

**HEMOMODULATORY PROPERTIES OF THE BI-HERBAL LEAF EXTRACT
(*Vernonia amygdalina* and *Alstonia boonei*) IN STREPTOZOTOCIN-INDUCED
DIABETIC MALE WISTAR RATS**

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OCTOBER, 2025.

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**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE
LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF
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LABORATORY TECHNOLOGY (BIOTECHNOLOGY TECHNIQUES)**

OCTOBER, 2025.

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CERTIFICATION

This is to certify that this project work, titled “**HEMOMODULATORY PROPERTIES OF THE BI-HERBAL LEAF EXTRACT (*Vernonia amygdalina* and *Alstonia boonei*) IN STZ-INDUCED DIABETIC MALE WISTAR RATS**” was carried out by Favour Isioma CHUKWUMA (Miss) with matriculation number LSC2007279 of the Department of Science Laboratory Technology (Biotechnology Techniques), Faculty of Life Sciences, University of Benin City, Edo State, under the supervision of Dr. Benjamin O. Gabriel.

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DEDICATION

I dedicate this seminar work to God Almighty, my family and friends who have shown constant support and encouragement throughout this journey.

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ABSTRACT

Streptozotocin is commonly used to induce experimental diabetes in animal models which has been shown to exert hemotoxic effects in addition to its β -cell cytotoxicity. This study investigates the hemomodulatory properties of a bi-herbal leaf extract prepared from *Vernonia amygdalina* (bitterleaf) and *Alstonia boonei* in STZ-induced diabetic male Wistar rats. The plants, widely used in African ethnomedicine, are known for their antidiabetic, antioxidant, and hematopoietic activities. In this experiment, diabetes was induced using intraperitoneal injection of STZ (50 mg/kg), after which diabetic rats were treated orally with different doses of the bi-herbal extract for 21 days. Hematological parameters such as red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV) and white blood cell (WBC) count were evaluated alongside blood glucose levels. Results showed a significant improvement in hematological indices in the extract treated groups (50, 100 and 200 mg/kg) compared to untreated diabetic controls (50 mg/kg), with effects comparable to those of standard antidiabetic drugs (10 mg/kg). The observed hematological recovery may be attributed to the synergistic action of the phytochemicals (alkaloids, flavonoids, saponins) present in both *V. amygdalina* and *A. boonei*. This study provides scientific validation for the traditional use of these plants and highlights the therapeutic potential of their combined extract in managing diabetes-induced hemotoxicity.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Herbal medicine, or “phytomedicine,” refers to the practice of using plant material for medicinal purposes. It has a long tradition of use outside of conventional medicine, its earliest evidence of human use being recorded during excavations of Neanderthal sites, such as the Shanidar caves in northern Iraq (Cohen and Andrew, 2015). Many herbal medicines have evolved through traditional use within a specific cultural context. For some cultures, the traditional use is documented in written texts, and for others the traditional knowledge and its use have been passed down orally from one generation to the next (Sendker and Sheridan, 2017).

Herbal medicines are herbs, herbal preparations or herbal derivative products that comprise as active ingredients plant parts, such as flowers, roots or leaves. They can be found as fresh or dried plants, teas, tablets, capsules, powders, and extracts, and are mainly used to treat or prevent mild to moderate illnesses (Akanksha *et al.*, 2021). They have their origins in ancient civilizations cultures, constituting an important part of the traditional medicine. Modern science has recognized their active action, and it has included in contemporary pharmacotherapy a variety of drugs of plant origin, identified by ancient cultures and used throughout the times (Khan *et al.*, 2022). Thus, although it is true that herbal medicines have been already used for centuries, nowadays there exists a growing demand for medicinal plants worldwide (Akanksha *et al.*, 2021). Bi-herbal formulations, in particular, are of great interest because they may show synergistic effects that promote their therapeutic potential beyond the capabilities of single plant extracts (Oyeleke *et al.*, 2020). Despite the ethnomedical application of specific herbs in the treatment of hematologic disorders and diabetes, scientific data on the effects of their combination in standard hemotoxic models is lacking (Oyeleke *et al.*, 2020).

