PREVENTIVE DIABETIC EFFECTS ON STREPTOZOCIN INDUCED DIABETIC RATS AFTER ADMINISTRATION OF BI-HERBAL MIXTURE OF *Ocimum gratissimum* AND *Murraya koenigii*

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus (DM) is the most common endocrine disorder, affecting over 100 million people worldwide, which accounts for approximately 6% of the global population. It is caused by either a deficiency in insulin production or the body's inability to effectively use insulin, leading to abnormal blood glucose levels. This condition can damage multiple body systems, particularly the blood vessels, eyes, kidneys, heart, and nerves (Ismail, 2019). Diabetes mellitus is classified into two main types: insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II). Type I diabetes is an autoimmune disorder characterized by inflammation and destruction of insulin producing cells in the pancreas, while Type II diabetes is marked by insulin resistance and impaired insulin secretion (Aurora *et al*., 2019).

The manifestation of DM substantially elevates susceptibility to numerous health complications, encompassing cardiovascular pathologies, peripheral circulatory disorders, cerebrovascular accidents, neural dysfunction, renal insufficiency, retinal pathology, vision loss, and limb amputations (Jothivel *et al*., 2017). The principal therapeutic objective involves preserving life and mitigating symptoms, while

supplementary aims encompass preventing chronic complications and enhancing longevity through addressing contributory risk elements. Insulin supplementation therapy remains crucial for Type I diabetes management, whereas modifications in lifestyle and nutritional habits constitute the cornerstone of Type II diabetes treatment (Bastaki, 2015). Furthermore, glucose-lowering medications including biguanides and sulfonylureas are utilized for diabetes treatment.

Nevertheless, these pharmaceutical agents present limitations due to their potential adverse reactions and diminishing efficacy with extended usage (Dixit and Joshi, 2015). A significant disadvantage of existing treatments involves their necessity for lifelong administration and accompanying adverse effects (Halin and Abroma, 2019).

In geographical areas where access to standard antidiabetic medications remains restricted, botanical medicines and their bioactive constituents present a viable alternative for DM management (Jothivel et al., 2017). Multiple experimental frameworks exist to assess the antidiabetic characteristics of plants (Grover *et al.,* 2017). This examination seeks to furnish comprehensive understanding of diabetes mellitus, encompassing its clinical manifestations, epidemiological information, complications, and existing therapeutic approaches.

Global estimates from 2011 indicated that 366 million individuals suffered from diabetes mellitus (DM), with projections suggesting an increase to 552 million by 2030. The occurrence of type 2 DM continues rising internationally, with 80% of affected individuals residing in economically developing nations. During 2011, DM accounted for 4.6 million fatalities (Olokoba *et al*., 2022). Projections estimate 439 million individuals will develop type 2 DM by 2030. The frequency of type 2 DM demonstrates

considerable variation across geographical regions attributed to environmental and lifestyle-associated risk elements (Zimmet and Alberti, 2015). Predictions indicate continued escalation in DM prevalence, particularly type 2 DM, throughout the forthcoming two decades, with predominant increases occurring in developing nations. Within these territories, the majority of patients range between 45 and 64 years of age (Wild *et al*., 2014).

1.2 EPIDEMIOLOGY

Global estimates from 2011 indicated that 366 million individuals suffered from diabetes mellitus (DM), with projections suggesting an increase to 552 million by 2030. The occurrence of type 2 DM continues rising internationally, with 80% of affected individuals residing in economically developing nations. During 2011, DM accounted for 4.6 million fatalities (Olokoba *et al*., 2022). Projections estimate 439 million individuals will develop type 2 DM by 2030. The frequency of type 2 DM demonstrates considerable variation across geographical regions attributed to environmental and lifestyle-associated risk elements (Zimmet and Alberti, 2015). Predictions indicate continued escalation in DM prevalence, particularly type 2 DM, throughout the forthcoming two decades, with predominant increases occurring in developing nations. Within these territories, the majority of patients range between 45 and 64 years of age (Wild *et al*., 2014).

1.3 TYPES OF DIABETES MELLITUS

Type 1 Diabetes:

Type 1 diabetes constitutes a persistent autoimmune disorder wherein the immune mechanism specifically eliminates pancreatic β-cells responsible for insulin synthesis (Ozougwu et al., 2023). In situations where pancreatic transplantation occurs between twin siblings without immunosuppressive therapy, difficulties emerge due to heterogeneous pancreatic tissue damage. The β-cells undergo rapid destruction, producing extensive insulitis triggered by infiltrating T lymphocytes, demonstrating an autoimmune mechanism (Deepti *et al*., 2017).

Type 1 diabetes is also known as insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes.

Symptoms:

Frequent urination, excessive thirst, weight loss, extreme fatigue, acetone breath, nausea, vomiting, blurred vision, and genital itching.

Type 2 Diabetes:

Type 2 diabetes mellitus, frequently termed adult-onset diabetes, demonstrates characteristics of progressive insulin secretion deterioration accompanied by insulin resistance. Affected individuals commonly display resistance to insulin's physiological actions (Singh *et al.,* 2016). Worldwide, this

condition affects 57% of the population and typically receives management through nutritional modifications, physical activity, and hypoglycemic pharmaceutical agents (Bastaki, 2015). This diabetic form demonstrates strong correlation with familial diabetes history, advancing age, excessive body weight, and insufficient physical activity (Baynest, 2015).

