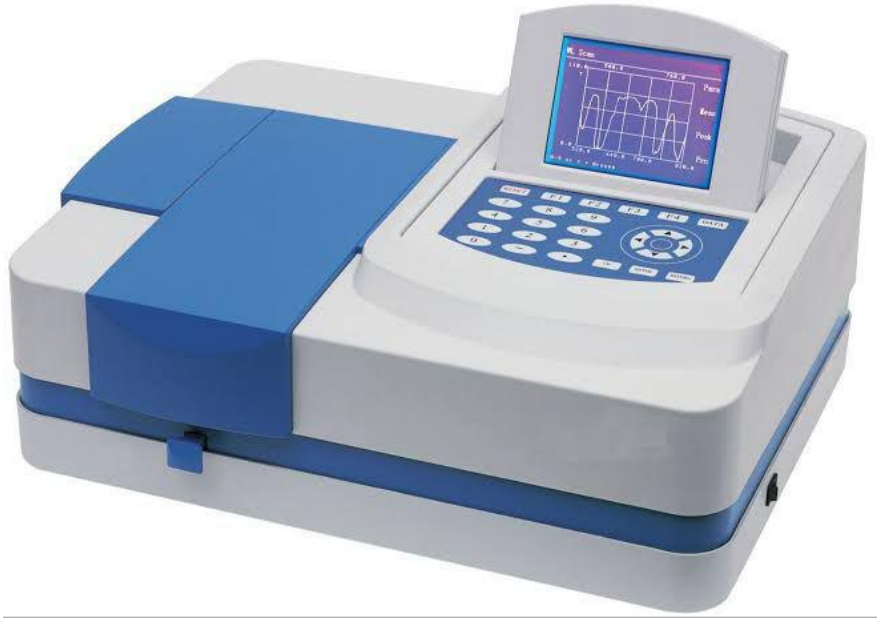


Figure 2.1. Spectrophotometer (Google)

Figure 2.2. Restrainer (BioPteB Laboratory)

Figure 2.3. Water Bath (BioPteB Laboratory)

Figure 2.4. Sensitive weight balance (Google)

Figure 2.5. Centrifuge (BioPteB Laboratory)

Figure 2.6. Glucometer and Test strips (BioPteB Laboratory)

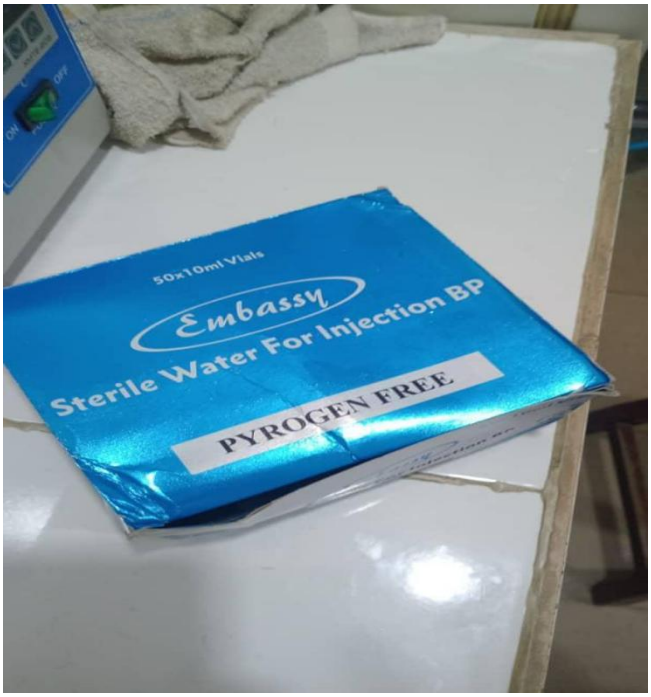


Figure 2.7. Sterile water (BioPteB Laboratory)

Figure 2.8. Urethane (BioPteB Laboratory)

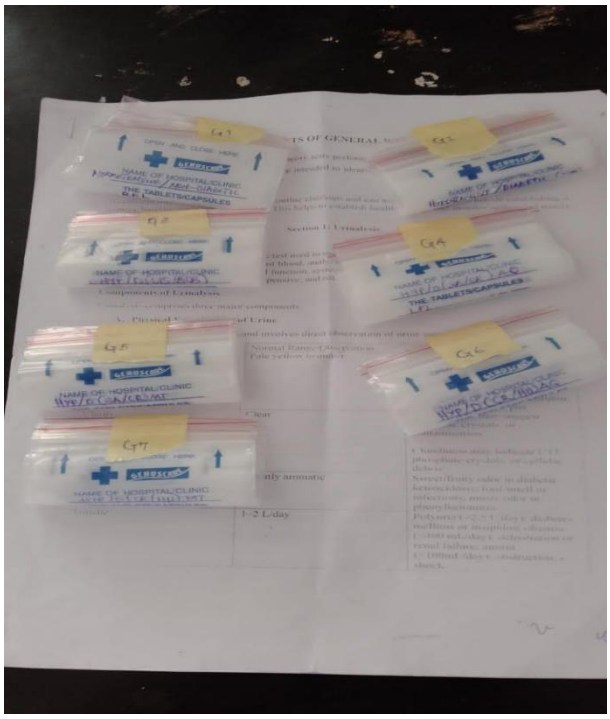


Figure 2.9. Tissue bags (BioPteB Laboratory)

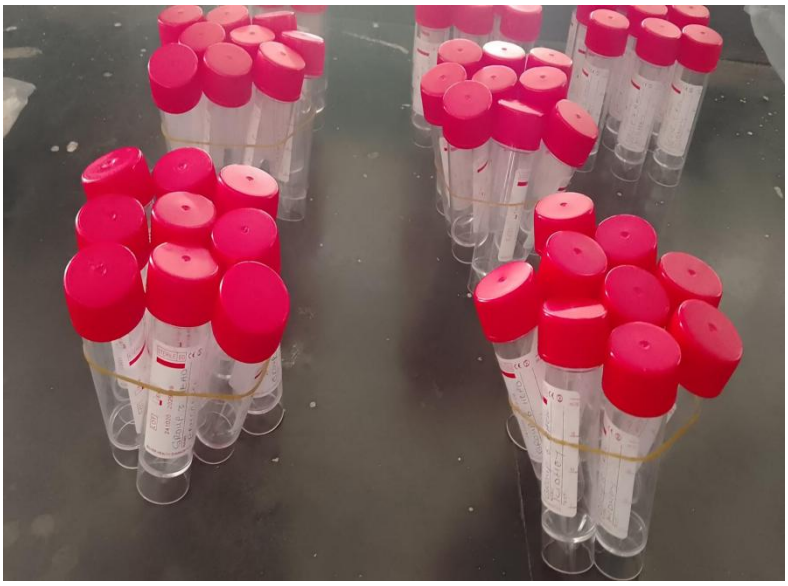


Figure 2.10. Universal Bottle for Histology (BioPteB Laboratory)

Figure 2.11. Gliberclamide (BioPteB Laboratory)

Figure 2.12. Lisinopril (BioPteB Laboratory)

Figure 2.13 MRBP System (BioPteB Laboratory)

2.4 EXPERIMENTAL DESIGN

The animals were categorized into seven distinct groups, each containing not less than 4 to 6 animals.

