THE COMPARATIVE EFFECT OF CO-ADMINISTRATION OF AQUEOUS FRACTION OF *CLEOME RUTIDOSPERMA/ HUNTERIA UMBELLATA* 50mg/kg OR METHANOL FRACTION 50mg/kg ON HEMATOPOEISIS OF INDUCED HYPERTENSIVE/DIABETIC MALE WISTAR RATS

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CERTIFICATION

This is to certify that this project work was carried out by **AZUBUIKE ONYEDIKA PETER** with the matriculation number **LSC2103715,** of the Department of Biochemistry, Faculty of Life Sciences, University of Benin in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) degree in biochemistry.

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DEDICATION

This work is dedicated to my mom, Chinwe Agidi Scholastica, and my elder brother, Ogechukwu Azubuike for their unconditional love and support both in cash and hand, and to God almighty for making this work a success.

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ABSTRACT

This study investigated the comparative effects of co-administration of aqueous and methanol fractions of *Cleome rutidosperma* and *Hunteria umbellata* on hematopoiesis in L-NAME/STZ-induced hypertensive and diabetic male Wistar rats. Hypertension and diabetes are chronic metabolic disorders that frequently coexist and contribute to cardiovascular complications through oxidative stress and hematological alterations. Traditional medicinal plants such as *Cleome rutidosperma* and *Hunteria umbellata* have been reported to possess antihypertensive and antidiabetic properties, yet their combined effects on blood cell formation remain underexplored. Hematological indices were evaluated to determine their effects on erythropoiesis, leukopoiesis, and thrombopoiesis. The findings revealed that both extracts produced beneficial effects on altered hematological parameters, with the methanol fraction showing greater hematoprotective efficacy. The methanol fraction significantly improved total lymphocyte, mid-sized cell percentage, and platelet indices, indicating enhanced immune response, erythropoietic stability, and hemostatic balance. These results suggest that the methanol fraction of *Cleome rutidosperma* and *Hunteria umbellata* exerts superior restorative effects on hematopoiesis in L-NAME/STZ-induced hypertensive and diabetic rats, highlighting its therapeutic potential in managing hematological disturbances associated with cardiometabolic disorders.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 HYPERTENSION

Hypertension, commonly known as high blood pressure, is a long-term health condition marked by consistently elevated arterial pressure. Clinically, it is defined as a systolic blood pressure (SBP) of 140 mmHg or higher and/or a diastolic blood pressure (DBP) of 90 mmHg or higher (Mancia *et al.,* 2007). The 2007 guidelines from the European Society of Hypertension (ESH) and European Society of Cardiology (ESC) further classify blood pressure into specific ranges: *optimal* (<120/80 mmHg), *normal* (120–129/80–84 mmHg), and *high-normal* (130–139/85–89 mmHg). Values beyond these thresholds are diagnosed as hypertension, categorized into three stages, along with a special category for isolated systolic hypertension (ISH). (Mancia *et al.,* 2007)

- Grade 1 (mild): SBP 140–159 mmHg and/or DBP 90–99 mmHg
- Grade 2 (moderate): SBP 160–179 mmHg and/or DBP 100–109 mmHg
- Grade 3 (severe): SBP ≥180 mmHg and/or DBP ≥110 mmHg
- Isolated systolic hypertension (ISH): SBP ≥140 mmHg with DBP <90 mmHg, graded by systolic level

When systolic and diastolic readings fall into different categories, the higher category is used to assess cardiovascular risk (Mancia *et al.,* 2007).

From an etiological standpoint, hypertension is divided into primary (essential) and secondary types. Primary hypertension is the dominant form, responsible for about 90–95% of cases (Carretero & Oparil, 2000). Its development is multifactorial, influenced by both genetic and environmental factors. While inherited traits significantly shape blood pressure regulation, lifestyle factors also play a major role. Risk factors include physical inactivity, obesity (Kyrou *et al.,* 2006), chronic stress, tobacco use, high alcohol intake, and excessive dietary salt (Lackland *et al.,* 2007). These influences often act together, intensifying the risk in genetically susceptible individuals.

On a biological level, primary hypertension is associated with several abnormalities: overactivation of the renin–angiotensin–aldosterone system (RAAS), excessive sympathetic nervous system stimulation, impaired function of natriuretic peptides, and reduced availability of vasodilators like nitric oxide (Mancia *et al.*, 2007). These disturbances promote vascular constriction, altered sodium balance in the kidneys, and persistently elevated blood pressure.

Secondary hypertension, which represents about 5–10% of cases, has distinct clinical relevance because it stems from identifiable and often reversible causes. Patients with resistant, sudden-onset, or early-onset hypertension, especially when accompanied by systemic disease, should be evaluated for secondary forms (Mancia *et al.,* 2007).

Common underlying causes include kidney disorders (e.g., chronic kidney disease, renal artery narrowing), endocrine abnormalities (such as primary aldosteronism, Cushing's syndrome, and pheochromocytoma), obstructive sleep apnea, and cardiovascular defects like aortic coarctation. Secondary hypertension may also arise during pregnancy (e.g., preeclampsia), from hormonal treatments, or as a side effect of certain medications. Identifying and treating these root causes can often normalize or significantly improve blood pressure outcomes (Mancia *et al.,* 2007).

1.1.1 PRIMARY HYPERTENSION

Primary hypertension, also known as essential hypertension, is a long-term disorder characterized by persistently high blood pressure with no identifiable underlying cause. It represents the most common type of hypertension, accounting for approximately 90–95% of all cases (Carey *et al.*, 2022). Its importance lies in being a major risk factor for cardiovascular disease, stroke, and kidney damage, thereby posing a significant global health burden.

Although the precise origin of primary hypertension is still unclear, it is understood to result from a multifaceted interaction between genetic, environmental, and lifestyle determinants. Family history plays a central role, with evidence indicating that individuals with hypertensive relatives are more likely to develop the condition (Wang *et al.,* 2023). Genes that regulate the renin–angiotensin–aldosterone system (RAAS), sodium handling, and vascular tone are particularly associated with blood pressure regulation (Patel *et al.,* 2021).

Lifestyle and environmental influences contribute significantly as well. Obesity elevates both blood volume and vascular resistance, making it strongly linked with hypertension (Nguyen *et al.,* 2024). Other contributing behaviours include physical inactivity, excessive alcohol use, and diets high in sodium but deficient in potassium, which are especially common in Western populations and closely associated with higher blood pressure (Smith & Jones, 2023).

1.1.2 SECONDARY HYPERTENSION

Secondary hypertension represents approximately 5–10% of all hypertension cases and differs from primary hypertension in that it results from an identifiable medical condition or external factor. Several underlying disorders can contribute to its development. Chronic kidney disease, for example, disrupts fluid and blood pressure regulation, while adrenal gland disorders—such as primary aldosteronism or pheochromocytoma—interfere with hormone balance that normally governs vascular function. Certain medications, including oral contraceptives and nonsteroidal anti-inflammatory drugs (NSAIDs), are also known to induce elevated blood pressure. Additional causes include congenital abnormalities such as coarctation of the aorta and endocrine disorders like thyroid dysfunction.

In patients with diabetes mellitus, secondary hypertension is particularly significant, as long-term complications frequently damage the kidneys, leading to renal artery stenosis and other nephropathies that subsequently elevate blood pressure. Unlike primary hypertension, this form can often be reversed or substantially improved when the underlying cause is addressed—for instance, through surgical removal of adrenal tumors or by altering the patient's medication regimen. However, accurate diagnosis requires careful clinical assessment, which can be especially challenging in individuals who also suffer from diabetes (Viera and Neutze, 2010).

1.1.3 HISTORY OF THE PREVALENCE OF HYPERTENSION

Hypertension is among the oldest documented medical conditions, with references dating as far back as *Nei Jin* by Huang Ti around 2600 BC. Throughout history, it has played a significant role in shaping human health and remains a major global concern. The complications that arise

from uncontrolled hypertension—such as myocardial infarction, stroke, and heart failure—are projected to become the leading cause of death worldwide in the near future.

Despite decades of extensive scientific investigation, the exact cause of hypertension is still not fully understood. Only about 5% of cases can be linked to a clearly identifiable underlying condition, leaving the vast majority categorized as primary (or essential) hypertension. Interestingly, some scholars, including Sir Geoffrey Rose, have argued that essential hypertension should not be regarded strictly as a disease, but rather as a condition defined by a blood pressure level where medical intervention yields more benefit than harm.

Ultimately, an individual's blood pressure is determined by the dynamic interaction between the heart and blood vessels, and gaining deeper insights into this complex relationship is essential for fully understanding the pathophysiology of hypertension. (Mayet, 2003).

1.1.4 TREATMENT OF HYPERTENSION

Non Pharmalogical Treatment

- **Weight Reduction**

Dietary interventions to lower body weight are often recommended for overweight people with mild hypertension. In people with hypertension, weight reducing diet has been shown to reduce blood pressure and body weight. A 4kg reduction in body weight would achieve a BP reduction of 4.5/3.2 mmHg. There is evidence that showed a reduction of 1kg in weight relates to 1 mmHg reduction in SBP.37 However, the long-term effect of weight loss on mortality and morbidity in people with hypertension is unknown.