Gestational Diabetes:

Gestational diabetes mellitus (GDM) develops when expectant mothers manifest diabetes resulting from hormonal alterations that diminish insulin sensitivity, producing insulin resistance. This category encompasses both women developing diabetes throughout pregnancy and those with previously undetected type 2 diabetes identified during gestation (Baynest, 2015). GDM possesses clinical importance as it correlates with elevated maternal and fetal health complications (Siddiqui *et al*., 2016)

Other Types of Diabetes:

1.4 Diabetes LADA (Latent Autoimmune Diabetes of Adults):

LADA represents an autoimmune diabetic variant manifesting in adult individuals. Characteristics include diabetes-related autoantibody presence without requiring immediate insulin therapy following diagnosis (Laugesen *et al.,* 2015). Certain individuals initially classified with type 2 diabetes may subsequently

require insulin, suggesting possible LADA diagnosis, representing a gradually progressive type 1 diabetes variant.

Diabetes MODY (Maturity Onset Diabetes of the Young):

MODY constitutes an autosomal dominant diabetic form resulting from genetic mutations affecting pancreatic β-cell maturation. Principal characteristics encompass early manifestation, autoimmune marker absence or insulin resistance, and maintained insulin production (Anik *et al*., 2015).

Double Diabetes:

This condition involves hyperglycemia in children and adolescents with characteristics of both type 1 and type 2 diabetes.

Brittle Diabetes:

A rare and severe form of type 1 diabetes, primarily affecting young women, characterized by extreme blood sugar instability, frequent hypoglycemia or ketoacidosis, and a significant impact on quality of life.

Diabetes Insipidus:

This condition produces excessive dilute urine excretion due to vasopressin insufficiency, resistance, or excessive fluid consumption. Polyuria characterization involves urine production surpassing 2 L/m²/24 hours in adults (Dilorgi *et al.*, 2017).

Neonatal Diabetes Mellitus:

Occurring within the first six months of life, this condition is caused by a single gene defect leading to insufficient insulin production. Affected infants often fail to gain weight and have high blood glucose levels, sometimes mistaken for type 1diabetes.

1.5 PATHOPHYSIOLOGY OF DIABETES: An Overview

Diabetes mellitus represents a persistent heterogeneous metabolic condition with intricate pathological development. Characterization includes elevated blood glucose concentrations or hyperglycemia, resulting from irregularities in insulin production or insulin function or both. Hyperglycemia manifests through various presentations causing carbohydrate, lipid, and protein metabolic dysfunction. Extended hyperglycemia frequently produces various microvascular and macrovascular diabetic complications, primarily responsible for diabetes-related illness and death. Hyperglycemia functions as the fundamental biomarker for diabetes diagnosis. This investigation focuses on diabetes classification and pathophysiological mechanisms including various subtypes (Mujeeb *et al.*, 2020).

1.6 SYMPTOMS AND CAUSES OF DIABETES

Symptoms:

Increased thirst and urination

Increased hunger

Fatigue

Blurred vision

Numbness or tingling in hands or feet

Slow healing sores

Unexplained weight loss

Ketones in urine (a byproduct of fat and muscle breakdown due to insulin deficiency)

Frequent infections (e.g., gum, skin, or vaginal infections)

Male sexual dysfunction

Causes:

Obesity

Excess glucocorticoids or growth hormone

Polycystic ovary syndrome

Insulin receptor mutations

Lipodystrophy (Ozougwu *et al*., 2023)

Causes of Type 1 Diabetes:

1. Genetic predisposition

2. Viral infections (e.g., German measles or mumps)

3. Environmental factors

Causes of Type 2 Diabetes:

Type 2 diabetes develops due to insulin resistance or insufficient insulin production by the pancreas.

Figure 1.2: Symptoms of diabetes mellitus (Ozougwu *et al*., 2023)

1.7 Diagnostic Tests for Diabetes:

Three primary blood tests are used to diagnose pre-diabetes and diabetes:

Casual Plasma Glucose (Random Plasma Test):

This assessment requires no fasting. A blood glucose concentration of 200 mg/dL or higher, combined with diabetic symptoms, establishes the diagnosis (Siddiqui et al., 2016; Harikumar et al., 2015).

2. Fasting Plasma Glucose (FPG):

Measures blood glucose after an overnight fast.

3. Oral Glucose Tolerance Test (OGTT): Evaluates blood glucose levels before and after consuming a glucose rich drink.

1.8 Diagnostic Tests for Diabetes:

Fasting Plasma Glucose (FPG):

A fasting plasma glucose concentration of 7.0 mmol/L demonstrates close correlation with a 2-hour plasma glucose measurement of ≥11.1 mmol/L in a 75g oral glucose tolerance examination (OGTT). Both measurements predict retinopathy development (Goldenberg and Punthakee, 2023). This assessment requires an 8-hour fasting period. A blood glucose concentration exceeding 126 mg/dL on two or more independent examinations confirms diabetes diagnosis (Baynest, 2015).