Group 1 consists of the Normotensive/Non-diabetic group (positive control).

Group 2 is the Hypertensive/Diabetic group (negative control).

Group 3 includes Hypertensive/Diabetic groups treated with Glibenclamide and Lisinopril at a dosage of 50 mg/kg.

Group 4 comprises Hypertensive/Diabetic groups treated with an aqueous extract of Sida acuta and Cleome rutidosperma, also at 50 mg/kg.

Group 5 consists of Hypertensive/Diabetic groups treated with a methanol extract of Sida acuta and Cleome rutidosperma, at the same dosage of 50 mg/kg.

Group 6 includes Hypertensive/Diabetic groups treated with an aqueous extract of Cleome rutidosperma and Hunteria umbellata, administered at 50 mg/kg.

Group 7 consists of Hypertensive/Diabetic groups treated with a methanol extract of Cleome rutidosperma and Hunteria umbellata, also at 50 mg/kg.

Additionally, the rats were marked with picric acid on various body parts, including the head, back, left hind limb (LH), right hind limb (RH), right fore limb (RF), and left fore limb (LF) within each group.

Figure 2.14 Labelling of the Rat Distribution into Groups (Animal House, University of Benin's Biochemistry Department).

2.5 PREPARATION OF MEDICINAL PLANTS FOR TREATMENT

The plant and seed samples were obtained from the surrounding vegetation around Ugbowo campus at the University of Benin and Oba market, Ringroad, Benin city. The samples were then air dried with minimal sunlight for about 7 weeks. Dried samples were pulverised and concentrations of methanol and distilled were prepared for extraction. The sample mixture was stirred/swirled and allowed to sit for 3 days before the citrate was recovered and fries dried to obtain extracts of methanol and water (aqueous extract).

2.6 ACCLIMATIZATION OF RAT

The rats underwent a three-week acclimatization period. This process guaranteed the stability of the rats during the collection of blood pressure measurements. Throughout the treatment and acclimation phases, the body weight of each rat was recorded. Acclimatization was done to ensure that the rats became accustomed to the temperature inside the MRBP machine which is used to take the blood pressure readings of the rats.

Figure 2.15 Acclimatization of a rat (BioPteB Laboratory).

2.7 QUANTIFICATION OF THE EXPERIMENTAL RAT BODY WEIGHT

Prior to the beginning of treatment on the rats, their body weight were recorded. Measurements were conducted over the course of one week preceding both the induction and treatment.

2.8 ASSESSING BLOOD GLUCOSE LEVELS OF THE RATS

Fasting Blood Sugar;

This assessment evaluates the blood glucose concentration following an overnight fast (typically 8-12 hours without any food intake).

Steps:

1. Preparation:

a. Confirm that the rat has not consumed any food or liquids other than water for a minimum of 8 hours before the test.

b. Gather the necessary supplies: glucose meter, test strips, lancet device, and alcohol swabs.

2. Thoroughly wash your hands with soap and water, then carefully clean the rat's tail and ensure it is completely dry. This procedure minimizes the risk of contamination and guarantees precise results.

3. Prepare the meter: Insert a test strip into the blood glucose meter.

4. Utilize the lancet device to select the site on the rat from which to draw a drop of blood.

5. Apply the drop of blood to the test strip, ensuring that there is sufficient blood for an accurate measurement.

6. Wait for the meter to indicate the blood glucose level.

Figure 2.16. Preparation of supplies for taking FBS reading (Animal House, University of Benin's Biochemistry Department).

2.9 PREPARATION AND EXTRACTION OF MEDICINAL PLANTS FOR TREATMENT

Fresh plant samples of; Cleome Rutidosperma and Sida Acuta were collected from the vicinity of Ugbowo Campus, University of Benin. On the other hand, the seeds of Hunteria Umbellata were purchased from Oba Market, Ring-Road, Benin City.

The ground sample was measured with a weighing balance, yielding the following results:

Cleome Rutidosperma: 189 g for the aqueous extract and 189 g for the methanol extract.

Hunteria umbellata: 330 g for the aqueous extract and 330 g for the methanol extract.

Sida acuta: 156 g for the aqueous extract, 156 g for the methanol extract.

After, the sample underwent extraction in both methanol and aqueous solutions.

Standard extraction ratios were as follows:

500 g of sample was dissolved in 2.5 liters.

Thus:

189 g of Cleome Rutidosperma was dissolved in 945 ml of both aqueous and methanol extracts.

156 g of Sida acuta was dissolved in 780 ml of both aqueous and methanol extracts.

330 g of Hunteria umbellata was dissolved in 1650 ml of both aqueous and methanol extracts.

Confirm that the samples are thoroughly dissolved and vortex every 2 hours.

Extract using Muslin cloth after a 24-hour period (Strain to collect sample)..

Re-submerge using the same volume of extract as mentioned above.

Decant using a hose and proceed to freeze-dry.

2.10 INDUCTION WITH STREPTOZOTOCIN (STZ) AND L-NAME

Each rat was subjected to intraperitoneal administration of Streptozotocin (STZ) during the second week of the experiment, continuing until diabetes was effectively induced. The dosage

given was based on the body weight of each rat, and blood glucose levels were measured to confirm the successful induction of diabetes.

L-NAME was administered orally starting from the second week of the experiment and continued until the final week before sacrifice to induce hypertension. The dosage was also determined by the body weight of each rat, and blood pressure readings were taken weekly using a blood pressure machine.

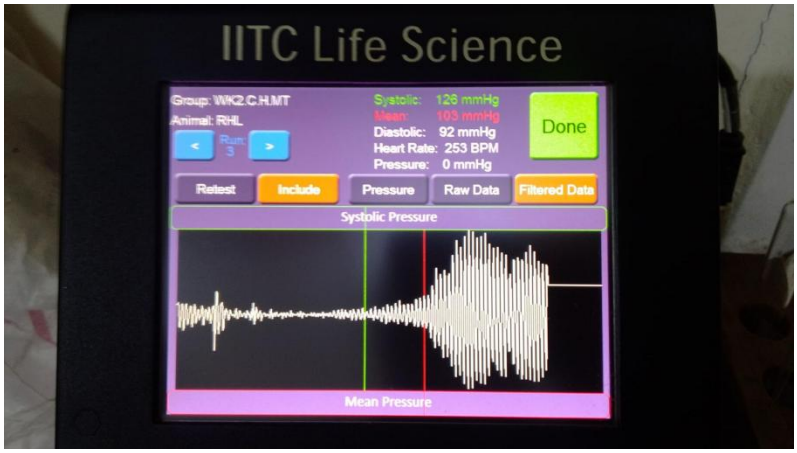


Figure 2.17. Blood Pressure Reading of an experimental rat using a standard Blood Pressure Machine (BioPteB Laboratory).