- **Healthy Eating**

A diet rich in fruits, vegetables and low fat dairy products with reduced saturated and total fat can substantially lower BP (11/6 mmHg in hypertensive patients and 4/2 mmHg in patients with high normal BP).60(Level I) A recent meta-analysis suggests that healthy dietary patterns such as the Dietary Approaches to Stop Hypertension (DASH), Nordic, and Mediterranean diet significantly lowered BP by 4.26/2.38 mmHg.61 These diets are rich in fruit, vegetables, whole grains, legumes, seeds, nuts, fish and dairy products and low in meat, sweets, and alcohol. A

recent cohort study that included Malaysian population without cardiovascular disease has shown that higher fruit, vegetable, and legume consumption was associated with a lower risk of non-cardiovascular and total mortality.

1.2 DIABETES

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyper-glycaemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases. (Bastaki, 2005).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. (American Diabetes Association, 2014).

1.2.1 TYPES OF DIABETES:

1.2.1.1 Insulin-Dependent Diabetes Mellitus (Type 1 Diabetes Mellitus)

Type 1 diabetes mellitus (T1DM), also called autoimmune diabetes, is a chronic condition in which the immune system mistakenly destroys the pancreatic beta cells that produce insulin. It often develops in children and young adults and typically presents suddenly, sometimes with life-threatening symptoms. Individuals with T1DM are more likely to have other autoimmune conditions such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease. The condition is usually associated with the presence of antibodies—including anti-glutamic acid decarboxylase (GAD), islet cell antibodies, or insulin autoantibodies—which confirm the

autoimmune mechanism behind beta-cell destruction (Savan *et al.,* 2024). The pace of beta-cell loss differs among individuals, progressing rapidly in some cases and gradually in others. As insulin production becomes severely impaired or absent, lifelong insulin therapy is required. At the time of diagnosis, most patients (85–90%) exhibit immune markers of beta-cell damage, even when fasting hyperglycaemia is first detected. While the precise cause of T1DM is still unclear, autoimmune processes are considered the primary driver (Savan *et al*., 2024).

1.2.1.2 Non–Insulin Dependent Diabetes Mellitus (Type 2 Diabetes Mellitus):

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes worldwide, accounting for 90–95% of cases. It develops through a complex pathophysiological process often described as the "ominous octet," with two central abnormalities: insulin resistance and progressive decline of beta-cell function (Alidrisi *et al.,* 2024). Unlike type 1, this form is not caused by autoimmune beta-cell destruction, and patients generally retain some endogenous insulin production. In its early stages, and often throughout life, individuals with T2DM may not require insulin therapy for survival. Risk factors vary, but obesity is a major contributor, as excess body fat strongly promotes insulin resistance. Although the exact causes of T2DM remain uncertain, most affected individuals present with overweight or obesity, which exacerbates metabolic dysfunction (American Diabetes Association, 2014).

1.2.1.3 Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) arises during pregnancy due to increased insulin resistance, primarily driven by placental hormones. This condition not only heightens the risk of maternal complications such as hypertensive disorders but also increases the likelihood of future type 2 diabetes in mothers. For infants, GDM is linked with adverse outcomes including perinatal complications, later obesity, and glucose intolerance. Management strategies typically begin with dietary modification, and in some cases, pharmacological treatments such as insulin, metformin, or glyburide may be used. Foetal monitoring helps determine whether therapy needs to be intensified. Postpartum glucose testing is essential to assess immediate diabetes risk, while ongoing annual monitoring of HbA1c and glucose levels helps predict the long-term development of type 2 diabetes. Preventive measures—such as adopting healthy lifestyle

practices, breastfeeding, and family planning—are strongly recommended to reduce risks in both mothers and children (Buchanan *et al.,* 2012).

1.2.2 HISTORY OF THE PREVALENCE OF DIABETES

Diabetes mellitus as a disease, for example a Dconstellation of symptoms, but not its pathogenesis, has been known by physicians for nearly 3,500 years in ancient Egypt. The Ebers papyrus dating from 1550 BC was found in a grave in Thebes region south of Egypt in 1862, and named after the Egyptologist Geary Ebers. The papyrus contains descriptions of various diseases, among them is a polyuric syndrome, presumably diabetes. The Egyptians suggested various remedies to this syndrome including a decoction of bowes, wheat and earth. The Verdic medical treatises from ancient India described, in detail, diabetes like conditions of 2 types: Congenital and late onset. Also, the Indians noticed the relation of diabetes to heredity, obesity, sedentary life and diet. They suggested the freshly harvested cereals and bituminous preparations containing benzoates and silica as a remedy for diabetes. The first time association of polyuria with a sweet-tasting substance was reported in the Indian literature from the 5th-6th century AD by Sushrant (a notable Indian physician). Araetus of Cappodocia (81-138 AD); who was best known for his differentiation between physical and mental disease, described diabetes as a polyuric wasting disease. Araetus said "Diabetes is a wonderful affection being a melting down of the flesh and limbs into urine. The patient never stops drinking water but the flow is incessant as if from the opening of adequeducts. The patient is short lived". Araetus used the Greek word diabetes, literally meaning to run through or siphon, to describe the disease. An Arab physician, Avicenna (960-1037) described accurately the clinical features and some complications of diabetes (peripheral neuropathy, gangrene and erectile dysfunction). Avicenna emphasized the idea of sweet taste of urine and may have introduced it to the European observers as his book (Kanon) had influenced the medical practice for several centuries. (Ahmed, 2002)

1.2.3 TREATMENT OF DIABETES

The general goals of the treatment of diabetes are to avoid acute decompensation, prevent or delay the appearance of late disease complications, decrease mortality, and maintain a good quality of life. As for chronic complications of the disease, it is clear that good control of

glycemia makes it possible to reduce the incidence of microvascular complications (retinopathy, nephropathy, and neuropathy) whereas good control of glycemia per se does not seem to be as determinant in the prevention of macrovascular complications (ischemic heart disease, cerebrovascular disease, peripheral arteriopathy). The diet –which generally must be low-calorie due to the frequency of associated obesity– and a program of regular exercise are the basis of the treatment of type 2 diabetes mellitus. When acceptable metabolic control is not achieved, either because the patient does not adapt to changes in life style or because, in spite of complying with the diet and exercising regularly, therapeutic objectives are not attained, pharmacological treatment must begin. (Simó and Hernández, 2002).

- **Pharamalogical Treatment**

In the mid-1950s the first sulfonylureas (SU) were developed for commercial use (carbutamide and tolbutamide).

The SUs stimulates the second phase of insulin secretion by pancreatic beta cells, that is to say, the release of preformed insulin. Therefore, the SUs requires the presence of a critical mass of beta cells with insulin secretory capacity in order to act. Therefore, the SUs will not be effective in patients who are pancreatectomized or have type 1 diabetes mellitus. The SUs act through high-affinity receptors located in the pancreatic beta cells. Binding to these receptors inhibits the opening of ATP-sensitive potassium channels and avoids potassium outflow from the cell, thus triggering cell membrane depolarization. As a result, the calcium channels open, increasing intracellular calcium content and calcium binding to calmodulin, which produces microfilament contraction and the exocytosis of insulin granules. In the heart and throughout the cardiovascular system there are also SU receptors and ATP-sensitive potassium channels, which have an important cardioprotective effect against ischemia. Closure of these channels by SUs could contribute to ischemia1 Nevertheless, although this possible harmful effect seems evident in experimental studies in which high doses of SUs are administered acutely, this does not seem to be clinically relevant, as has been shown in the UKDPS study (Simó and Hernández, 2002).

1.3 HYPERTENSION AND DIABETES UNDER CORMORBIDITY DISORDER

Comorbidity has a myriad of definitions especially in health care, but the commonly acceptable definition is the presence of one or more disorders in addition to the index disorder or diseases. Alternatively, it is the presence of two or more conditions simultaneously in one person, with no special regard to their pathology or being interdependent with each other. In this literature review, concentration is on comorbidity of diabetes and hypertension regardless of whether diabetes or hypertension is the primary disorder. Diabetes mellitus and hypertension are commonly known as concordant co morbid conditions, although at some point can occur independently. Comorbidities can have profound effects on patients' ability to manage their self-care and comorbid illnesses can sap the financial resources of people with diabetes by increasing their outof-pocket costs for medical care. Health care systems in developed and developing countries have a huge responsibility of health service delivery to people with comorbid or multiple chronic conditions, who are prone to increased utilization of health care services. Lack of adherence to clinical guidelines by health care providers on management of comorbid conditions has been found to be a factor (Ahmed *et al.,* 2017).

Recent studies have shown that, up to 75% of adults with diabetes also have hypertension in USA alone and patients with hypertension alone often show evidence of insulin resistance. In most developing countries it is estimated that comorbid diabetes and hypertension is a 40-60% among patients with chronic diabetes and hypertension affecting the most productive age groups of below 60years of age. The disease may precede diabetes mellitus, be diagnosed at same time as diabetes or may be as a result of diabetic nephropathy. With a global shift from communicable disease to non-communicable disease and from premature death due to years lived with disability, Sub-Saharan Africa where Eastern Africa countries exist lacks literature on treatment outcomes of patients with comorbid hypertension and diabetes mellitus especially in primary health care settings (PHC) (Ahmed *et al.,* 2017).