Oral Glucose Tolerance Test (OGTT) :

A two-hour blood glucose concentration of 200 mg/dL or higher signifies diabetes, whereas concentrations between 140-199 mg/dL suggest pre-diabetes (Harikumar *et al*., 2015)

Hemoglobin A1C (HbA1c) :

.HbA1c measures average blood glucose levels over the past 23 months and can be taken at any time of day, making it more convenient than FPG or OGTT. An HbA1c level ≥6.5% confirms diabetes, while ≤5.7% rules it out (Goldenberg and Punthakee, 2023)

1.9 Diagnostic Test for Gestational Diabetes:

O'Sullivan Test:

This test detects gestational diabetes by administering 50g of glucose to a fasting patient and measuring blood glucose levels after one hour. Levels above 1500 mg/L suggest gestational diabetes (Ngugi *et al*., 2017).

1.10 Treatment for Diabetes:

Treatment varies depending on the type of diabetes and individual patient factors.

1.10.1 Treatment for Type 1 Diabetes:

Patients with type 1 diabetes cannot produce insulin and rely on external insulin administration to survive.

1.10.2 Insulin Therapy:

Conventional Therapy: Two daily injections of mixed insulin (rapid/short-acting and intermediate-acting) before breakfast and dinner.

Split Night Time Dose: Mixed insulin before breakfast, rapid/short-acting insulin before dinner, and intermediate acting insulin before bedtime.

Multiple Daily Injections (MDI): Rapid/short-acting insulin before meals and intermediate/long-acting insulin once or twice daily.

Continuous Subcutaneous Insulin Infusion (CSII or Insulin Pump): A bolus dose is administered before meals based on carbohydrate intake and blood glucose levels.

1.10.3 Treatment for Type 2 Diabetes:

Treatment depends on factors such as body weight, eating habits, physical activity, symptom severity, blood glucose levels, and diabetes duration. Options include diet, exercise, medication, and insulin therapy.

1.10.4 Self-Care:

Physical exercise

Smoking cessation

Weight loss

Nutritional counseling

Diabetic diet and dietary fiber

Medications:

Antidiabetic drugs

Blood thinners

Statins

Insulin

1.11 Preventative Measures:

Influenza vaccine

Pneumococcal vaccine

1.12 Pharmacological Treatments for Diabetes:

1.12.1 Oral Anti-Diabetic Drugs:

These drugs regulate blood glucose by affecting glucose absorption, peripheral uptake, hepatic glucose output, and insulin secretion.

1. Sulfonylureas:

The first widely used oral hypoglycemic agents.

Bind to pancreatic βcell receptors to increase insulin secretion.

Taken 15-30 minutes before meals (Harikumar *et al*., 2015).

2. Meglitinide Analogues:

Non-sulfonylurea insulin secretagogues (e.g. repaglinide, netaglinide).

Act on βcell receptors to enhance insulin secretion.

3. Biguanides:

Reduce hepatic glucose output and increase peripheral glucose uptake.

Metformin is the most commonly used biguanide (Harikumar *et al*., 2015).

4. Alpha Glucosidase Inhibitors:

Inhibit enzymes that break down carbohydrates in the small intestine.

Acarbose is taken with the first bite of food, starting at 25-50 mg daily.

5. Thiazolidinediones (Glitazones) :

Improve insulin sensitivity in adipose tissue and skeletal muscle.

Pioglitazone and rosiglitazone are examples, with effects observed within 24 weeks and peaking at 8-12 weeks.

1.4 Aim

This study aims to investigate the normoglycemic and anti-diabetic effects of the methanol extract of *Ocimum gratissimum* and *Murraya koenigii* in normal and Streptozotocin (STZ) induced diabetic rats.

1.5 Objectives

1. Assess the effect of the methanol extract of *Ocimum gratissimum* and *Murraya koenigii* on blood glucose levels in STZ induced diabetic rats to determine its potential to restore normoglycemia.

2. Evaluate the impact of the extract on insulin secretion and insulin sensitivity in diabetic rats to understand its mechanisms in modulating glucose metabolism.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BIOLOGY OF *Ocimum gratissimum*

Scent leaf (*Ocimum gratissimum*), also referred to as African basil, clove basil, or efirin in Yoruba, is a multifaceted herb indigenous to Africa, Madagascar, and certain parts of Asia. Renowned for its aromatic qualities, it enhances various dishes and has a long-standing history in

traditional medicine. In the last decade, many researchers have focused on medicinal plants and their bioactive compounds due to their promise in treating and preventing serious conditions, including arthritis, diabetes, cancer, and stroke.

Plate 2.1 an image showing *Ocimum gratissimum* Photo credit: Olabisi Abe

This perennial herb demonstrates recognition not solely for its potent scent but additionally for its commercial value. Scent leaf belongs to the Lamiaceae family. Beyond serving as a flavor enhancer for fish, meat, soups, and stews, it finds application in treating multiple ailments including pain, fever, inflammation, anaemia, diarrhoea, and infections produced by fungi and bacteria. It comprises substantial quantities of macronutrients advantageous for physiological function,

Symptoms:

Enhanced thirst and urinary output

Amplified appetite

Physical exhaustion

Vision impairm

and when ingested in suitable quantities, the leaves deliver considerable nutritional advantages (Chaachouay *et al*., 2022).