2.11 PREPARATION OF STERILE CITRATE BUFFER

Reagents required: Citric acid, Sodium citrate and sterile water.

Procedure: To prepare a 100ml solution of 0.1M sterile citrate buffer at PH 4.5;

1. Dissolve 1.355g of citric acid and 1.029g of sodium citrate in sterile water.
2. Adjust the final volume to 100ml.
3. Check and adjust the PH if necessary.

(pH = 4.5 Concentration = 0.1M)

Using Henderson Hassel Bach equation

$$pH = pKa + \text{Log}\frac{[conjugate\ base]}{[conjugate\ acid]}$$

$\text{Log}\ \frac{[sodium\ citrate]}{[citric\ acid]} = \text{pH} - \text{pKa}$

$\text{Log}\ \frac{[sodium\ citrate]}{[citric\ acid]} = 4.5 - 4.76$

$\text{Log}\ \frac{[sodium\ citrate]}{[citric\ acid]} = -0.26$

$\frac{[sodium\ citrate]}{[citric\ acid]} = 10^{-0.26} = 0.549$

$\frac{[sodium\ citrate]}{[citric\ acid]} = \frac{0.549}{1}$

Therefore, $[sodium\ citrate] + [citric\ acid] = 1 + 0.549 = 1.549$

Since the concentration is 0.1M, it therefore means that

$$0.1mol \leftrightarrow 1000ml$$

$$xmol \leftrightarrow 100ml$$

$x = \frac{100 \times 0.1}{1000} = 0.01\text{M}$

$$Sodium\ citrate = \frac{0.549}{1.549} \times 0.01 = 0.0035M$$

$$Citric\ acid = \frac{1}{1.549} \times 0.01 = 0.00645M$$

Molecular Weight:

$$Citric\ acid \rightarrow 210.14g$$

$$Sodium\ Citrate \rightarrow 294.10g$$

Reacting mass (m) = $c \times M$

$$m(sodium\ citrate) = 0.0035 \times 294.10 = 1.02935g \approx 1.03g$$

$$m(citrate) = 0.00645 \times 210.14 = 1.355403g \approx 1.36g$$

2.12 PREPARATION OF FORMAL SALINE

1. Dissolve 0.9g of sodium chloride salt in 90ml of distilled water.

2. Mix one sodium chloride solution with 10ML of 40% formalin to obtain a final volume of 100ml.
3. To prepare 40% formalin, 40ml of formalin is measured and then distilled water is added to make it up to 100ml.

2.14 ANIMAL SACRIFICE

After a five-week treatment period, the animals underwent an overnight fast prior to the day of sacrifice. Urethane was administered to each rat as an anesthetic, tailored to their respective body weights. The thoracic and abdominal cavities were incised, and a 5ml syringe was utilized to extract blood from the heart, which was subsequently stored in both EDTA and plain sample bottles. The heart was excised, weighed, and preserved in ice blocks until the time for homogenization.

Rat ID	**Normotensive/Non-diabetic (+ve control)**		**Hypertensive/Diabetic (-ve control)**		**Hypertensive/Diabetic +Lisinopril/Glibenclamide**		**Hypertensive/Diabetic +Sida acuta/Cleome ruditosperma Methanol**	
	Dose (g)	**Dose (ml)**	**Dose (g)**	**Dose (ml)**	**Dose (g)**	**Dose (ml)**	**Dose (g)**	**Dose (ml)**
HEAD	**0.185**	**0.615**	**0.210**	**0.670**	**0.167**	**0.555**	**0.2265**	**0.610**
BACK	**-**	**-**	**0.146**	**0.485**	**0.200**	**0.665**	**0.2145**	**0.605**
R.H. LIM	**0.218**	**0.725**	**0.156**	**0.520**	**0.150**	**0.500**	**0.2415**	**0.500**

B								
L.H. LIMB	**0.183**	**0.610**	**0.204**	**0.680**	**0.213**	**0.710**	**-**	**-**
R.F. LIMB	**0.164**	**0.545**	**0.165**	**0.550**	**0.222**	**0.740**	**0.216**	**0.72**
L.F. LIMB	-	-	-	-	-	-	-	-

Table 2.1. URETHANE ADMINISTRATION CHART

2.15 ANIMALS MORTALITY

In total, 9 rats were lost during the period of the experiment.

Figure 2.18. Animals lost during experiment (BioPteB laboratory).

RAT I.D/DRUG	Hypertensive/Diabetic (-ve control) (ml)		Hypertensive/Diabetic+ Lisinopril(10mg/kg)/ Glibenclamide(5mg/kg) (ml)				Hypertensive/Diabetic+*Sida acuta/Cleome rutidosperma* Aqueous (50mg/kg) (ml)			Hypertensive/Diabetic+ *Sida acuta/Cleome rutidosperma* Methanol extract (50mg/kg) (ml)			Hypertensive/Diabetic+*Cleome rutidosperma/ Hunteria umbellata(seed)* Aqueous (50mg/kg) (ml)			Hypertensive/Diabetic+ *Cleome rutidosperma/ Hunteria umbellata(seed)* Methanol extract (50mg/kg) (ml)		
	L-NAME	STZ	LIS	GLIB	L-NAME	STZ	AQ	L-NAME	STZ	MET EX	L-NAME	STZ	AQ	L-NAME	STZ	MET EX	L-NAME	STZ
HEAD	0.588	0.735	1.31	0.655	0.524	0.655	0.525	0.420	0.525	0.735	0.588	0.735	0.590	0.472	0.590	0.500	0.400	0.500
BACK	0.432	0.540	1.51	0.755	0.604	0.755	0.665	0.532	0.665	0.490	0.392	0.490	0.570	0.456	0.570	0.400	0.320	0.400
R.H LIMB	0.452	0.565	1.17	0.585	0.468	0.585	0.575	0.460	0.575	0.565	0.452	0.565	0.640	0.512	0.640	0.590	0.472	0.590
L.H LIMB	0.672	0.840	1.40	0.700	0.560	0.700	0.620	0.496	0.620				0.535	0.428	0.535	0.685	0.548	0.685
R.F LIMB	0.448	0.560	1.56	0.780	0.624	0.780	0.510	0.408	0.510	0.560	0.448	0.560	0.645	0.516	0.645	0.500	0.400	0.500
L.F LIMB							0.650	0.520	0.650									

Table 2.2. DOSAGE ADMINISTRATION CHART.