The coexistence of hypertension and diabetes is a major contributor to the development and progression of diabetes complications. Up to 80% of people with diabetes will die of cardiovascular disease. The comorbid hypertension in diabetic patients is attributed to the risk of death and cardiovascular disease (CVD) events by 44% and 41%, respectively, as compared to 7% and 9% of these risks in people with diabetes alone. Furthermore, the development of

hypertensive in diabetic patients increases healthcare costs and complicates treatment strategy. Diabetes approximately doubles risk of cardiovascular diseases and concomitant hypertension nearly doubles that risk again. Moreover, diabetes related renal dysfunction, and increased arterial stiffness have been proposed as contributing factors for the development of hypertension in diabetics. Focusing on detecting and managing hypertension in patients with diabetes is one of the most effective things that can be done to prevent diabetes complications. Overweight/obesity, older age, lack of physical exercise, unhealthy diet, smoking and family history of hypertension are major risk factors for hypertension development among diabetic patients (Long and Daggo, 2011).

1.4 CHALLENGES ASSOCIATED WITH THE COEXISTENCE OF HYPERTENSION AND DIABETES

Hypertension and diabetes mellitus are known to be the two most common diseases in the westernized world and the risk of both diseases increases with age. Statistically, about 2.5 to 3 million Americans have been diagnosed with both hypertension and diabetes. Hypertension is reported in over two-thirds of patients with diabetes Coexistence of Hypertension with Diabetes Mellitus and Its Pharmacotherapy specifically type 2 diabetes. Hypertension is prevalent among patients with diabetes. However, this depends on the duration and type of diabetes, sex, age, ethnicity and race of the patient, body mass index (BMI) of the patient, presence of other diseases such as kidney disease, history of glycemic control and many other factors. Greater body mass, longer duration of diabetes, and the presence of chronic proteinuria are all key factors of higher blood pressure, particularly systolic pressure, in the diabetic patients. Hypertension in diabetic patients is more frequent in men than in women before the fifth decade and more frequent in women thereafter. (Moke *et al.,* 2023). The coexistence of both diseases is more prevalent among blacks than whites; the socioeconomically disadvantaged have a higher occurrence of both conditions. However, other unknown factors are likely to play a role in the higher prevalence of hypertension in patients with diabetes mellitus. (Moke *et al.,* 2023). The common coexistence of hypertension and diabetes is not a mere coincidence but rather due to some intertwined pathogenic mechanisms. Patients with diabetes are twice likely to develop hypertension as compared to non-diabetic patients and patients with hypertension are more likely to develop diabetes as compared to normotensive people. (Wang *et al.,* 2021).

Some of the challenges of coexistence of hypertension and diabetes are;

- **Heart-related complications**

Comorbidity increases the risk of heart failure, stroke, and coronary artery disease (CAD). The combined effects of diabetes and hypertension accelerate atherosclerosis and left ventricular hypertrophy, increasing cardiovascular morbidity and death by two to four times (Buse *et al.,* 2007).

- **Issues with the kidneys**

Diabetes and high blood pressure are the two primary causes of chronic kidney disease (CKD). Diabetic nephropathy and hypertensive nephrosclerosis exacerbate glomerular damage, resulting in proteinuria and the onset of end-stage renal disease (ESRD) (Reutens, 2013).

1.5 THE EFFICACY OF MEDICINAL PLANTS IN TREATING HYPERTENSION AND DIABETES

Hypertension and diabetes are among the fastest-rising noncommunicable diseases in sub-Saharan Africa, imposing a heavy burden on healthcare systems. According to recent estimates, roughly 19 million African adults have diabetes, (Frimpong *et al.,* 2024) and hypertension rates are equally alarming. In many African communities, limited access to modern drugs and the high cost of care have led patients to rely on traditional herbal remedies. Indigenous herbal therapies are widely used for these chronic conditions. For example, a 2024 review of African diabetes care reported that oral herbal decoctions prepared from leaves or barks are a common first line of treatment (Frimpong *et al.,* 2024) Traditional medicine practitioners often cite plants such as Allium sativum (garlic), Olea europaea (olive), Azadirachta indica (neem), Allium cepa (onion) and Moringa oleifera as key antidiabetic remedies. Similarly, herbs like *Hibiscus sabdariffa* (bissap) and *Combretum micranthum* (kinkeliba) are popular for blood pressure control.

1.6 REGIMEN FOR THE TREATEMNT OF HYPERTENSION AND DIABETES

Angiotensin-converting enzyme (ACE) inhibitors are widely prescribed medications for the management of hypertension as well as chronic conditions such as heart failure and kidney disease. Their mechanism of action involves inhibiting the enzyme responsible for converting angiotensin I to angiotensin II — a potent vasoconstrictor that narrows blood vessels. By blocking this conversion, ACE inhibitors promote vasodilation (widening of blood vessels), enhance sodium and water excretion (natriuresis), and suppress sympathetic nervous activity. These combined effects result in a significant reduction in blood pressure.

Because of their proven efficacy and safety, ACE inhibitors are often considered first-line agents for treating high blood pressure. They are also commonly used in combination with other drug classes such as thiazide diuretics, calcium channel blockers, or angiotensin receptor blockers (ARBs) to achieve optimal blood pressure control (Cutrell *et al.,* 2023).

Beyond blood pressure reduction, ACE inhibitors offer cardiovascular protection through additional mechanisms. They inhibit the degradation of bradykinin, a peptide that stimulates nitric oxide production and promotes improved circulation. Bradykinin also plays an important role in reducing inflammation, oxidative stress, and the risk of thrombosis within blood vessels. Furthermore, ACE inhibitors enhance fibrinolysis—the body's natural process of dissolving clots—thereby lowering cardiovascular risk. By alleviating vasoconstriction and supporting endothelial function, these drugs contribute to the prevention of major cardiovascular events such as myocardial infarction and stroke (Strauss *et al.,* 2023).

1.7 SIDA ACUTA

Sida acuta (*S. acuta*), commonly known as broom weed, is a tropical plant with notable medicinal potential and is widely distributed across tropical regions. It is a drought-resistant perennial shrub that grows abundantly in various environments. *S. acuta* is an erect and branched plant, reaching up to 1 meter in height, with a woody taproot and fibrous, hairy stems—particularly when young. The leaves are simple and alternately arranged, while the inflorescences are solitary and axillary, borne on stalks up to 1.3 cm long and jointed midway. The plant produces yellow flowers with five petals and a capsular fruit composed of five to six carpels. Owing to its abundance and pharmacological potential, studies such as that of (Shittu *et al.,* 2020) have examined its phytochemical constituents to promote its medicinal utilization among local populations.

Sida acuta is indigenous to pantropical regions and has been traditionally employed in the treatment of various ailments including asthma, inflammation, fever, ulcers, and the common cold. The plant typically grows in cultivated fields, along roadsides, waste areas, and drainage channels, where it is sometimes referred to as “Sengh.” In certain regions, chewing the leaves of *S. acuta* is traditionally used in the treatment of gonorrhea (Paarakh *et al.*, 2023).

Fig 1.1: Sida Acuta (Hyde *et al.,* 2025) (Source: University of Benin football field)

1.8 CLEOME RUTIDOSPERMA

Cleome rutidosperma, commonly known as the fringed spider flower, is a flowering herbaceous plant belonging to the family *Cleomaceae*. It is an erect herb with a sparsely branched stem, typically reaching a height of 50–100 cm, and is believed to have originated from West Africa. The species is now widely distributed across Southeast Asia and several tropical regions of the Americas. Although *Cleome rutidosperma* often grows as a weed, it is occasionally cultivated as a potherb in West Africa, where its leaves are used in soups or consumed as cooked vegetables.

In traditional Indian medicine, *Cleome rutidosperma* is reputed for its therapeutic properties and is used in the management of paralysis, convulsions, epilepsy, pain, spasms, and skin diseases. Similarly, in the Democratic Republic of Congo, Ghana, and Gabon, the leaf sap is applied in the treatment of deafness and earaches, while in Nigeria, the leaf extract is traditionally used to treat convulsions. Although several studies have isolated and identified bioactive compounds from this plant, information regarding its nutritional composition remains limited. Hence, recent research has sought to determine the phytochemical, mineral, and proximate compositions of *C. rutidosperma*, which could provide valuable insights into its medicinal potential and nutritional value (Akinsola & Oluwafemi, 2022).

Fig 1.2: Cleome Rutidosperma (Source: University of Benin football field)

1.9 HUNTERIA UMBRELLATA

Hunteria umbellata (Family: *Apocynaceae*) is a glabrous tree native to West Africa, commonly known as *Demonuain* in French and *Abeere* among the Yoruba people of southwestern Nigeria. In African traditional medicine, a water decoction prepared from the dried seeds of *H. umbellata* is highly valued for its therapeutic effects in managing pain, swellings, infections, gastric ulcers, diabetes mellitus, obesity, and for inducing labour at term (Ajiboye *et al.*, 2017). The plant is reputed for its wide range of medicinal applications, including the treatment of fever, leprosy sores, stomach and liver disorders, and as an anthelmintic, particularly effective against intestinal worms (Nwaogwugwu *et al.*, 2022).