2.2 TAXONOMY OF *Ocimum gratissimum*

Scent leaf is classified within the Plantae kingdom, belonging to the Lamiaceae family (commonly known as the mint family) and is identified as the species *Ocimum gratissimum*. This perennial shrub can reach heights of 2 to 5 meters. It is known by various names including Efinrin (in Yoruba), Nchanwu (in Igbo), and Afeu (in Efik).

Scientific Name: *Ocimum gratissimum*

Kingdom: Plantae

Phylum: Anthophyta

Class: Dicotyledoneae

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: gratissimum

2.2. Table 2.1 showing the nutrition data of *Ocimum gratissimum* and their amount.

S/N	NUTRIENTS	AMOUNT(g/mg)
1.	Water	73.483g
2.	Calories	93.613g
3.	Fats	1.197g
4.	Proteins	8.48g
5.	Carbohydrates	8.783g
6.	Fiber	6.895g
7.	Iron	1.0005mg
8.	Potassium	319 6mg
9.	Phosphorus	209mg
10.	Sodium	50.47mg
11.	Zinc	1.09mg
12.	Vitamin C	18.64mg

2.3. PHYTOCHEMICALS OF *Ocimum gratissimum*

The plant produces various compounds, encompassing Thymol, eugenol, methyl chavicol, gratissimol, alkaloids, tannins, flavonoids, oligosaccharides, P-cymene, γ-terpene, trans-sabiene hydrate, 1,8 cineole,

linalool, methyl eugenol, and additional compounds. Seeds yield pentoses, hexoses, uronic acid, lipids, thymol, and eugenol. Leaves supply eugenol, methyl eugenol, cis-ocimene, pinene, camphor, germacrene-D, trans-caryophyllene, farnesene, 1-bisabolene, bisaboline, thymol, citral, ethyl cinnamate, linalool, terpinene, p-cymene, limonene, terpinolene, 1,8-cineole, and oleanolic acid. The predominant phytochemicals identified in Scent leaf comprise flavonoids, terpenes, tannins, and phenols, which may contribute significantly to its diverse health advantages (Shirwaikar, 2008).

2.4. PHARMACOLOGICAL USES OF *Ocimum gratissimum*

1. Anti- microbial, Anti- bacteria and Anti-fungal: Scent Leaf is also known as the "fever" leaf because of the antiseptic, anti-fungal and antibacterial qualities of its oil. Scent Leaf is a remedy for skin ailments because of the presence of antiseptic, anti-fungal and antibacterial qualities. It is fights skin diseases such as ringworm when ground into paste for skin care.

2. Anti-inflammatory, Anti-Ulcer and Analgesic:The particular constituents in scent leaf extract believed responsible for its anti-ulcer functionality are eugenols and carvacrol, both representing phenolic compounds. Eugenols constitute natural compounds demonstrated to exhibit anti-

inflammatory and antioxidant characteristics. These compounds inhibit cyclooxygenase-2 (COX-2) activity, an enzyme performing crucial roles in ulcer development.

Carvacrol represents another phenolic compound identified in scent leaf extract demonstrated to possess antimicrobial, antioxidant, and anti-inflammatory characteristics, believed to safeguard the gastric mucosa by augmenting mucus synthesis and diminishing gastric acid production. Collectively, these compounds function synergistically to protect the gastric mucosa from damage and facilitate healing of existing ulcers (Ajayi *et al.*, 2017).

3.Anti-oxidants effect and stress relief :High concentration of vitamins present in scent leaf especially ascorbic acid and alpha – tocopherol helps to scavenge free radicals in the body thereby reducing oxidative stress leading to stress relief, cancer prevention and prevention from certain heart diseases.

4. Cardiovascular effect: it aids the heart to function correctly by removing toxic and cholesterol which the body does not need.

5. Anti- Viral and Anti-Cancer: It contains bioactive compounds, including eugenols, thymol, and rosmarinic acid that have been shown to possess antiviral properties. These compounds may

help to inhibit the replication of certain viruses, including herpes simplex virus, HIV, may also help to boost the immune system, which can enhance the body's ability to fight viral infections.

6. Anticonvulsant:

Research identified that scent leaf extracts possess anticonvulsant characteristics because they postpone the onset of PTZ-induced seizures and protect treated mice from seizure-related mortality. PTZ produces convulsions by competing with the chloride (Cl)-channel complex at the γ-aminobutyric acid (GABA) A receptor to diminish GABA-dependent inhibition (Zakaryan *et al.*, 2017).

7. Anti-diarrhoea property and calming effect:Scent leaf has received utilization for centuries to support digestive wellness, relieving stomach conditions including bloating, discomfort, and digestive delays. Its calming characteristics additionally facilitate soothing stomach aches, vomiting, diarrhoea, and dysentery, promoting timely digestion and relief from gastrointestinal distress (Ezekwesili et al., 2004).