CHAPTER THREE

RESULTS

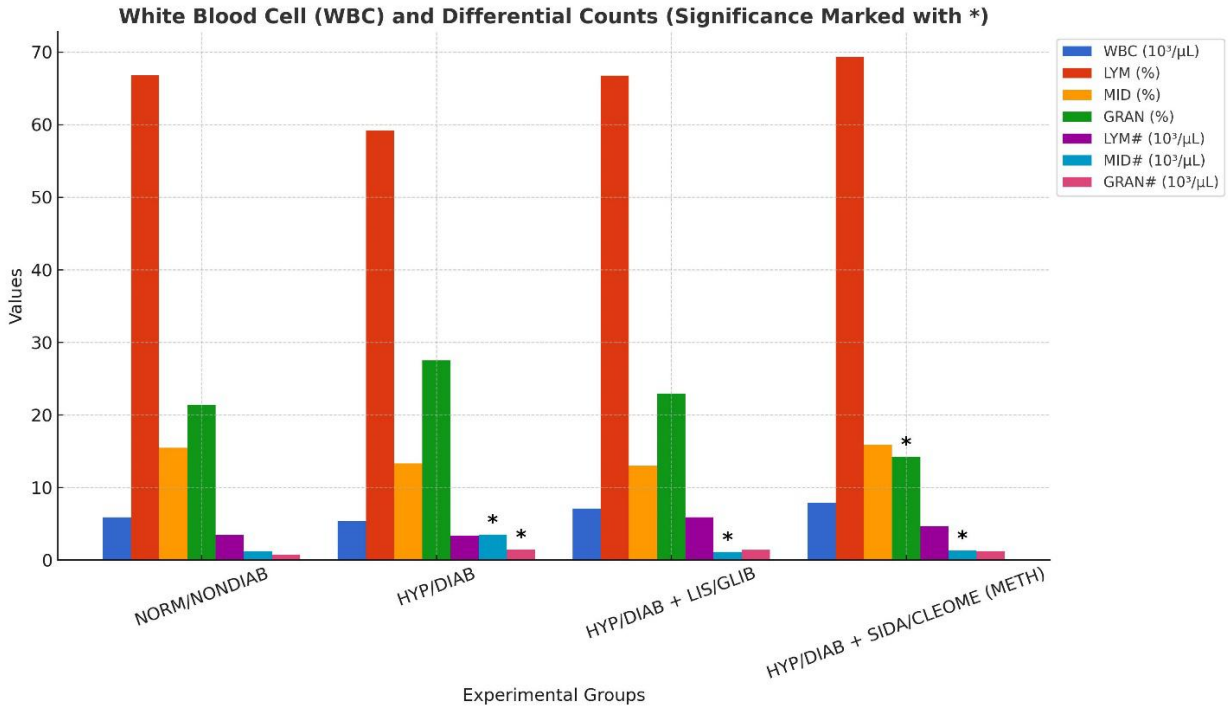
3.1 HEMATOLOGICAL PARAMETERS OF GROUPS.

This chapter presents the findings of a study assessing the effects of co-administration of Methanol extracts from Sida Acuta/Cleome Rutidosperma (SIDA/CLEOME(METH)) and the drugs Lisinopril/Glibenclamide (LIS/GLB) on blood cell formation in hypertensive and diabetic (HYP/DIAB) male Wistar rats. The data include white blood cell counts, differential counts, red blood cell counts, and various hematological and blood clotting parameters. Results are shown in graphs as mean ± standard deviation and analyzed by Turkey HSD. Statistically significant differences between groups are marked with an asterisk (*) when $p \leq 0.05$, while non-significant differences are unmarked at $p \geq 0.05$.

3.2 WHITE BLOOD CELL (WBC) AND DIFFERENTIAL COUNTS

Figure 3.1 presents the results of WBC counts and differential counts such as lymphocyte counts (LYM#) and proportion (LYM%), granulocyte count (GRAN#) and proportion (GRAN%) and MID (mid-sized cells containing monocytes, eosinophils, basophils) counts and proportion across the experimental groups.

Figure 3.1 : White Blood Cell (WBC) and Differential Counts



Comparative analysis of White Blood Cell (WBC) and Differential Counts across groups

1. The comparative analysis shows that white blood cell (WBC) counts between NORM/NONDIAB and HYP/DIAB did not significantly differ, indicating similar immune cell levels. Treatment with LIS/GLIB or SIDA/CLEOME (METH) resulted in higher WBC counts compared to hypertensive/diabetic controls, but these increases were not statistically significant. The findings imply no substantial alteration in WBC counts due to hypertension, diabetes, or the tested treatments in this study

2. The lymphocyte percentage (LYMC%) comparison in Table 3.2 shows no significant difference among NORM/NONDIAB, HYP/DIAB, and treatment groups LIS/GLIB or SIDA/CLEOME (METH) with p-values of 0.315, 0.289, and 0.167 respectively. This indicates that the lymphocyte levels remained fairly stable across all groups without significant changes due to hypertension, diabetes, or the treatments administered.

3. There was no significant differences in monocyte percentage (MID%) across the groups compared: NORM/NONDIAB, HYP/DIAB, and treatment groups with LIS/GLB or SIDA/CLEOME (METH). The p-values for these comparisons were 0.324, 0.988, and 0.199

respectively, showing that monocyte percentages remained statistically unchanged regardless of hypertension, diabetes, or treatment status.

4. Granulocyte percentages did not significantly differ between the NORM/NONDIAB and HYP/DIAB groups ($p > 0.170$), nor between HYP/DIAB and LIS/GLB treatment groups ($p > 0.277$). However, there was a significant decrease in granulocyte percentage in the o SIDA/CLEOME (METH group compared to the HYP/DIAB ($p = 0.003$), with granulocytes dropping from $27.50 \pm 4.06\%$ to $14.20 \pm 1.01\%$. This suggests that while hypertension and diabetes themselves do not significantly alter granulocyte counts, treatment with this plant extract notably reduces granulocyte levels in this model.

5. Absolute lymphocyte counts did not significantly differ among NORM/NONDIAB, HYP/DIAB and treated groups with LIS/GLB or SIDA/CLEOME (METH). The p-values were all greater than 0.05, indicating stable lymphocyte counts regardless of disease status or treatment.