Botanically, *Hunteria umbellata* may grow as a shrub or small tree reaching heights of up to 22 meters with a trunk diameter of approximately 40 cm. Its flowers are characterized by a white, creamy, or pale-yellow corolla, while the fruit is smooth and yellow when mature. The species typically thrives in forest habitats ranging from sea level to altitudes of about 600 meters. The genus *Hunteria*, belonging to the family *Apocynaceae*, was first described in 1824 and includes species native to both Africa and parts of South and Southeast Asia.

Fig 1.3: Hunteria umbellata (Source: Oba Market)

1.10 AIM AND OBJECTIVES

The aim of the study is to evaluate the antihypertensive/antidiabetic or pancreatic-protective effects of *Sida acuta, Cleome rutidosperma* and *Hunteria umbellata* in male wistar rats.

1.10.1 OBJECTIVES

1. To induce hypertension and diabetes using L-NAME (ω-Nitro-L-arginine methyl ester) and streptozotocin respectively.
2. To investigate the antihypertensive/antidiabetic effect of Cleome rutidosperma/Hunteria umbellata on L-NAME (ω-Nitro-L-arginine methyl ester) and Streptozotocin induced hypertension/diabetes of both aqueous and methanol group

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Plants: *Sida acuta* and *Cleome rutidosperma* plants were harvested around the Department of Biochemistry environment, while the seed plants, *Hunteria umbellate* was in Oba market at ring-road and New Benin Market, Benin City, Edo State. The plants were all conveyed firstly to the Department of Plant Biology and Biotechnology (PBB) for identification by Prof Akinnibosun Adewale Henry. Voucher Numbers UBH-S454 (*sida acuta*), UBH-C148 (*Cleome rutidosperma*), and UBH-H637 (*Hunteria umbellata*) were given to the individual plants for reference purpose and deposited at the herbarium for a while. Thereafter, the plants were taken to the Department of Biochemistry Laboratory of about 20 days for air-drying and then pulverized at the Department of Pharmacology Laboratory in the University of Benin.

Animal: Thirty-five (35) Healthy adult male Wistar rats with weights ranging from 120g-150g were purchased and taken to the animal house in the Department of Biochemistry, Faculty of Life Sciences, University of Benin. The Wistar rats were acclimatized for two weeks in the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Edo State. The animals were kept in a cage at room temperature and had free access to water and standard feed (grower pellet) throughout the duration of acclimatization and administration. Sanitation of the cage and its surroundings was ensured in order to prevent infection and contamination.

2.1.1 PREPARATION OF PLANT EXTRACT

A 156g of *sida acuta* was weighed and immersed in aqueous extract and another 156g of the same plant was weighed and also submerged in 99.8% methanol extract. Upon standardizing, it was deduced that if 500g of the sample (plant) is dissolved in 2.5L of the extract (aqueous or methanol), therefore each 156g of *Sida acuta* will be dissolved in 0.78L which is approximately equal to 780ml of aqueous extract and methanol extract respectively. Following the same procedure, a 189g of *Cleome rutidosperma* weighed and submerged in aqueous extract and

another 189g of the same plant was weighed and also submerged in 99.8% methanol extract. By standardization, if 500g of the plant is dissolved in 2.5L of the extract, therefore each 189g of *Cleome rutidosperma* will be *Hunteria umbellata* plant seed was weighed to be submerged in aqueous extract and another 330g of the same plant seed for 99.8% methanol extract. By standardizing, if 500g of the plant seed (*Hunteria umbellata)* is dissolved in 2.5L of the extract, therefore each 330g of *Hunteria umbellata* seed plant was dissolved in 1.65L ≡ 1650mL of aqueous extract and methanol extract respectively. These samples (plants) were well dissolved in their respective extracts and were continuously vortexed at an interval of 2 hours within 24 hours. After 24 hours, the samples were extracted using muslin cloth to obtain the filtrate and were re-submerged using the same volume of extract used before for another 24 hours. The same procedure was carried out after the 24 hours and the filtrate were obtained. The filtered extract was duly freeze-dried in the University of Benin Energy Centre to obtain the extracts of aqueous and methanol for the respective plants and seeds. These extracts obtained were kept and stored in sterile bottles and kept in the refrigerator at -18ºC temperature until they were needed for analysis.

2.1.2 Drugs

Drugs obtained for this project are streptozotocin (STZ), L-NAME (Nitro-L arginine methyl ester). While Glibenclamide and Lisinopril were used for treatment of Diabetes and Hypertension respectively.

Fig 2.1 Lisinopril (Source: Osagie-Eweka Laboratory)

Figure 2.2 Glibenclamide (Source: Osagie-Eweka Laboratory)

2.1.3 MATERIALS AND APPARATUS

39 males wistar rats, Lisinopril, Glibenclamide, 99.8% methanol, Sterile water, Methanol, Restrainer, Glucometer, Glucose strips, MRBP, Paper towel, Rat feed, Test tubes, Picric acid, centrifuge, spectrophotometer, masking tapes, distilled and sterile water, EDTA bottles, dissecting sets, plain bottles, plates, Sensitive Balance, Water bath, Hand gloves, Pipette, Cotton wool, pH meter, Refrigerator, Syringes (1ml and 5ml), Universal bottles, assay kits (Cholesterol, triglycerides, LDL, ALT, AST, sodium, potassium, GGT, Creatinine randox & Urea), Citric acid, Sodium citrate, 40% formalin, gavage, syringes (1ml and 5ml), beakers, measuring cylinder (10ml, 100ml and 1000ml), fan, tissue bag, morning fresh soap, mortar and pestle, tissue bag, foil paper, normal saline, 9g of sodium chloride, muslin cloth, nose masks and shower cap.

2.2 METHODS

2.2.1 EXPERIMENTAL DESGIN AND ADMINSTRATION

The experiment was performed on thirty-five (35) male Wistar rats. The thirty-nine rats were divided into seven (7) groups unequally. The extracts (aqueous and methanol) were administered to the rats orally with respect to their body weight. Based on this research, seven (7) groups were studied and they are;

Group 1: NORMOTENSIVE / NON-DIABETIC (POSITIVE CONTROL)

Rats in this group were fed with normal fed and clean tap water daily with no administration of L-NAME, streptozotocin (STZ), aqueous or methanol extracts.

Group 2: HYPERTENSIVE / DIABETIC (NEGATIVE CONTROL)

Rats in the group were fed with normal feed, clean tap water and induced with L-NAME/STZ without administering the extracts or treatments.

Group 3: HYPERTENSIVE / DIABETIC + Treated with Lisinopril (10mg/kg) / Glibenclamide (5mg/kg)

Rats in this group were fed with normal feed, clean tap water daily and induced with L-NAME/STZ, then treated with Lisinopril and Glibenclamide and no extracts.

Group 4: HYPERTENSIVE / DIABETIC + *Sida acuta* + *Cleome rutidosperma* Aqueous extract (50mg/kg).

Rats in this group were fed with normal feed, clean tap water daily and induced with L-NAME/STZ, then treated with the aqueous extracts (*Sida acuta* + *Cleome rutidosperma*).

Group 5: HYPERTENSIVE / DIABETIC + *Sida acuta* + *Cleome rutidosperma* Methanol extract (50mg/kg).

Rats in this group were fed with normal feed, clean tap water daily and induced with L-NAME/STZ, then treated with the methanol extracts (*Sida acuta* + *Cleome rutidosperma*).

Group 6: HYPERTENSIVE / DIABETIC + *Cleome rutidosperma* + *Hunteria Umbellata* Aqueous extract (50mg/kg).

Rats in this group were fed with normal feed, clean tap water daily and induced with L-NAME/STZ, then treated with the aqueous extracts (*Cleome rutidosperma* + *Hunteria Umbellata*).

Group 7: HYPERTENSIVE / DIABETIC + *Cleome rutidosperma* + *Hunteria Umbellata* Methanol extract (50mg/kg).

Rats in this group were fed with normal feed, clean tap water daily, induced with L-NAME/STZ, and then treated with the methanol extracts (*Cleome rutidosperma* + *Hunteria Umbellata*).

Figure 2.3 Grouping of the rat cage

2.2.2 MEASUREMENT OF BODY WEIGHT

The weight (baseline) of each rat for all groups were measured first week before acclimatization using the sensitive weighing balance. After acclimatization and treatment, the weight of all the rats were measured again. The average weight of the rats before acclimatization was gotten to be 119g while after treatment was gotten to be 122g.

2.2.3 ACCLIMATIZATION AND RESTRAINING OF WISTAR RATS

A total of Thirty-five (35) Wistar rats were procured and housed under standard conditions, with regular feeding and daily cleaning of their cages. The animals were allowed a two-week acclimatization period in the Animal House of the Department of Biochemistry, University of Benin, to adapt to their new environment. Following this, the rats were gradually familiarized with the experimental procedures by being restrained in a restrainer and exposed to a hand fan. This step was carried out to further acclimatize them to the operational conditions of the MRBP system and to ensure reliable measurement outcomes.