2.5. **TRADITIONAL BENEFITS**

Ocimum gratissimum represents a widely available plant utilized by communities for treating various ailments, as emphasized in numerous ethno-pharmacological investigations (Ajayi et al., 2017). This perennial, aromatic plant currently exists on all continents and receives recognition for its medicinal characteristics. Its therapeutic capacity in Africa proves extensive and varies by geographical region (Kpoviessi *et al*., 2014).

In Cameroon, its infusions receive consideration as tonic and advantageous for chest conditions, while leaf juice finds employment to relieve dizziness, headaches, colds, and coughs. In Côte d'Ivoire, various preparations of this plant receive utilization to address ear infections, skin pathologies, and eye infections. In Nigeria, recommendations exist for treating diarrhea and suggested utilization for respiratory conditions and as an anthelminthic (Kpoviessi *et al*., 2014).

The plant has additionally received utilization for addressing headaches, fevers, eye and skin complications, as well as pneumonitis. In Togo, its infusion facilitates cough alleviation. Fresh juice from leaves provides advantages against diarrhea and dysentery, while aqueous maceration finds employment to manage conditions including hematuria and purulent urethritis (Kpoviessi et al., 2014). In the Benin Republic, aqueous maceration of its pulp or aerial portions receives utilization to treat conditions including dystopia, pelvic pain, colic, candidiasis, digestive dysmenorrhea, vomiting, hemorrhoids, and diarrhoea. A decoction of its stems receives indication for hepatitis, cough, asthma, and wound infections (Chah et al., 2006; Kpoviessi *et al*., 2014). Furthermore, leaf juice receives utilization to address angina, headaches, fever, and malnutrition, while its flowers receive utilization to enhance the flavor of various culinary preparations.

2.6. TOXICITY STUDY

Scent leaf *(Ocimum gratissimum)* is generally considered safe for consumption in normal culinary amounts. However, like many other herbs and plants, excessive consumption may lead to some negative side effects. Studies haven't found significant toxicity with scent leaf consumption. In fact, some research suggests it may even have protective effects against certain toxins.

2.6.1. Here are some potential side effects of excessive scent leaf consumption

Allergies: Avoid using fragrance leaves if you have any allergies to plants in the mint or basil family. So someone might develop swelling, hives or trouble in breathing.

Anti-fertility: Scent Leave has a potential anti fertility property. It contains compounds that may interfere with the levels of certain hormones in the body including oestrogen and progesterone, which play a key role in regulating the menstrual cycle and fertility. Scent leaf may also have a negative effect on sperm function, inhibiting its ability to fertilize an egg.

Guidelines (2011) and institutional regulations.

3.3. Acute Toxicity Study

The acute toxicity studies were performed in compliance with the guidelines set forth by the Organization for Economic Co-operation and Development (OECD, 2018).

Three (3) male mice and 3 female mice each were administered 5000 mg/kg of BHFOGIG respectively and observed for 72 hours for possible signs of toxicity, mortality or morbidi

2.4 Nutritional Uses *Murraya koenigii* and *Occimum gratissimum*

The bi-herbal mixture of *Murraya koenigii* (Curry Leaf) and *Occimum gratissimum* (Scent Leaf) has been traditionally used for its nutritional benefits. Here are some of the nutritional uses of the bi-herbal mixture: •Rich in Antioxidants: The mixture is rich in antioxidants, including flavonoids, phenolic acids, and terpenoids, which can help protect against oxidative stress and cell damage.

• Good Source of Vitamins and Minerals: The mixture is a good source of vitamins A, C, and E, as well as minerals like calcium, iron, potassium, and zinc (Shimizu, 2024).

•Digestive Health: The mixture may help support digestive health due to its carminative and anti-inflammatory properties.

•Immune System Support_: The mixture may help support the immune system due to its antioxidant and anti-inflammatory properties.

•Anti-Inflammatory Compounds*: Both Curry Leaf and Scent Leaf contain anti-inflammatory compounds that may help reduce inflammation and improve overall health.

• High in Fiber:Both herbs are high in dietary fiber, which can help promote digestive health and support healthy blood sugar levels

•Antimicrobial Properties:The mixture has antimicrobial properties, which can help protect against infections and promote wound healing.

•High in Fiber: Both herbs are high in dietary fiber, which can help promote digestive health and support healthy blood sugar levels Medicinal uses the bi-herbal mixture of *Murraya koenigii* (Curry Leaf) and *Ocimum gratissimum* (Scent Leaf) has been traditionally used in various cultures for its medicinal properties. Here are some of the medicinal uses of the bi-herbal mixture:

1. Digestive issues: The mixture may help alleviate digestive issues such as bloating, gas, and indigestion due to its carminative and anti-inflammatory properties.

2. Respiratory problems: The mixture may help relieve respiratory problems such as bronchitis, asthma, and coughs due to its expectorant and anti-inflammatory properties.

3. Skin and wound healing: The mixture may help promote skin and wound healing due to its antimicrobial and anti-inflammatory properties.

•Fever and malaria: The mixture may help reduce fever and alleviate symptoms of malaria due to its antipyretic and anti-parasitic properties.

•Pain relief: The mixture may help alleviate pain and inflammation due to its analgesic and anti-inflammatory properties.

4. Antibacterial and anti-fungal infections: The mixture may help treat bacterial and fungal infections such as diarrhea, dysentery, and ringworm due to its antimicrobial properties.