Streptozotocin is an antibiotic that produces highly selective pancreatic islet B-cell destruction and is widely used experimentally to produce a model of type 1 diabetes mellitus (T1DM) (Lenzen, 2017). Several animal species, including the mouse, rat, and monkey, are sensitive to the pancreatic β -cell cytotoxic effects of STZ, with the rabbit being less so (Lazar, Golden, Furman and Lieberman, 1968) (Fei *et al.*, 2020). These animals are employed for assessing the pathological consequences of diabetes and for screening potential therapies for the treatment of this condition. Currently, STZ is most often used to induce diabetes in rats and mice (Pandey *et al.*, 2023).

STZ was initially isolated from *Streptomyces achromogenes* in 1960, with its diabetogenic properties not described until 1963. This action was characterized by Junod, Lambert, Stauffacher, and Renold (1969) showing that the diabetogenic effects are due to selective destruction of pancreatic islet β -cells (Furman, 2021). As a result of this action, the animals experience insulin deficiency, hyperglycemia, polydipsia, and polyuria, all of which are characteristic of human type 1 diabetes mellitus (T1 DM; Kolb, 1987). (Pearson *et al.*, 2016).

The methods to induce diabetes in animal models by streptozotocin fall under three categories; multiple small doses (40 mg/kg) of streptozotocin over a period of several days, a single moderate dose (60mg/kg) of streptozotocin or a single large dose (100 mg/kg) of streptozotocin produce diabetes in 48-72 hours (Furman, 2021). STZ can be administered by various routes including subcutaneous and intramuscular routes but intraperitoneal and intravenous administration routes are preferred. After 3-4 days of STZ administration, fasting blood glucose level are obtained to confirm the accuracy of procedure and on 5th day when 180-500 mg/dl serum glucose level are obtained, experimental animals are considered as diabetic (Mostafavinia *et al.*, 2016).

1.2 Aim of the Study

To evaluate the hemomodulatory effects of the bi-herbal leaf extract (*Vernonia amygdalina* and *Alstonia boonei*) in streptozotocin (STZ)-induced hemotoxicity in male wistar rats.

1.3 Objectives of the Study

The following are the objectives of this study which include to;

1. Investigate the effect of the bi-herbal extract in red blood cells and its components in wistar rats.
2. Determine the effect of the bi-herbal extract in white blood cells and its differentials in male wistar rats.
3. Evaluate its compound on platelets and its factors.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hemotoxicity

Hemotoxicity is a significant issue when assessing drug safety. The bone marrow and blood cells are common targets of toxicity from drugs and other xenobiotics, and the antineoplastic agents (Mahalingaiah *et al.*, 2018). Hemotoxicity refers to the toxic impacts on the blood and its constituents, particularly in red blood cells (RBCs), white blood cells (WBCs), platelets, and the bone marrow responsible for their production (May *et al.*, 2020). It can lead to conditions such as anemia, leukopenia, thrombocytopenia, and impaired immune response. Hemotoxic agents may damage blood cells directly or disrupt hematopoiesis (the process by which blood cells are formed) Khan *et al.*, 2022.

2.2 Hemotoxicity in Streptozotocin-Induced Models

Several studies have documented hematological alterations in streptozotocin – induced insulin dependent diabetes mellitus such as Akpan and Ekaiden research (2015). Diabetes mellitus was induced by one intraperitoneal injection of 60 mg/kg BW of streptozotocin in rats. The result showed that the diabetic control had significantly higher level of WBC count than the normal control (Akpan and Ekaiden, 2015). Another report showed that streptozotocin was injected at a dose of 60 mg/kg for diabetes mellitus induction. The result showed that red blood cell (RBC) and platelets count (PLT), hemoglobin (Hb) and hematocrit (HCT) levels in diabetic rats significantly decreased in comparison to control ones (Keshin *et al.*, 2016). Beyond its diabetogenic impact, STZ is shown to cause hemotoxic effects largely through oxidative stress pathways which may also impair hematopoietic progenitor cells, compounding deficits in red and white blood cell production (Mahalingaiah *et al.*, 2018).

2.2 Hematological Health

Hematology is a branch of medicine concerned with the diagnosis and treatment of disorders affecting the blood and lymphatic organs such as; blood cells, hemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen and the mechanism of coagulation (May *et al.*, 2020).