2.2.4 EXPERIMENTAL INDUCTION OF HYPERTENSION AND DIABETES IN MALE WISTAR RAT

To establish experimental models of hypertension and diabetes, several pharmacological agents are commonly employed. In this study, hypertension is induced using L-NAME (Nω-nitro-L-arginine methyl ester), a nitric oxide synthase inhibitor that blocks nitric oxide synthesis. This inhibitor leads to increased vascular resistance and elevated systolic blood pressure (above 80mmHg) due to the reduced vasodilatory effect of nitric oxide. The model effectively reproduces hypertensive pathophysiology observed in humans, such as vascular remodeling and heightened peripheral resistance.

Diabetes on the other hand, is experimentally induced using Streptozotocin (STZ), a glucosamine derivative of nitrosourea naturally produced by the fungus Streptomyces achromogenes. STZ exhibits antibacterial properties and selectively destroy pancreatic β-cells by generating free radicals and nitric oxide, leading to cellular necrosis. Upon entering pancreatic cells via the glucose transporter GLUT2, it initiates β-cell death, resulting in hyperglycemia characteristic of diabetes.

Figure 2.4 MRBP System

(Source: Osagie-Eweka Laboratory)

Figure 2.5 Weighing balance

(Source: Osagie-Eweka Laboratory)

Figure 2.6: Sterile water

(Source: Osagie-Eweka Laboratory)

Figure 2.7: Glucometer and test strips

(Source: Osagie-Eweka Laboratory)

Figure 2.8: Centrifuge

(Source: Osagie-Eweka Laboratory)

Figure 2.9: Restrainer

(Source: Osagie-Eweka Laboratory)

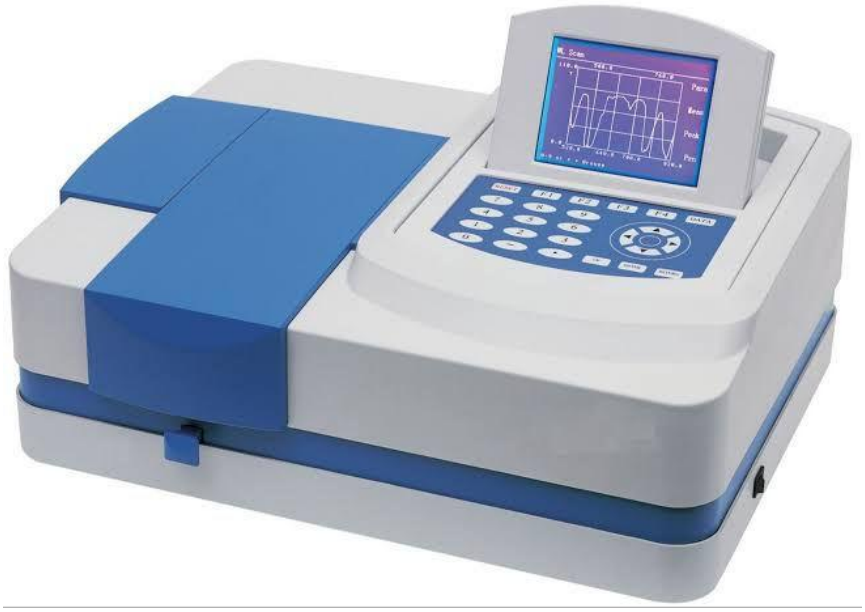

Figure 2.10: Spectrophotometer

(Source: Osagie-Eweka Laboratory)

2.2.5 PROCEDURES FOR TESTING BLOOD GLUCOSE LEVEL

Rats tail was warmed up carefully to ease flow of blood and visibility of the veins. This was carried out for about five (5) to ten (10) minutes. Cotton wool soaked in methylated spirit was used to clean the tail, preventing any form of contamination before pricking. The tail was pricked with the provided lancet. Then the blood was applied from the right side of the test strip to allow the blood flow to the remaining part of the strip via capillary action. A beep was observed indicating proper flow of blood into the test strip. After eight (8) seconds, a reading showing the blood glucose level of the rat was observed.

2.2.6 PROCEDURES FOR TAKING BLOOD PRESSURE

The rats were acclimatized to minimize stress, ensuring more reliable and accurate values. Before taking the values, the MRBP machine were preheated for one hour, and the system was properly calibrated. The temperature within the MRBP chamber was maintained at 34ºC to provide suitable conditions for accurate hemodynamic readings once the rats were placed inside.

2.2.7 PREPARATION OF STERILE CITRATE BUFFER

Reagents used: Citric acid, sodium citate, sterile water

(pH=4.5, Concentration= 0.1m)

Using Henderson Hasselbech Equation

$$pH = pka + \text{Log}\frac{[\boldsymbol{Conjugate\ base}]}{[\boldsymbol{Conjugate\ acid}]}$$

$$\text{Log}\frac{[\boldsymbol{Sodium\ Citrate}]}{[\boldsymbol{Citric\ acid}]} = 4.5 - 4.76$$

$$\frac{[\boldsymbol{Sodium\ Citrate}]}{[\boldsymbol{Citric\ acid}]} = -\ 0.26$$

$$\frac{[\boldsymbol{Sodium\ Citrate}]}{[\boldsymbol{Citric\ acid}]} = 10^{-0.26} = 0.54 = \frac{\mathbf{0.54}}{\mathbf{1}}$$

Therefore [Sodium Citrate] + [Citric acid] = 1 + 0.549

[Sodium Citrate] + [Citric acid] = 1.549

If 0.1mol is equivalent to 1000ml, therefore 0.01mol will be equivalent to 100ml

Therefore;

$$\text{Sodium citrate} = \frac{0.549}{1.549} \text{ x } 100 = 0.0035\text{M}$$

$$\text{Citric acid} = \frac{1}{1.549} \text{ x } 100 = 0.00645\text{M}$$

Molecular weight;

Citric acid = 210.14g, Sodium citrate = 294.10g

Reacting mass = $\frac{Concentration}{Molecular\ Wt}$

M (Citric acid) = 0.00645 x 210.14 = 1.355g

M (Sodium citrate) = 0.0035 x 294.10 = 1.029g

Procedure:

To prepare a 100ml solution of 0.1M sterile citrate buffer at pH 4.5. Dissolve 1.355g of citrate acid and 1.029g of sodium citrate in sterile water Adjust the final volume to 100ML. Check and adjust the pH if necessary.

2.2.8 PREPARATION OF FORMAL SALINE

Weigh 9g of Nacl and dissolve in 900ml of distilled water, measure 40ml of formal saline and the add 60ml of distilled to make it up to 100ml. Add the 100ml of formal saline solution to the 900ml of Nacl solution to make it a 1000ml formal saline solution.

2.2.9 SACRIFICE OF ANIMALS

After treatment for two (2) weeks, the animals were fasted overnight (before the sacrifice day) and sacrificed. Each of the rats were anesthetized with Urethane according to their body weight. While under anesthesia, the thoracic and abdominal region were opened and the blood was taken from the heart using a 5ml syringe and kept in sample bottles and plain sample bottles. The heart was harvested and weighed, and stored in formal saline universal sample bottles for histology.

DOSAGE ADMINISTRATION CHART

RAT I.D	HYPERTENSIVE/ DIABETIC (-VE CONTROL)		HYPERTENSIVE/DIABETIC + LISINOPRIL (10mg/kg)/ GLIBENCLAMIDE (5mg/kg) (ml)				HYPERTENSIVE/DIABETIC + *SIDA ACUTA/CLEOME RUTIDOSPERMA* AQUEOUS EXTRACT (50mg/kg) (ml)		
	L-NAME	STZ	LIS	GLIB	L-NAME	STZ	AQ	L-NAME	STZ
HEAD	0.588	0.735	1.31	0.655	0.524	0.650	0.525	0.420	0.525
BACK	0.432	0.540	1.51	0.755	0.604	0.750	0.665	0.532	0.665
R.H LIMB	0.452	0.565	1.17	0.585	0.468	0.585	0.575	0.460	0.575
L.H LIMB	0.672	0.840	1.40	0.700	0.560	0.700	0.620	0.496	0.620
R.F LIMB	0.448	0.560	1.56	0.780	0.624	0.780	0.510	0.408	0.510
L.F LIMB	-	-	-	-	-	-	0.650	0.520	0.650

HYPERTENSIVE/DIABETIC + *SIDA ACUTA/CLEOME RUTIDOSPERMA* METHANOL EXTRACT (50mg/kg) (ml)			HYPERTENSIVE/DIABETIC + CLEOME RUTIDOSPERMA/ HUNTERIA UMBELLATA AQUEOUS EXTRACT (50mg/kg) (ml)			HYPERTENSIVE/DIABETIC + CLEOME RUTIDOSPERMA/ HUNTERIA UMBELLATA METHANOL EXTRACT (50mg/kg) (ml)		
MTH	L-NAME	STZ	AQ	L-NAME	STZ	MTH	L-NAME	STZ
0.735	0.588	0.735	0.590	0.472	0.590	0.500	0.400	0.500
0.490	0.392	0.490	0.570	0.456	0.570	0.400	0.320	0.400
0.565	0.452	0.565	0.640	0.512	0.640	0.590	0.472	0.590
-	-	-	0.535	0.428	0.535	0.685	0.548	0.685
0.560	0.448	0.560	0.645	0.516	0.645	0.500	0.400	0.500
-	-	-	-	-	-	-	-	-

CHAPTER THREE

RESULTS

3.1 HEMATOLOGICAL PARAMETERS OF GROUPS

This chapter presents the results of the comparative effects of aqueous fraction of *Cleome rutidosperma / Hunteria umbellata* or *Cleome rutidosperma / Hunteria umbellata* Methanol fraction on Hematopoesis of Hypertensive/Diabetic male Wistar Rats. The findings include the effect on white blood cells (WBC) counts and differential counts, red blood cell (RBC) counts and other related hematological and coagulation parameters. Results are shown in tabular form, where the data are expressed in mean ẋ ± standard deviation (SD) and analyzed by one way ANOVA followed by post hoc test. Superscript letters (a, b, c, bc) denote significant differences between groups where applicable ($p < 0.05$), while no significant difference is indicated where $p > 0.05$.