5. Oral health: The mixture may help treat oral health issues such as toothache, gum inflammation, and bad breath due to its antimicrobial and anti-inflammatory properties.

6. Antioxidant and anti-aging: The mixture may help protect against oxidative stress and cell damage, promoting overall health and well-being.

7. Immune system support: The mixture may help support the immune system, reducing the risk of illness and infection.

Mechanism of action Antimicrobial properties: The bioactive compounds present in the mixture, such as eugenol, beta-caryophyllene, and alpha-humulene, may disrupt the cell membrane of microorganisms, leading to cell lysis and death. Here's the detailed explanation of the mechanism of action for each of the properties: Antioxidant Property:The bi-herbal mixture of *Murraya koenigii* and *Ocimum gratissimum* exhibits antioxidant properties due to the presence of various phytochemicals, including flavonoids, phenolic acids, and terpenoids. These compounds can scavenge free radicals, reduce oxidative stress, and protect cells from damage by neutralizing free radicals and reducing the production of reactive oxygen species (ROS). This helps to maintain cellular homeostasis and prevent oxidative damage to cells. ImmuneImprovement Property The bi-herbal mixture of *Murraya koenigii* and *Ocimum* gratissimum has been shown to exhibit immunomodulatory effects, which can help improve immune function. This is achieved by activating immune cells, such as macrophages and natural killer cells, which can help eliminate pathogens and tumor cells. The bi-herbal mixture also enhances the production of cytokines, such as interleukin-2 (IL-2) and interferon-gamma (IFN- γ), which can help coordinate the immune response. By modulating the immune response, the bi-herbal mixture can help reduce inflammation and oxidative stress, thereby preventing tissue damage.

Murraya koenigii

Murraya koenigii, commonly recognized as Curry Leaf, represents a tropical tree native to India and Southeast Asia. It belongs to the Rutaceae family and receives widespread cultivation in numerous global regions for its aromatic leaves. The principal characteristics of *Murraya koenigii* encompass its scientific designation, *Murraya koenigii*, common designation, Curry Leaf, family, Rutaceae, native origin, India and Southeast Asia, and cultivation for its aromatic leaves. Traditionally, *Murraya koenigii* has received utilization in various applications. In Indian and Sri Lankan cuisine, it receives utilization as a flavoring agent, contributing unique aroma and taste to culinary preparations. In traditional medicine, *Murraya koenigii* finds employment for its anti-inflammatory and antioxidant characteristics, providing relief from various health conditions. Furthermore, it receives utilization in rituals and ceremonies for its spiritual significance, emphasizing its cultural importance in numerous communities (Kumar and Clark, 2017).

Murraya koenigii* and *Ocimum gratissimum

Murraya koenigii and *Ocimum gratissimum* has been shown to exhibit anti-diabetic effects, which can help regulate blood sugar levels. This is achieved by inhibiting the activity of alpha-glucosidase, an enzyme that breaks down carbohydrates into glucose, thereby reducing postprandial blood sugar levels. The bi-herbal mixture also enhances insulin sensitivity by increasing the expression of insulin receptors and glucose transporters, thereby improving glucose uptake in cells.

Plate 2.2 an image showing *Ocimum gratissimum* Photo credit: Esosa Imhontu

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION OF RESEARCH MATERIALS

The Fruits of *Occimum gratissimum* and *Murraya* k*oenigii* were purchased from of Oba market in Benin City, Edo State, Nigeria. They were cleaned and air dried at room temperature for 2 weeks and transferred to an oven to completely dry for 24 hours at 45^0C after which they were weighed and pulverised into powdery form and stored in air tight container respectively (Obaro *et al*., 2024).

3.2 Extraction of Plant Material

The powdered sample was extracted by method of cold maceration. 200 g of the powdered samples were soaked in 1000 ml of methanol solvent and scooped vigorously in an air tight jar and wrapped with black cloth which was kept in a cupboard and monitored regularly and scooped for 72 hours. After 72 hours, cheese cloth was used to separate the plant fiber from the concentrate and transferred to the oven to dry for seven (7) days at a controlled temperature of 45^0C. The dried sample was weighed and the yield was calculated.

3.3 Experimental Animals

Albino mice weighing 25 to 30 g and rats weighing 200 to 250 g were purchased from the Animal House of the Department of Anatomy, School of Basic Medicine,University of Benin.

3.3.1 Drugs /Chemicals/Materials

Streptozotocin, Glibenclamide, distilled water, rat restrainer, pelletized feed, cotton wool, methylated spirit, pocket P^H meter, glass slide, cover slips, microscope, centrifuge, hand gloves, plastic cages, syringe, oral gavage, glucometer**.**

3.3.2 Acute Toxicity Study

Acute toxicity study was carried out by methods of (OECD, 2018) Organisation of economic cooperation development guidelines.

Three (3) male mice and 3 female mice each were administered 5000 mg/kg of the aqueous extract of unripe plantain peel respectively and observed for 72 hours for possible signs of toxicity, mortality or morbidity.

3.3.3 Acute Normoglycemic Study

Study Design Before the induction of diabetes, six normoglycemic rats were administered single of 1000 mg/kg of the Methanol fruit extract of *Ocimum gratissimum* and *Murraya koenigii* (MOCGMK) orally and the blood sugar level were evaluated using blood samples obtained from tail vein.