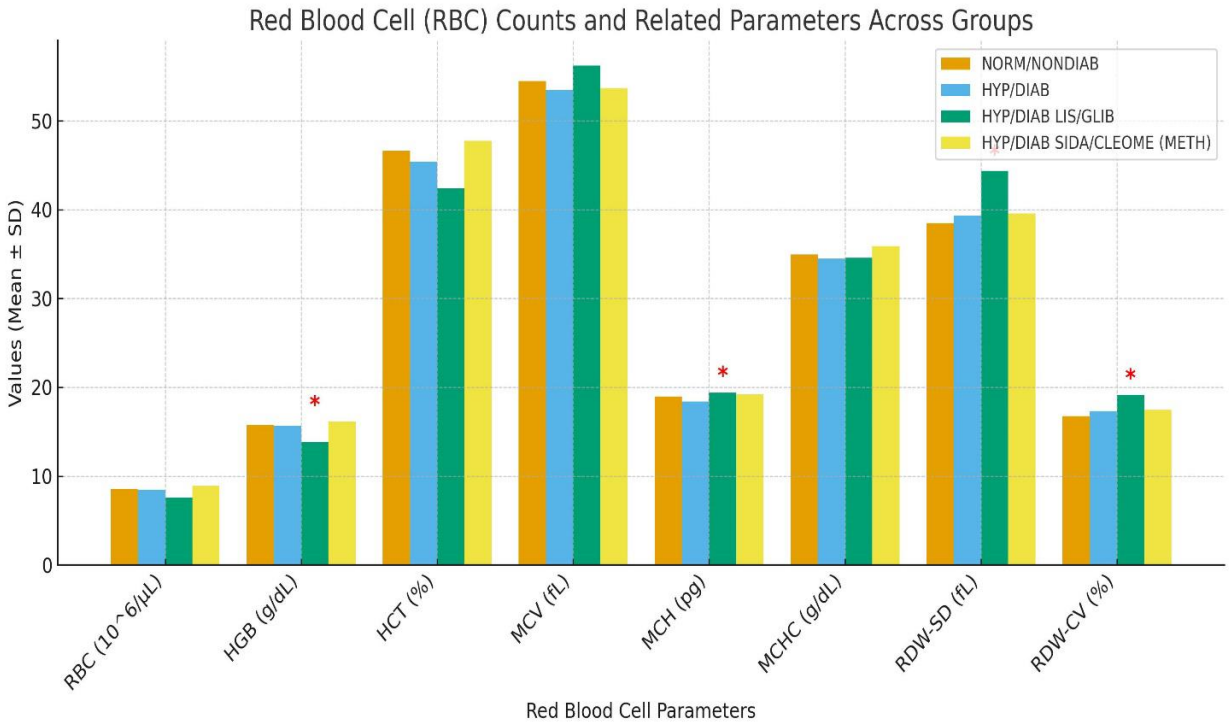
6. There was a highly significant increase in monocyte counts in HYP/DIAB rats compared to NORM/NONDIAB controls ($p < 0.001$). Treatment with LIS/GLB or SIDA/CLEOME (METH) resulted in a marked and statistically significant reduction in monocyte count, bringing levels closer to those of healthy controls ($p < 0.001$ for both).

7. There was a statistically significant increase in granulocyte counts in HYP/DIAB rats compared to NORM/NONDIAB controls ($p < 0.028$). However, there were no significant differences in granulocyte counts between the hypertensive/diabetic group and those treated with LIS/GLB or SIDA/CLEOME (METH) ($p > 0.997$ and $p > 0.630$, respectively).

3.3 RED BLOOD CELL (RBC) COUNTS AND RELATED PARAMETERS

Table 3.2 summarizes the results of RBC counts and related parameters such as hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), standard deviation and coefficient of variation for range of width of RBC (RDW-SD and RDW-CV) across the experimental groups.

Figure 3.2 Red Blood Cell (RBC) Counts and Related Parameters



Comparative analysis of Red Blood Cell (RBC) and Differential Counts across group

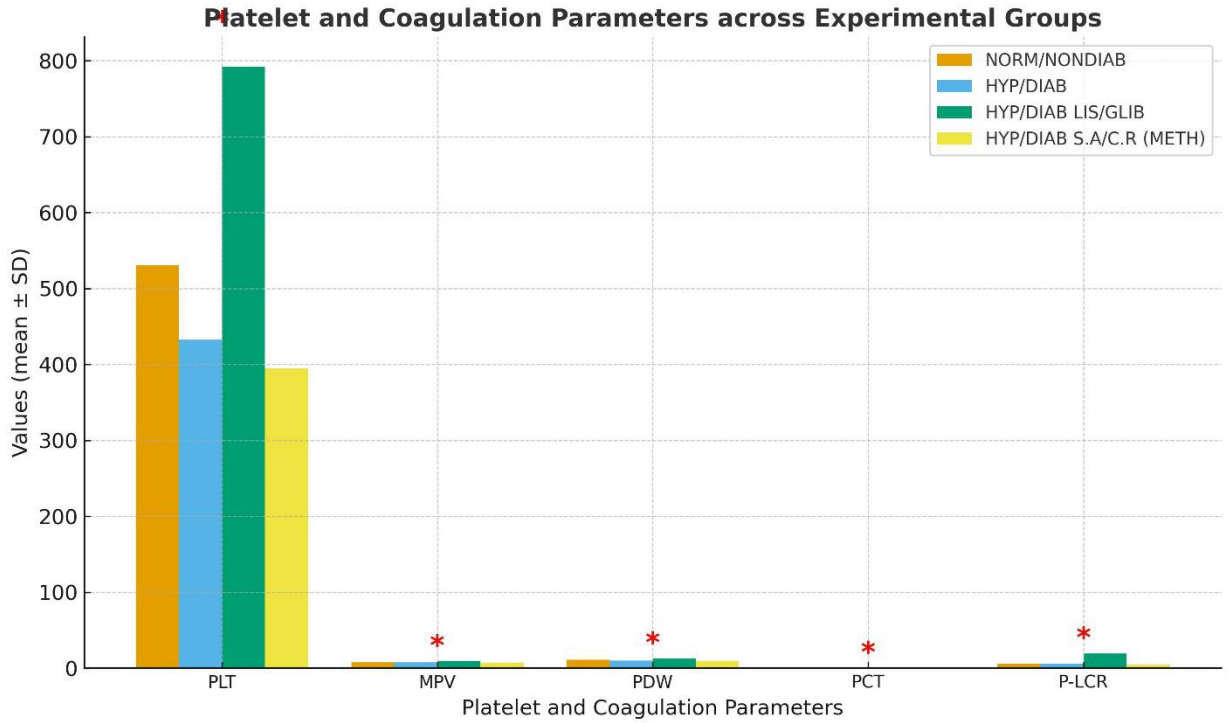
1. RBC Count (10^6/µL): No significant difference between NORM/NONDIAB (8.57 ± 0.46) and HYP/DIAB (8.49 ± 0.35), nor between HYP/DIAB and treatments with LIS/GLB (7.58 ± 1.09) or SIDA/CLEOME (METH) (8.90 ± 0.41) ($p > 0.18$)
2. Hemoglobin (HGB, g/dL): Similar levels between NORM/NONDIAB (15.77 ± 0.15) and HYP/DIAB (15.68 ± 0.97). Treatment with LIS/GLB showed a statistically significant reduction in HGB (13.82 ± 0.59; $p = 0.016$), while SIDA/CLEOME (METH) treatment did not differ significantly (16.15 ± 0.78; $p = 0.869$)
3. Hematocrit (HCT %): Values were comparable across groups with no significant differences.
4. Mean Corpuscular Volume (MCV,fL): No significant differences among groups.
5. Mean Corpuscular Hemoglobin (MCH, pg): Statistically significant increase in the LIS/GLB group (19.40 ± 0.42, $p = 0.034$) compared to others, no significance in SIDA/CLEOME (METH).
6. Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL): No significant differences.

7. Red Cell Distribution Width - Standard Deviation (RDW-SD, fL) and Coefficient of Variation (RDW-CV, %): LIS/GLB treatment showed significant changes (p = 0.000), while other comparisons were not significant.

3.4 PLATELETS AND COAGULATION PARAMETERS

Table 3.3 details the platelets and coagulation parameters such as PLT, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet large cell ratio (P-LCR), all across the experimental groups.