3.2 WHITE BLOOD CELL (WBC) AND DIFFERENTIAL COUNTS

Table 3.1 shows the outcomes of WBC and differential counts, including lymphocyte counts (LYM#) and percentages (LYM%), granulocyte counts (GRAN#) and percentages (GRAN%), as well as MID (mid-sized cells such as monocytes, eosinophils and basophils) counts and percentages across the different experimental groups.

Table 3.1 White Blood Cell (WBC) and Differential Counts

GROUP	N/ND	HYP/D	CR/HU (AQ)	CR/HU (MTH)
WBC (x 10^3 /µL)	**6.67 ± 1.46^{a}**	**5.36 ± 0.85^{a}**	**7.60 ± 1.13^{a}**	**7.83 ± 1.01^{a}**
LYM (%)	**66.8 ± 4.53^{a}**	**59.2 ± 3.17^{a}**	**66.5 ± 5.73^{a}**	**62.4 ± 4.66^{a}**
MID (%)	**16.23 ± 1.57^{a}**	**13.33 ± 2.26^{a}**	**16.65 ± 0.64^{a}**	**23.6 ± 0.45^{bc}**
GRAN (%)	**21.4 ± 0.99^{a}**	**27.5 ± 4.06^{a}**	**16.8 ± 5.09^{a}**	**16.73 ± 1.40^{b}**

GROUP	N/ND	HYP/D	CR/HU (AQ)	CR/HU (MTH)
LYM# (x 10^3 /µL)	**4.60 ± 1.20[a]**	**3.22 ± 0.41[a]**	**5.33 ± 0.55[b]**	**4.97 ± 0.55[a]**
MID# (x 10^3 /µL)	**1.20 ± 0.00[a]**	**0.73 ± 0.21[a]**	**1.25 ± 0.21[a]**	**1.15 ± 0.70**
GRAN# (x 10^3 /µL)	**0.75 ± 0.07[a]**	**1.43 ± 0.25[a]**	**1.30 ± 0.57[a]**	**1.40 ± 0.20[a]**

Values are expressed as mean ± SD. Mean values in the same row with different subscript (a, b, c, bc) differ significantly ($p < 0.05$) using Bonferroni post hoc test (a) = non-significant, (b) significant to normotensive, (c) = significant when compared to the hypertensive/diabetic group and (bc) = significant when compared to normotensive and hypertensive/diabetic group.

3.2.1 INTERPRETATION OF WHITE BLOOD PARAMETERS

A significant increase was observed in (MID%, 23.6 ± 0.45) on the *Cleome rutidosperma / Hunteria umbellata* methanol extract when compared to the N/ND and HYP/D group. Although there was also a slight increase observed in the *Cleome rutidosperma / Hunteria umbellata* aqueous extract when compared to the hypertensive/diabetic group although not siginificant. However, there was a significant decrease in (GRAN%; 16.73 ± 1.40) in the methanol treated group compared to the HYP/D group, while (LYM#; 5.33 ± 0.55) exhibited a significant increase in the aqueous treated group compared to the HYP/D.

3.3 RED BLOOD CELL (RBC) AND ITS PARAMETERS

Table 3.2 summarizes red blood cell (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), along with standard

deviation and coefficient of variations for range of width of RBC (RDW-SD and RDW-CV) across the experimental groups.

Table 3.2 Red Blood Cell (RBC) and Related parameters

GROUP	N/ND	HYP/D	CR/HU (AQ)	CR/HU (MTH)
RBC (10^6/µL)	8.35 ± 0.12[a]	8.36 ± 0.24[a]	8.15 ± 0.53[a]	7.47 ± 0.16[bc]
HGB (g/dL)	15.8 ± 0.15[a]	15.0 ± 0.36[a]	16.6 ± 0.49[a]	14.0 ± 0.77[b]
HCT (%)	45.5 ± 1.27[a]	45.4 ± 2.40[a]	44.2 ± 4.34[a]	39.9 ± 2.43[a]
MCV (fL)	54.5 ± 1.32[a]	53.3 ± 1.19[a]	54.2 ± 2.37[a]	54.8 ± 1.87[a]

GROUP	N/ND	HYP/D	CR/HU (AQ)	CR/HU (MTH)
MCH (pg)	18.9 ± 0.34[a]	18.6 ± 0.37[a]	19.1 ± 0.75[a]	19.5 ± 0.40[a]
MCHC (g/dL)	34.9 ± 0.83[a]	34.5 ± 0.65[a]	35.4 ± 1.46[a]	35.0 ± 0.32[a]
RDW-SD (fL)	38.5 ± 0.00[a]	39.3 ± 1.92[a]	41.2 ± 2.99[a]	41.3 ± 1.21[a]
RDW-CV (%)	16.8 ± 0.26[a]	17.32 ± 0.57[a]	17.6 ± 0.28[a]	17.68 ± 0.52[a]

Values are expressed as mean ± SD. Mean values in the same row with different subscript (a, b, c, bc) differ significantly ($p < 0.05$) using Bonferroni post hoc test (a) = non-significant, (b) significant to normotensive, (c) = significant when compared to the hypertensive/diabetic group and (bc) = significant when compared to normotensive and hypertensive/diabetic group.

3.3.1 INTERPRETATIONS OF RED BLOOD CELLS AND RELATED PARAMETERS

The results shows that *Cleome rutidoserma* and *Hunteria umbellata* methanol treated group exhibited a significant decrease in red blood cell (RBC) count ($7.47 \pm 0.16 \times 10^6$ µL, $p < 0.05$) and hemoglobin (HGB, 14.0 ± 0.77 g/dL), suggesting potential anemia effect. Conversely, the *Cleome rutidoserma* and *Hunteria umbellata* aqueous treated group also exhibited an increase in hemoglobin (16.6 ± 0.49) in comparison to HYP/D group, though non-statistically significant.

3.4 PLATELETS AND COAGULATION PARAMETERS

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

Table 3.3 Platelets count (PLT) and Related parameters

GROUP	N/ND	HYP/D	CR/HU (AQ)	CR/HU (MTH)
PLT (10^3 /µL)	530.67 ± 75.27^{a}	432.33 ± 88.71^{a}	390.00 ± 15.56^{a}	325.33 ± 4.93^{b}
PCT (%)	0.39 ± 0.06^{a}	0.36 ± 0.01^{a}	0.28 ± 0.01^{a}	0.26 ± 0.02^{b}
P-LCR (%)	5.70 ± 0.71^{a}	7.30 ± 1.01^{a}	2.45 ± 0.35^{bc}	5.25 ± 0.35^{a}
MPV (fL)	7.43 ± 0.25^{a}	7.48 ± 0.26^{a}	7.33 ± 0.15^{a}	7.90 ± 0.45^{a}
PDW (%)	10.83 ± 0.58^{a}	10.05 ± 0.67^{a}	9.30 ± 0.17^{b}	9.70 ± 0.00^{a}

Values are expressed as mean ± SD. Mean values in the same row with different subscript (a, b, c, bc) differ significantly ($p < 0.05$) using Bonferroni post hoc test (a) = non-significant, (b) significant to normotensive, (c) = significant when compared to the hypertensive/diabetic group and (bc) = significant when compared to normotensive and hypertensive/diabetic group.

3.4.1 INTERPRETATIONS OF PLATELETS AND COAGULATION PARAMETERS

In L-NAME/STZ-induced hypertensive/diabetic rats, platelet indices showed significant differences ($p < 0.05$) in platelet count (PLT), plateletcrit (PCT), platelet large cell ratio (P-LCR), and platelet distribution width (PDW), but not in mean platelet volume (MPV). PLT and PCT were significantly lowest in the methanol-treated group (325.33 ± 4.93; 0.26 ± 0.02) compared to HYP/D. P_LCR and PDW was significantly reduced in the aqueous group (2.45 ± 0.35; 9.30 ± 0.17) relative to the HYP/D.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

This study investigated the comparative effects of co-administration of aqueous and methanol fractions of *Cleome rutidosperma* and *Hunteria umbellata* on hematopoiesis in L-NAME/STZ-induced hypertensive/diabetic male Wistar rats. The results showed that both plants fractions produced beneficial effects on several hematological parameters that were altered in the disease condition, though the methanol fraction demonstrated more pronounced hematoprotective effects.