3.3.4 Anti-Diabetic Study

Control Group: Rats of this group were administered distilled water (2 ml//kg/body weight) for 14 days.

Diabetic Group: In this group, rats were induced to become diabetic through a single intramuscular injection of STZ at a dose of 40 mg/kg of body weight. These diabetic rats received oral administration of distilled water for 14 days at a dose of 1 mL/kg/d body weight.

MOCGMK Treated Diabetic Group: Diabetic rats in this group were treated with methanol fruit extract of *Occimum gratissimum* and *Murraya koenigii* at doses of 100 and 250 mg/kg of body weight/d for 14 days while in a fasting state.

Glibenclamide Treated Diabetic Group: Rats in this group, which were also diabetic, received treatment with glibenclamide at a dose of 20 mg/kg of body weight/d for 14 days.

A single dose of Methanol extract of *Occimum gratissimum* and *Murraya koenigii* **MOCGMK** was administered orally every day in the morning (at 10.00 AM) using an orogastric gavage for a duration of 14 days. After 14 days of extract treatment animals were left untreated for 7 days (24^{th} day of STZ-injection), blood glucose levels were measured, and all animals were euthanized using chloroform anesthesia. Blood was subsequently drawn, and serum was separated by centrifugation at 3000 rpm for 10 minutes to perform serum biochemical and lipid profile tests. The right kidney were dissected out from each group and fixed in 10% formalin for histological studies.

While administering glibenclamide treatment, the FBG levels in all the test rats were assessed after 7 and 14 days of treatment through blood samples obtained from the tail vein were analyzed using an AccuCheck glucometer.

3.3.5 Blood Glucometer Check

Accucheck active glucometer and blood glucose test strips, manufactured by Roche Diagnostic GmbH in Mannheim, Germany, were employed to measure fasting blood glucose levels.

3.3.6 Histological Study

Wistar rats used for the anti-diabetic studies were anaesthetised and had their Kidneys excised. The excised organs were histologically prepared for further microscopic study.

3.3.7 Statistical Analysis

The results from the studies were expressed as mean ± SEM. Statistical analysis were carried out using graph pad prism 8 version software (UK). Comparisons between the control and treated groups were analysed using one-way ANOVA and, Dunnett's multiple comparisms test a and d = $P \leq 0.05$, and 0.0001 was regarded as indicating significant difference.

3.4 Experimental Grouping

Group 1 (G1) Control group

Group 2 (G2) the rats were given

Group 3 (G3) the rats were given

3.5 Experimental design

The rabbits were housed in wooden cages. The rabbits were fed 10% body weight with their respective food twice daily.

Group 1 rabbits were fed with commercial growers mash (10 am and 6 pm), Group 2 rabbits were fed with ripe plantain peel (10 am and 6 pm) while Group 3 rabbits were fed with unripe plantain peel (10 am and 6 pm). Cleaning and changing of the rabbit's beddings and enclosures was done twice daily at 10 hours (10 am and 6pm) in order to keep the environment clean for the rabbits to prevent infections and out breaks. Regular feed of the rabbits and cleaning of utensils as well as routine management practices was ensured.

3.6 Data collection

Data was collected at the beginning of the experiment regarded as day zero (1) and subsequently on weekly basis by taking measurements body weight. The body weight was measured using a Meteller electronic scale to the nearest 1000 g, while hematological parameters were measured using capillary tube and hematocrits reader. The average weekly weight gain was determined by the mean weight gains or loss. Twenty four (24) hours after the last feeding blood was drawn from the vein in the ear for Haematology and biochemical tests.

3.7 Statistical analysis

Every value was represented as Mean ± Standard Error of Mean (SEM). Using the UK's Graph Pad Prism 8.2 software, oneway ANOVA was used to analyze the data. $P \leq 0.05$ was used to define significance for differences.

CHAPTER FOUR

RESULTS

4.1 Effect of Methanol fruit extract of *Occimum gratissimum* and *Murraya koenigii* on Acute Toxicity Study

Administration of a single dose of 1000, 2000 and 5000 mg/kg of Methanol extract of *Ocimum gratissimum* and *Murraya koenigii in* mice resulted in no signs of toxicity such as: Grooming, nausea and writhing.

Table 4.1: Effect of Methanol fruit extract of *Ocimum gratissimum* and *Murraya koenigii o***n Acute Toxicity Study 4.2 EFFECT OF Methanol extract of *Ocimum gratissimum* and *Murraya koenigii* ON NORMOGLYCEMIC BLOOD SUGAR LEVEL**

Administration of a single dose of 1000 mg/kg of **MOCGMoK** to normoglycemic rats resulted in significant ($p \leq 0.005$) reduction of blood sugar levels of rats after 24, 36 and 72 hours (85.0 ± 1.0, 79.3±1.2 and 66.0±2.1 mg/dl) when compared with 0 hour (90.2±1.3 mg/dl)

Table 4.2: Effect of Methanol fruit extract of *Occimum gratissimum* and *Murraya koenigii* (MOCGMK) on normoglycemic blood sugar level

4.3 EFFECT OF METHANOL EXTRACT *Ocimum gratissimum* and *Murraya koenigii* ON BLOOD SUGAR LEVEL (BSL) OF STREPTOZOTOCIN INDUCED DIABETIC RATS.