Figure 3.3 Platelets and Coagulation Parameters



Comparative analysis of platelets and Coagulation parameters across group

1. Platelet Count (PLT, × 10^3/µL): NORM/NONDIAB group had a mean platelet count of 530.66 ± 75.27. The HYP/DIAB group exhibited a reduced platelet count of 432.33 ± 88.71; however, this difference was not statistically significant (p = 0.367). Treatment with LIS/GLB significantly increased platelet count to 791.66 ± 67.72 (p = 0.001*), indicating a likely restoration or stimulation of platelet production/function. Conversely, treatment with

SIDA/CLEOME METH resulted in a platelet count of 394.50 ± 50.95 which was not significantly different from the untreated HYP/DIAB group (p = 0.891).

2. Mean Platelet Volume (MPV, fL): There were no significant differences between NORM/NONDIAB (7.78 ± 0.71) and HYP/DIAB (7.60 ± 0.36) groups (p = 0.935). LIS/GLB treatment caused a significant increase in MPV (9.20 ± 0.36, p = 0.002*), suggesting the presence of larger or more reactive platelets. The SIDA/CLEOME METH group had an MPV of 7.50 ± 0.16, not significantly different from HYP/DIAB (p = 0.986).

3. Platelet Distribution Width (PDW, %): PDW was similar between NORM/NONDIAB (10.83 ± 0.58) and HYP/DIAB (10.05 ± 0.67) groups (p = 0.521). LIS/GLB treatment significantly increased PDW to 12.63 ± 1.06 (p = 0.004*), indicating increased variability in platelet size. SIDA/CLEOME METH treatment did not significantly change PDW (9.28 ± 0.59, p = 0.469).

4. Plateletcrit (PCT, %): PCT values showed no significant difference between NORM/NONDIAB (0.39 ± 0.06) and HYP/DIAB (0.32 ± 0.06) (p = 0.399). LIS/GLB treatment significantly elevated PCT to 0.6 ± 0.00 (p = 0.000*), whereas SIDA/CLEOME METH treatment did not significantly affect PCT (0.30 ± 0.02, p = 0.096).

5. Platelet Large Cell Ratio (P-LCR, %): P-LCR was stable between NORM/NONDIAB (5.70 ± 0.071) and HYP/DIAB (6.37 ± 0.75) (p = 0.635). LIS/GLB treatment drastically increased P-LCR to 19.15 ± 0.35 (p = 0.000*), suggesting a higher proportion of large platelets, potentially more reactive or newly formed. The SIDA/CLEOME METH group showed a non-significant decrease in P-LCR (4.50 ± 0.00, p = 0.06).

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The present study on "Comparative Hematopoietic Modulation by Lisinopril/Glibenclamide and Methanol Fraction of Sida acuta / Cleome rutidosperma" compared standard pharmacologic treatment (lisinopril + glibenclamide) with a combined methanol fraction of Sida acuta and Cleome rutidosperma (the plant extract) for their effects on hematological indices in hypertensive/diabetic male Wistar rats. Overall, the two treatment strategies produced distinct hematopoietic and immuno-hematologic responses: lisinopril/glibenclamide tended to alter red cell and platelet indices in ways consistent with ACE-inhibitor and sulfonylurea effects reported in the literature, while the plant extract showed relative preservation of erythroid indices and evidence of anti-inflammatory modulation (reduced granulocyte/monocyte percentages) consistent with hematoprotective effects reported for these plants.

White blood cell (WBC) and differential counts are essential indicators of immune function and inflammation. In this study, total WBC and lymphocyte counts showed no significant differences among all groups, suggesting that neither hypertension/diabetes nor the treatments caused major leukocytic disturbances.

Monocyte counts were significantly elevated in hypertensive/diabetic rats compared to normal controls, reflecting low-grade inflammation typically associated with metabolic and vascular dysfunction. Both treatments—lisinopril/glibenclamide and Sida acuta/Cleome rutidosperma—significantly reduced monocyte levels ($p < 0.001$), suggesting that both regimens exerted anti-inflammatory effects capable of restoring immune balance. This aligns with reports that lisinopril reduces vascular inflammation via suppression of angiotensin II-mediated immune activation, while phytochemicals from Sida acuta and Cleome rutidosperma possess antioxidant and immunomodulatory properties.

However, the methanol fraction of Sida acuta/Cleome rutidosperma significantly reduced granulocyte percentage ($p = 0.003$), while lisinopril/glibenclamide had no significant effect. This reduction in granulocytes may indicate a potential anti-inflammatory or immunomodulatory property of the plant extract, as granulocytes, particularly neutrophils, play key roles in oxidative and inflammatory responses. This result is concordant with multiple preclinical reports that Sida acuta and Cleome species contain flavonoids, alkaloids and other antioxidants/anti-inflammatory

phytochemicals Cleome species have documented antioxidant and anti-inflammatory phytochemistry that can plausibly reduce granulocyte/monocyte activation and infiltration (Enechi, et al. 2021).

Red blood cell (RBC) indices provide insight into oxygen-carrying capacity and erythropoietic function. There were no significant differences in RBC count, hematocrit, or most red cell indices across groups, indicating that hypertension and diabetes did not drastically impair erythropoiesis within the study duration. However, treatment with lisinopril/glibenclamide significantly decreased hemoglobin concentration ($p = 0.016$), which may reflect mild drug-induced anemia or hemodilution, a known side effect associated with long-term ACE inhibitor use.

Interestingly, the methanol fraction of Sida acuta/Cleome rutidosperma maintained hemoglobin levels comparable to normal controls, suggesting a hematoprotective effect that may be due to antioxidant constituents enhancing erythrocyte stability or reducing oxidative hemolysis.

Sida acuta ethanol/methanol extracts have been reported to ameliorate anemia and protect hematological indices in rodent toxicity and infection models (Cheungpasitporn, W et al. 2015) likely through antioxidant protection of erythrocytes and indirect support of bone-marrow function; this explains why the extract group maintained hemoglobin and RBC indices in this study. Furthermore, mean corpuscular hemoglobin (MCH) was significantly increased in the lisinopril/glibenclamide group ($p = 0.034$), possibly compensating for reduced RBC mass, whereas the extract-treated group maintained normal indices, indicating better preservation of erythrocyte structure and function.

The platelet profile revealed notable differences between treatments. Hypertensive and diabetic rats exhibited reduced platelet counts relative to the normal control group, consistent with the thrombocytopenia commonly associated with chronic hyperglycemia and vascular stress. Treatment with lisinopril and glibenclamide significantly increased platelet count ($p = 0.001$), plateletcrit ($p = 0.000$), mean platelet volume ($p = 0.002$), platelet distribution width ($p = 0.004$), and platelet large cell ratio ($p = 0.000$).

These findings suggest enhanced thrombopoietic activity and increased platelet reactivity, potentially reflecting improved bone marrow function or compensatory platelet production in response to hematologic stress. In contrast, administration of the methanol fraction of Sida acuta

and Cleome rutidosperma did not significantly alter platelet indices relative to the hypertensive/diabetic control group.

However, the slight increase in platelet count (394.50 ± 50.95 vs. 432.33 ± 88.71) indicates mild hematopoietic modulation. This suggests that while the plant extract supports hematopoietic balance, it does not overstimulate thrombopoiesis—an advantage in managing cardiovascular risk where excessive platelet activity may predispose to thrombotic events.