In this study, the hypertensive/diabetic control rats (Group 2) showed a general reduction in total monocyte (MID%) and lymphocyte (LYM#) counts compared to the normotensive group. This decrease may be linked to the toxic effects of nitric oxide synthase inhibition by L-NAME and β-cell destruction of the pancreas by streptozotocin (STZ), which often results in oxidative stress and immune suppression. The lymphocyte counts (LYM#) showed an increase when treated with the extracts in comparison with the hypertensive/diabetic group. This improvement/increase was especially evident in the aqueous fraction-treated rats, which showed a significant increase in absolute lymphocyte count (LYM#) suggesting that the aqueous extract stimulate lymphopoiesis by protecting the bone marrow cells from oxidative damage and by promoting the release of growth factors such as interleukins and colony-stimulating factors more than the methanol treated group.

Administration of the methanol fraction of the *Cleome rutidosperma* and *Hunteria umbellata* demonstrated notable impacts, with an increase in the percentage of MID cells. The elevation of MID% imply enhanced recruitment or proliferation of monocytes and other mid-cell leukocytes, which play a crucial role in chronic inflammatory resolution and immunomodulation.

The reduction in granulocyte percentage (GRAN%) observed in the aqueous and methanol treated groups may reflect a normalization of inflammatory status. Elevated granulocyte levels are often a sign of oxidative inflammation in hypertensive and diabetic conditions. The extracts, particularly the aqueous fraction lowered inflammatory stress by scavenging reactive oxygen species (ROS) because of the presence of antioxidant phytochemicals (flavonoids, phenolics) and downregulate pro-inflammatory cytokines.

These findings agree with earlier reports that *Cleome rutidosperma* and *Hunteria umbellata* possess immunomodulatory and antioxidant compounds, including flavonoids, alkaloids, and phenolic acids, which can improve immune cell production and function (Adejowun *et al.,* 2011; Niwoye *et al.,* 2019).

The red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT) values are indicators of oxygen transport and erythropoietic function. In this study, significant differences were observed among groups for RBC and HGB. The hypertensive/diabetic control rats had slightly increased RBC and slightly reduced hemoglobin (HGB) levels, which can be attributed to oxidative damage to red blood cells, suppression of erythropoietin production, and increased lipid peroxidation associated with L-NAME and STZ toxicity. In the *Cleome rutidosperma* and *Hunteria umbellata* aqueous extract group, there was a slight reduction compared to the methanol extract group which had a pronounced decrease. The reduction in red blood cell (RBC) reflects potential suppressive or hemolytic effects. Administration of the methanol extract significantly improved hemoglobin effectively than aqueous extract. This improvement suggests that the methanol fraction enhances erythropoiesis, possibly through antioxidant protection of erythroid progenitor cells and stimulation of erythropoietin synthesis.

The total platelet count (PLT) decreased in the hypertensive/diabetic control group compared to the normontensive/non-diabetic group, suggesting that the induction of L-NAME/STZ suppressed thrombopoiesis which may be attributed to oxidative and inflammatory damage to the bone marrow megakaryocytes. Administration of the plants extracts further reduced the platelet count. Correspondingly, platelets (PCT) – the volume fraction of blood occupied by platelets - showed an elevated PCT relative to the non-diabetic group, consistent with increased thrombosis risk in diabetes. Treatment with the extracts reduced PLT and PCT values, for both treated

groups which is effective in the reduction of oxidative damage and platelet activation and produced less hyperactive platelet pool because hyperactive platelets raise thrombosis risk. The ANOVA result of PLT and PCT indicated that the methanol extract differed significantly from the control, showing a strong regulatory effect on thrombocytosis. The P_LCR was highest in the hypertensive/diabetic group suggesting platelet hyperactivation and turnover. Treatment significantly reduced this parameter to aqueous extract and methanol extract, with the aqueous extract being effective in preventing formation of large, reactive platelets – a hallmark for oxidative damage and vascular injury.

The reduction in Platelet Distribution Width (PDW) of both extracts and methanol, implies a normalization of platelet morphology and decreased heterogeneity in platelet size. This points to the extract's ability to stabilize platelet production and suppress hyper-reactivity. The aqueous fraction showed a better regulation of megakaryocyte activity and platelet release, due to high content of hydrophilic antioxidants.

4.2 CONCLUSION

The co-administration of methanol fractions of *Cleome rutidosperma* and *Hunteria umbellata* proved to be the more effective intervention in restoring hematopoietic balance in L-NAME/STZ-induced hypertensive/diabetic male wistar rats. It significantly increased mid-sized cell percentage (MID%) while markedly reducing granulocyte percentage (GRAN%), indicating potent anti-inflammatory an immunomodulatory effect. Although aqueous fraction of *Cleome rutidosperma* and *Hunteria umbellata* improved RBC and HGB, it suppressed platelets and lack differential modulation of MID% and GRAN%. Thus, methanol fraction offers a superior hematopoietic support than aqueous fraction.

REFERENCES

Adeneye A. Adejuwon, Sofidiya M. Oluwatoyin and Adenekan O. Sunday, (2011). Anti-inflammatory and Antioxidant Activities of Hunteria umbellate seed fractions. *Pharmacologia*, **2**, 165-171.

Ahmed, A. M., (2002). History of diabetes mellitus. *Saudi Medical Journal,* **23**(4), 373-378.

Ahmed, M. M., El Degwy, H. M., Ali, M. I. and Hegazy, N. H., (2017). The effect of educational intervention on knowledge, attitude and glycemic control in patients with type 2 diabetes mellitus. *International Journal of Community Medicine and Public Health*, **2**(3), 302–307.

Ajiboye, T.O., Hussaini, A.A., Nafiu, B.Y., and Ibitoye, O.B., (2017). Aqueous seed extract of *Hunteria umbellata* (Apocynaceae) palliates hyperglycemia, insulin resistance, dyslipidemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome in rats. *Journal of Ethnopharmacology*, **198**, 84–193.

Akinsola, A.F. and Oluwafemi, A.G., (2022). Phytochemical analysis, proximate composition and mineral profile of *Cleome rutidosperma* found in Ado-Ekiti, Nigeria. *International Journal of Advanced Biochemistry Research*, **6**(2), 94–100.

Alidrisi, H.A., Odhaib, S.A., Nwayyir, H.A. and Almomin, A.M.S., (2024)**.** Effectiveness and safety of add-on once-daily liraglutide (1.2 mg) in type 2 diabetes patients with obesity: data from a real-world cohort of Iraqi patients. *Clinical Diabetology*, **13(**3), 140–147.

American Diabetes Association, (2014). Standards of medical care in diabetes. *Diabetes Care*, https://doi.org/10.2337/dc14-S014

Bastaki, S.M., (2005)**.** Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism*, **13**(3), 111–134.

Buchanan, T.A., Xiang, A.H. and Page, K.A., (2012). Gestational diabetes mellitus: risks and management during and after pregnancy. *Nature Reviews Endocrinology*, **8(**11), 639–649.

Carey, R.M., Muntner, P. and Whelton, P.K., (2022). Prevention, detection, evaluation, and management of high blood pressure in adults: Synopsis of the 2022 American Heart Association/American College of Cardiology hypertension guideline. *Annals of Internal Medicine*, **175**(7), 994–1007.

Carretero, O.A. and Oparil, S., (2000). Essential hypertension: Part I: Definition and etiology. *Circulation*, **101**(3), 329–335

Cutrell, E.B., Johnson, R.P. and Patel, K.D., (2023). Angiotensin-converting enzyme (ACE) inhibitors: mechanisms, therapeutic applications, and recent advances. *Journal of Cardiovascular Pharmacology and Therapeutics*, **28(**5), 455–468.

Frimpong, C., Mensah, J., Boateng, S. and Agyekum, F., (2024). Ethnopharmacological review of African medicinal plants with antidiabetic potential. *Journal of Ethnopharmacology*, 319, 117248.

Hyde, M.A., Wursten, B.T., Ballings, P. and Coates Palgrave, M. (2025) *Flora of Zimbabwe: Species information: individual images: Sida acuta.* Available at: https://www.zimbabweflora.co.zw/speciesdata/image- 3 (Accessed: 28 October 2025).

Kyrou, I. and Tsigos, C. (2006). Hypothalamic–pituitary–adrenal axis, cytokines and metabolic syndrome. *Obesity Metabolism*, **2**,116–126.

Lackland, D.T., Voeks, J.H., and Egan, B.M., (2007). Epidemiology of hypertension: classification and risk factors. *Journal of Clinical Hypertension*, **9**(9), 676–683.

Long, A.N. and Dagogo-Jack, S., (2011). Comorbidities of diabetes and hypertension: mechanisms and approach to target organ protection. *Journal of Clinical Hypertension (Greenwich)*, **13**(4), 244–251.

Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., and Zanchetti, A., 2007) Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of

Hypertension (ESH) and of the European Society of Cardiology (ESC*)*, *European Heart Journal*, **28**(12), 1462–1536.

Marwa, I.N., Gugu, G. and Mtshali, G., (2017). Comorbidity of diabetes and hypertension and available management strategies in Eastern African region. *IOSR Journal of Nursing and Health Science*, **6**(6),1

Mayet, J. and Hughes, A., (2003). Cardiac and vascular pathophysiology in hypertension. *Heart*, **89**(9), 1104–1109.