Table 4.3: Effect of Methanol extract of *Occimum gratissimum* and *Murraya koenigii* on blood sugar level (BSL) of Streptozotocin induced diabetic rats.

4.4Effect of Methanol extract of *Ocimum gratissimum* and *Murraya koenigii* on Renal and Hepatic Function

4.4.1Aspartate aminotransferase

Figure 4.1: The aspartate reducing effect of methanol extract of *Ocimum gratissimum* and *Murraya koenigii*

4.4.2 Effect of Methanol fruit extract of *Ocimum gratissimum* and *Murraya koenigii* on Alanine Aminotransferase

Figure 4.2: effect of ripe and unripe plantain peels on alanine aminotransferse

4.4.3 Effect of Methanol extract of *Ocimum gratissimum* and *Murraya koenigii* on Alkaline phosphatase

figure 4.3: Effect of 100 and 250 mg/kg on alkaline phosphatase

Key: = $p< 0.01$, =$p<0.05$

4.4.4 Effect of Methanol extract of *Occimum gratissimum* and *Murraya Koenigii* on Creatinine level

4.5 Effect of Methanol extract of *Occimum gratissimum* and *Murraya koenigii* on Blood urea nitrogen

Figure 4.5: Effect *Occimum gratissimum* and *Murraya koenigii* on Blood creatinine level

Key: "= $p < 0.05$.

CHAPTER FIVE

5.0 DISCUSSION

Occimum gratissimum and *Murraya koenigii* (**MOCGMK**) commonly known as the sent leaves, has long been used in traditional medicine for its potential anti-diabetic properties. Recent studies on the methanol extract of its fruit have provided valuable insights into its normoglycemic and anti-diabetic effects, particularly in Streptozotocin (STZ) induced diabetic rat models ("Rattus norvegicus ") (Amjad *et al*., 2023). Acute toxicity studies have demonstrated that the methanol extract is relatively safe, with no fatalities recorded even at high doses (up to 5000 mg/kg), indicating a favorable safety profile for therapeutic use.The results from Table 4.2 show that administration of a single dose of 1000 mg/kg of the extract to normoglycemic rats significantly reduced blood sugar levels after 24, 36, and 72 hours (85.0 ± 1.0, 79.3 ± 1.2, and 66.0 ± 2.1 mg/dL, respectively) compared to the baseline (90.2 ± 1.3 mg/dL), confirming its normoglycemic effect. Similarly, Table 4.3 indicates that administering 100 mg and 250 mg of the methanol fruit extract to STZinduced diabetic rats significantly reduced blood sugar levels after 7, 14, and 28 days (100 mg: 168.4 ± 2.7, 62.1 ± 1.1, and 97.2 ± 6.3 mg/dL; 250 mg: 179.5 ± 1.8, 28.6 ± 0.8, and 75.1 ± 6.0 mg/dL), demonstrating its anti-diabetic potential.

Phytochemical analysis of the methanol extract revealed the presence of bioactive compounds such as alkaloids, flavonoids, saponins, glycosides, tannins, and terpenoids, which are known for their medicinal properties and likely contribute to the plant's anti-diabetic effects (Bever and Zahad, 2019). While the exact mechanisms remain unclear, flavonoids and saponins are thought to play a key role. Flavonoids may enhance glucose uptake in peripheral tissues, while saponins could improve insulin sensitivity and glucose metabolism (Boosenberg and Van, 2018).

In addition to **MOCGMK**, have been studied for their impact on renal and hepatic functions. **MOCGMK** rich in starch, iron, calcium, and antioxidants like polyphenols and flavonoids, while ripe peels have higher moisture, crude protein, fiber, ash, and potassium content. The antioxidant properties of **MOCGMK** may protect against oxidative stress in renal and hepatic tissues. For instance, Figures 4.1 and 4.2 show that *Ocimum gratissimum* significantly reduced aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, respectively, compared to the control group ($p \leq 0.05$). Similarly, Figures 4.3, 4.4, and 4.5 demonstrate that **MEOCGK** extract significantly reduced alkaline phosphatase (ALP) and blood creatinine levels at doses of 150 mg/kg and 500 mg/kg ($p < 0.05$, $p < 0.01$, and $p < 0.001$). In accordance with journal of pharmacognosy and phytochemistry shows that the plants extract have favourable effects in lowering the severity of diabetics together with hepatic protection Kumar (2018).

5.2 Conclusion

The methanol fruit extract of **MOCGMK** demonstrates significant normoglycemic and anti-diabetic effects in STZinduced diabetic rat models. The presence of bioactive compounds such as flavonoids, saponins, and terpenoids likely contributes to these effects. However, further research is needed to fully elucidate the mechanisms of action and to evaluate the longterm safety and efficacy of the extract for diabetes management. Additionally, MOCGMK with their rich nutrient and antioxidant profiles, show potential benefits for renal and hepatic health, highlighting the importance of exploring natural products for therapeutic applications.

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