Notably, the plant extract did not induce the degree of platelet activation or thrombopoietic stimulation observed with lisinopril/glibenclamide. This moderated effect could be beneficial clinically, as exaggerated platelet reactivity in diabetes and hypertension contributes to pro-thrombotic states. Therefore, an agent that stabilizes platelet function while mitigating inflammation, such as the Sida acuta/Cleome rutidosperma extract, may lower thrombotic risk without promoting bleeding complications.

4.2 CONCLUSION

Overall, lisinopril/glibenclamide treatment showed strong stimulatory effects on platelet parameters, suggesting hematopoietic activation but also possible hyper-reactivity. In contrast, the methanol fraction of Sida acuta/Cleome rutidosperma demonstrated a stabilizing and protective influence across hematological indices—reducing granulocyte and monocyte counts, preserving hemoglobin, and maintaining platelet homeostasis. This pattern indicates that the plant extract exerts a balanced hematopoietic modulation, possibly through antioxidative, anti-inflammatory, and cytoprotective mechanisms rather than direct stimulation. The combined evidence points to Sida acuta/Cleome rutidosperma as a promising natural alternative or adjunct therapy for mitigating hematological disturbances associated with hypertension and diabetes, offering comparable anti-inflammatory benefits to conventional drugs but with fewer hematotoxic effects.

REFERENCES

Ajibola, O.O.E., Akinmegha, T.S., Nwaiwu, O. & Balogun, O.J. (2018) 'Biometric analysis of *Hunteria umbellata* (K. Schum.) Hallier f and metformin in the treatment of diabetes', *Journal of Applied Science and Environmental Management*, **22(**4):561–564.

Altamash, S. (2017) 'A practical approach to hypertension management in diabetes', *Diabetes Therapy*, 8: 981–989.

American Diabetes Association (ADA) (2008) 'Economic costs of diabetes in the U.S. in 2007', *Diabetes Care*, **31(**3): 596–615.

American Diabetes Association (2009) 'Diagnosis and classification of diabetes mellitus', *Diabetes Care*, **32**(1): S62–S67.

Banting, F.G., Best, C.H., Collip, J.B. & Macleod, J.J.R. (1922) 'The internal secretion of the pancreas', *Journal of Laboratory and Clinical Medicine*, **7**(5): 251–266.

Bernard, M., Cheung, Y. & Li, C. (2012) 'Diabetes and hypertension: Is there a common metabolic pathway?', *Current Atherosclerosis Reports*, 14: 160–166.

Centers for Disease Control and Prevention (CDC) (2014) *National diabetes statistics report: Estimates of diabetes and its burden in the United States, 2014*. Atlanta, GA: U.S. Department of Health and Human Services.

Cheungpasitporn, W., Thongprayoon, C., Chiasakul, T., Korpaisarn, S. & Erickson, S.B. (2015) 'Renin-angiotensin system inhibitors linked to anemia: a systematic review and meta-analysis', *QJM: An International Journal of Medicine*, **108**(11): 879–884.

Donkor, A.M., Ahenkorah, B., Wallah, T.A. & Yakubu, A. (2023) 'Evaluation of extracts from *Sida acuta*, *Phyllanthus amarus*, *Parkia biglobosa* and their herbal ointment for therapeutic and biological activities', *Heliyon*, **9**(9): 19316.

Enechi, O.C., Amah, C.C., Okagu, I.U., Ononiwu, P.C., Nweke, A.C., Ugwuanyi, T.C., Ajibo, E.A., Nweze, A.C. & Chukwurah, B.C. (2021) '*Sida acuta* leaf extract ameliorates haematological and biochemical alterations in alloxan-induced diabetic rats', *Clinical Phytoscience*, **7**(78).

Ezeja, M.I., Anaga, A.O. & Asuzu, I.U. (2015) 'Anti-diabetic, anti-hyperlipidemic and antioxidant activities of *Gouania longipetala* methanol leaf extract in alloxan-induced diabetic rats', *Journal of Intercultural Ethnopharmacology*, **4**(1): 60–68.

International Diabetes Federation (IDF) (2013) *IDF diabetes atlas*, 6th ed. Brussels: International Diabetes Federation.

Janaka, K. & Luigi, G. (2016) 'Diabetes mellitus: A global epidemic', *Journal of Diabetes and Metabolic Disorders*, **15(**31): 1–5.

Karamanou, M., Protogerou, A., Tsoucalas, G., Androutsos, G. & Poulakou-Rebelakou, E. (2016) 'Milestones in the history of diabetes mellitus: The main contributors', *World Journal of*

Diabetes, **7**(1): 1–7.

Kenneth, S.P. (2012) 'The past 200 years in diabetes', *New England Journal of Medicine*, **367**(14): 1–8.

Menter, P., Carey, R.M., Gidding, S., Jones, D.W., Taler, S.J., Wright, J.T. & Whelton, P.K. (2018) 'Potential U.S. population impact of the 2017 ACC/AHA high blood pressure guideline', *Journal of the American College of Cardiology*, **71**(2): 109–118.

Pranali, M.W. (2024) 'Hypertension and comorbidities: A silent threat to global health', *Hypertension Comorbidity*, **1**(1): 17.

Ramesh Babu, H.N., Rajeshwari, N. & Manjunath, K.M. (2024) 'Antioxidant and cytotoxic properties of *Sida acuta* (Burm. f.) GCMS profile', *Journal of Applied Biology and Biotechnology*, **19**(2): 250–257.

Sanger, F. (1955) 'The amino acid sequence of insulin', *Biochemical Journal*, **60**(4): 556–574.

Sharpey-Schafer, E.A. (1910) *The endocrine organs in health and disease, with an historical review. Harben Lectures*. London: Constable.

Sowjanya, N., Gardner, M.J., Khangura, D., Kurukulasuriya, L.R. & Sowers, J.R. (2021) 'Hypertension in diabetes', *National Library of Medicine, National Institutes of Health*.

Sutherland, E.W. & Rall, T.W. (1958) 'Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles', *Journal of Biological Chemistry*, **232**(2): 1077–1091.

Trang, N. (2023) ‘Phytochemical composition, antioxidant, anti-inflammatory, anticancer, and antidiabetic activities of *Cleome rutidosperma* DC: A review of pharmacological evidence’.

Trang, N. (2023) ‘A review on bioactive compounds and pharmacological properties of *Cleome rutidosperma* DC’, *Journal of Tropical Life Science*, **13**(3): 615–624.

Whelton, P.K., Carey, R.M., Aronow, W.S., Casey, D.E., Collins, K.J., Dennison Himmelfarb, C., De Palma, S.M., Gidding, S., Jamerson, K.A., Jones, D.W., Mac Laughlin, E.J., Muntner, P., Ovbiagele, B., Smith, S.C., Spencer, C.C., Stafford, R.S., Taler, S.J., Thomas, R.J., Williams, K.A., Williamson, J.D. & Wright, J.T. (2018) ‘A guideline for the prevention, detection, evaluation, and management of high blood pressure in adults’, *Journal of the American College of Cardiology*, **71**(19): 127–248.