Moke, E. G., Demaki, W. E., Daubry, T. M. E., Ataikir, O. M., Agbonifo-Chijiokwu, E., Ofulue, O. O., Ekuerhare, B., Akpoyovwere, O., Edje, K., and Isibor, N. O., (2023). Coexistence of hypertension and diabetes mellitus and its pharmacotherapy. Scientia Africana, **22(**2), 135-154.

Nguyen, T., Lopez, J. and Martinez, A., (2024). Obesity and hypertension: Mechanistic links and clinical implications. *Current Hypertension Reports*, **26**(2), 115–128.

Niwoye, A. A,. Ndaman, S. A., Akanya, H. H. O. and Egwim, E. E. C. (2019). Antioxidant and hypoglycemic effect of some medicinal plants. *GSC Biological and Pharmaceutical Sciences*, **8(**2), 70-80

Nwaogwugwu, J.C., Nosiri, C.I., Aguwamba, C., Friday, A.C. and Ike, U.W., (2022). Effect of *Hunteria umbellata* Methanolic Seed Extracts on Streptozotocin-induced Diabetic Albino Rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, **12**(3), 14–27.

Paarakh, P.M., Karthik, M., Kaviarasu, S., Kavya, N., Kavya, S.S., Kavya, M. and Muthukumar, A., (2023). *Sida acuta* Burm. f.: a comprehensive review. *YMer*, **22**(11). [online] Available at: YMer Digital [Accessed 29 August 2025].

Patel, R.S., Shah, T. and Kandula, P., (2021). Role of the renin-angiotensin-aldosterone system and genetic factors in the pathophysiology of primary hypertension. *Journal of Human Hypertension*, **35**(6), 560–572.

Savan, C., Viroja, D., and Kyada, A., (2024). An updated review on diabetes mellitus: Exploring its etiology, pathophysiology, complications and treatment approach. IP international *Journal of Comprehensive and Advanced Pharmacology*, **9(**1), 31-36.

Shittu, M.D. and Alagbe, J.O., (2020). Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. *International Journal on Integrated Education*, **3**(11), 119–124.

Simó, R. and Hernández, C., (2002). Treatment of diabetes mellitus: general goals, and clinical practice management. *Revista espanola de cardiologia*, **55**(8), 845-860.

Smith, A. and Jones, M., (2023). Dietary sodium and potassium imbalance as determinants of blood pressure: A review of recent evidence. *Nutrients*, **15**(4), 912.

Strauss, M., Becker, H. and Lin, C., (2023). Bradykinin-mediated vascular protection and fibrinolytic effects of ACE inhibitors in cardiovascular therapy. *European Heart Journal*, **44**(9), 765–778.

Unger, T., Borghi, C., Charchar, F., Khan, N.A., Poulter, N.R., Prabhakaran, D., Ramirez, A., Schlaich, M., Stergiou, G.S., Tomaszewski, M., Wainford, R.D., Williams, B. and Schutte, A.E., (2020). International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension*, **75**(6), 1334–1357.

Viera, A. J. and Neutze, D. M., (2010). Diagnosis of secondary hypertension: an age-based approach. *American Family Physician***, 82**(12),1471–1478.

Wang, J., Sun, H. and Xu, Y., (2023). Genetic predisposition and the risk of essential hypertension: Insights from genome-wide association studies. *Hypertension Research*, **46**(3), 456–468.

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)
N/ND	6.50 5.30 8.20	62.50 71.20 63.30 70.20	15.40 18.50 16.00 15.00	22.10 20.70	4.60 3.40 5.80	1.20 1.20	0.80 0.70
$\bar{X}$±SD	**6.67 ± 1.46**	**66.8 ± 4.53**	**16.23 ± 1.57**	**21.4 ± 0.99**	**4.60 ± 1.20**	**1.20 ± 0.00**	**0.75 ± 0.07**
HYP/D	5.70 4.40 6.00	55.50 61.00 61.00	13.10 11.20 15.70	31.40 27.80 23.30	3.20 2.70 3.30 3.70	0.80 0.50 0.90	1.70 1.20 1.40
$\bar{X}$±SD	**5.36 ± 0.85**	**59.2 ± 3.17**	**13.33 ± 2.26**	**27.5 ± 4.06**	**3.22 ± 0.41**	**0.73 ± 0.21**	**1.43 ± 0.25**
CR/HU (AQU)	6.80 8.40	70.60 62.50	16.20 17.10	13.20 20.40	4.80 5.30 5.90	1.10 1.40	0.90 1.70
$\bar{X}$±SD	**7.60 ± 1.13**	**66.5 ± 5.73**	**16.65 ± 0.64**	**16.8 ± 5.09**	**5.33 ± 0.55**	**1.25 ± 0.21**	**1.30 ± 0.57**
CR/HU (MTH)	7.30 9.00 7.20	68.90 61.50 61.40 57.80	23.10 24.00	16.60 15.40 18.20	5.00 5.50 4.40	1.10 1.20	1.20 1.40 1.60
$\bar{X}$±SD	**7.83 ± 1.01**	**62.4 ± 4.666**	**23.6 ± 0.45**	**16.73 ± 1.40**	**4.97 ± 0.55**	**1.15 ± 0.70**	**1.40 ± 0.20**

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 (%)
N/ND	8.23	15.80	46.10	56.10	19.10	34.20	38.50	16.50
	8.32	15.60	44.00	54.30	19.40	35.90	38.50	16.80
	8.47	15.90	46.30	52.90	18.70	35.40	38.50	17.10
				54.70	18.70	34.30	38.50	16.60
$\bar{X}$±SD	**8.35 ± 0.12**	**15.8 ± 0.15**	**45.5 ± 1.27**	**54.5 ± 1.32**	**18.9 ± 0.34**	**34.9 ± 0.83**	**38.5 ± 0.00**	**16.8 ± 0.26**
HYP/D	8.71	14.90	46.90	53.90	19.10	35.60	38.50	16.80
	8.17	14.70	43.90	53.80	18.20	33.90	40.60	17.80
	8.31	15.40	42.70	51.40	18.50	34.40	36.30	16.60
	8.27		48.70	54.20	18.60	34.20	40.60	17.70
			44.80	54.20		34.30	40.60	17.70
$\bar{X}$±SD	**8.36 ± 0.24**	**15.0 ± 0.36**	**45.4 ± 2.40**	**53.3 ± 1.19**	**18.6 ± 0.37**	**34.5 ± 0.65**	**39.3 ± 1.92**	**17.3 ± 0.57**
CR/HU (AQU)	8.51	16.90	45.70	53.80	19.80	36.90	40.60	17.80
	8.41	16.20	47.60	56.70	19.20	34.00	44.40	17.40
	7.54		39.30	52.00	18.30	35.20	38.50	
$\bar{X}$±SD	**8.15 ± 0.53**	**16.6 ± 0.49**	**44.2 ± 4.34**	**54.2 ± 2.37**	**19.1 ± 0.75**	**35.4 ± 1.46**	**41.2 ± 2.99**	**17.6 ± 0.28**
CR/HU (MTH)	7.55	13.80	39.60	52.50	19.40	34.80	42.70	17.30
	7.57	14.70	42.30	56.00	19.10	34.70	40.60	18.40
	7.28	13.00	36.70	54.10	19.90	35.40	40.60	17.70
		14.50	41.20	56.60		35.10		17.30
$\bar{X}$±SD	**7.47 ± 0.16**	**14.0 ± 0.77**	**39.9 ± 2.43**	**54.8 ± 1.87**	**19.5 ± 0.40**	**35.0 ± 0.32**	**41.3 ± 1.21**	**17.68 ± 0.52**

PLATELETS AND ITS DIFFERENTIAL COUNTS

GROUPS	PLT (10^3/µL)	PCT (%)	P-LCR (%)	MPV (%)	PDW (%)
N/ND	452.00 538.00 602.00	0.32 0.41 0.44	6.20 5.20	7.20 7.70 7.40	10.50 10.50 11.50
$\bar{X}$±SD	**530.67 ± 75.27**	**0.39 ± 0.06**	**5.70 ± 0.71**	**7.43 ± 0.25**	**10.83 ± 0.58**
HYP/D	331.00 470.00 496.00	0.35 0.37	7.10 6.40 8.40	7.70 7.10 7.60 7.50	9.50 9.70 10.00 11.00
$\bar{X}$±SD	**432.33 ± 88.71**	**0.36 ± 0.01**	**7.37 ± 1.01**	**7.48 ±0.26**	**10.05 ± 0.67**
CR/HU (AQU)	401.00 379.00	0.29 0.27	2.70 2.20	7.30 7.50 7.20	9.50 9.20 9.20
$\bar{X}$±SD	**390.00 ± 15.56**	**0.28 ± 0.01**	**2.45 ± 0.35**	**7.33 ± 0.15**	**9.30 ± 0.17**
CR/HU (MTH)	323.00 322.00 331.00	0.24 0.25 0.28	5.00 5.50	7.50 8.00 7.60 8.50	9.70 9.70
$\bar{X}$±SD	**325.33 ± 4.93**	**0.26 ± 0.02**	**5.25 ±0.35**	**7.90 ± 0.45**	**9.70 ± 0.00**