APPENDIX

HEMATOLOGICAL DATA

WHITE BLOOD CELLS AND ITS DIFFERENTIAL COUNTS

GROUPS	WBC ($x\ 10^3$ /µL)	LYM%	MID%	GRAN %	LYM# ($x\ 10^3$ /µL)	MID# ($x\ 10^3$ /µL)	GRAN# ($x\ 10^3$ /µL)
NORM/NON	6.50	62.50	15.40	22.10	2.4	1.20	0.80
DIAB	5.30	71.20	16.00	20.70	4.60	1.20	0.70
		63.30	15.00		3.40		
		70.20					
X̄±SD	**5.90 ± 0.85**	**66.8 ± 4.53**	**15.47 ± 0.50**	**21.4 ± 0.99**	**3.47 ± 1.10**	**1.20 ± 0.00**	**0.75 ± 0.07**
HYP/DIAB	5.70	55.50	13.10	31.40	3.20	0.80	1.70
	4.40	61.00	11.20	27.80	3.30	4.0	1.20
	6.00	61.00	15.70	23.30	3.70	3.0	1.40
X̄±SD	**5.37 ± 0.85**	**59.17 ± 3.18**	**13.33 ± 2.26**	**27.5 ± 4.06**	**3.22 ± 0.41**	**3.50 ± 0.71**	**1.43 ± 0.25**
LIS/GLIB	6.30	63.3	14.2	19.4	4.0	1.10	1.2

	8.5 6.5	69.6 62.6 78.9 59.2	12.1 12.6	25.3 24.1	6.7 5.3 7.6	1.0 1.2 1.1	1.5 1.5
X̄±SD	**7.90 ± 1.22**	**66.72 ± 7.78**	**12.97 ± 1.10**	**22.93 ± 3.12**	**5.90 ± 1.58**	**1.10 ± 0.08**	**1.40 ± 0.17**
SIDA/CLEOME (METH)	8.90 8.1 6.7	70.4 70.5 59.6 67.0	14.5 16.4 16.8	15.1 13.1 14.4	4.8 4.5	1.30 1.40 1.20	1.30 1.4 1.0
X̄±SD	**7.90 ± 1.11**	**69.30 ± 1.99**	**15.90 ± 1.23**	**14.20 ± 1.01**	**4.65 ± 0.21**	**1.30 ± 0.10**	**1.23 ± 0.21**

RED BLOOD CELLS AND ITS DIFFERENTIAL COUNTS

GROUPS	**RBC (10^6/µL)**	**HGB (g/dL)**	**HCT (%)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**	**ROW-SD (fL)**	**ROW-CV (%)**
NORM/NON DIAB	8.23 9.24 8.32 8.47	15.80 15.60 15.90	46.10 50.1 44.00 46.30	56.10 54.30 52.90 54.70	19.10 19.40 18.70 18.70	34.20 35.90 35.40 34.30	38.50 38.50 38.50 38.50	16.50 16.80 17.10 16.60
X̄±SD	**8.57 ±**	**15.68 ±**	**46.63**	**54.5 ±**	**18.98**	**34.95 ±**	**38.5 ±**	**16.75 ±**

	0.46	**1.15**	**± 2.54**	**1.32**	**± 0.34**	**0.83**	**0.00**	**0.26**
HYP/DIAB	8.71 8.17 8.31 9.00 8.27	16.7 14.90 14.70 16.7 15.40	46.90 43.90 42.70 48.70 44.80	53.90 53.80 51.40 54.20 54.20	19.10 18.20 17.6 18.50 18.60	35.60 33.90 34.40 34.20 34.30	38.50 40.60 36.30 40.60 40.60	16.80 17.80 16.60 17.70 17.70
X±SD	**8.49 ± 0.35**	**15.68± 0.97**	**45.4 ± 2.40**	**53.3 ± 1.19**	**18.40 ± 0.55**	**34.48 ± 0.65**	**39.3 ± 1.92**	**17.3 ± 0.57**
LIS/GLIB	6.96 6.53 7.45 9.38 7.56	13.6 13.1 14.3 14.3 16.20	40.3 39.6 39.9 50.8 41.3	58.0 60.7 53.6 54.2 54.7	19.50 20.0 19.1 19.4 18.9	33.7 33.0 35.8 35.8 34.6	44.9 44.9 42.7 44.9	18.7 19.8 18.6 19.4
X±SD	**7.58 ± 1.09**	**13.82 ± 0.59**	**42.38 ± 4.75**	**56.24 ± 3.02**	**19.40 ± 0.42**	**34.58 ± 1.25**	**44.35 ± 1.10**	**19.13 ± 0.57**
SIDA/CLEOME (METH)	9.40 8.96 8.41 8.83	15.6 16.7	50.1 50.6 44.3 46.0	53.4 56.5 52.7 52.1	19.50 20.00 18.5 18.9	36.7 35.5 35.2 36.3	40.6 40.6 38.5 38.5	18.0 17.3 17.2 17.4

X̄±SD	**8.90 ± 0.41**	**16.15 ± 0.78**	**45.36± 3.72**	**53.68 ± 1.96**	**19.22 ± 0.66**	**35.93 ± 1.00**	**41.3 ± 1.21**	**17.48 ± 0.36**

GROUPS	**PLT (10^3/µL)**	**PCT (%)**	**P-LCR (%)**	**MPV (fL)**	**PDW (%)**
NORM/NON	452.00	0.32	6.20	7.20	10.50
DIAB	538.00	0.41	5.20	8.8	10.50
	602.00	0.44		7.70	11.50
				7.40	
X̄±SD	**530.67 ± 75.27**	**0.39 ± 0.06**	**5.70 ± 0.71**	**7.78 ± 0.71**	**10.83 ± 0.58**
HYP/DIAB	331.00	0.25	5.60	7.70	9.50
	470.00	0.35	7.10	7.10	9.70
	496.00	0.37	6.40	8.1	10.00
				7.60	11.00
				7.50	
X̄±SD	**432.33 ± 88.71**	**0.32 ± 0.06**	**6.37 ± 0.75**	**7.60 ±0.36**	**10.05 ± 0.67**
LIS/GLIB	743	0.67	18.9	9.1	13.6
	869	0.67	19.4	9.6	12.8
	763			8.9	11.5
X̄±SD	**791.67 ±**	**0.67 ± 0.00**	**19.15 ± 0.35**	**9.20 ± 0.36**	**12.63 ± 1.06**

	67.72				
SIDA/CLEOME (METH)	328 452.00 396.00 402	0.32 0.29 0.30	4.5 4.5 3.9	7.7 7.3 7.5 7.5	8.4 9.7 9.5 9.5
$\bar{X}$±SD	**394 ± 50.95**	**0.30 ± 0.02**	**4.50 ±0.00**	**7.50 ± 0.16**	**9.28 ± 0.59**

PLATELETS AND ITS DIFFERENTIAL COUNTS