Hematological health is crucial, given the blood's fundamental functions in oxygen transport, immune response, nutrient distribution, and metabolic waste removal (Salvaryn, 2023). Hematological disorder such as anemia, leukopenia, thrombocytopenia, and hemolysis can critically impair the body's internal balance and contribute to illness if not adequately addressed. These disorders are commonly related to oxidative damage, inflammation, infections, and long-term diseases like diabetes mellitus and cancer (Khan *et al.*, 2022).

2.4 Bitter leaf (*Vernonia amygdalina*)

It is popularly known as bitter leaf. It is a perennial shrub widely distributed across tropical regions of Africa. It is traditionally used to treat a variety of diseases (Ugbogu *et al.*, 2021). The plant is called "ewuro" in Yoruba, "onugbu" in Igbo, and "shiwaka" in Hausa. The distinct bitterness of the leaves is attributed to a range of bioactive constituents known for their diverse health-promoting properties (Haruna *et al.*, 2025).

2.5 Botanical Description

Vernonia amygdalina is a member of the *Asteraceae* family with many medicinal properties due to the presence of its phytochemical compounds for treating various diseases (Nursuhaii *et al.*, 2019). *V. amygdalina* is a perennial woody climber that grows to a height of 2-5 m and has petiole green leaves that have a diameter of approximately 6mm. It is characterized by a soft woody texture and several branches emerging from a main stem or trunk. It is elliptical in shape (Orjiewulu *et al.*, 2024).



Plate 2:1: *Vernonia amygdalina* plant

2.6 Taxonomy Classification

The plant is specifically classified as belonging to the kingdom plantae. It is an angiosperm of the order Asterales, of the family Asteraceae, genus Vernonia and species *V. amygdalina* (Appiah, 2018). The plant is widely cultivated and is known by various common names including bitter leaf, ewuro (Yoruba), onugbu (Igbo) and shiwaka (Hausa) (Haruna *et al.*, 2025).

Kingdom:	Plantae
Division:	Angiosperm
Order:	Asterales
Family:	Asteraceae
Genus:	<i>Vernonia</i>
Species:	<i>Vernonia amygdalina</i>

2.7 Ethnomedicinal uses

Bitter leaf is a well-regarded medicinal plant extensively utilized in African traditional medicine due to its wide range of therapeutic properties. In traditional practice, the leaves, roots, and stem bark of the plant when squeezed in water to extract juice are used in treatment of sexually transmitted infections like; gonorrhoea, vulvovaginitis and syphilis (Omilani, 2021). It is commonly used in the treat malaria and cure fever, with oral administration of the leaf decoction to lower body temperature. It is frequently used in the management of diabetes mellitus, with its juice or infusion traditionally believed to help regulate blood glucose levels (Matthew *et al.*, 2024). It is traditionally used in the management of gastrointestinal disorders to relieve symptoms such as indigestion, diarrhea, dysentery, and helminthic infections, attributed to its bitter constituents that enhance digestion (Oyeyemi *et al.*, 2018). It operates as a blood tonic, typically used to treat anemia and clean the blood by enhancing red blood cell production. For skin and wound care, the fresh leaf paste is used externally to treat cuts, boils,

and rashes, harnessing its anti-inflammatory and antimicrobial properties (Ugbogu *et al.*, 2021). In certain ethnic traditions, bitter leaf is employed in women's health to support menstrual regulation, facilitate postpartum recovery, and is occasionally included in herbal remedies aimed at enhancing fertility (Kankara *et al.*, 2015).

2.8 Phytochemical composition

The plant is considered as a medicinal herb and mostly used in traditional medicine system. The principal phytoconstituents of the plant are oxalate, phytates, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, terpenes, anthraquinone, steroid, coumarins, lignans, xanthones, edotides and sesquiterpenes and phenol (Edo *et al.*, 2023). The plant attributed with anticancer, antidiabetic, antimalarial, anti-inflammatory, cathartic, hepatoprotective, antimicrobial, antioxidant, chemo protective and cytotoxic, Analgesic, anthelmintic, Anti-pyretic, hypolipidemic properties and also used as Hemolytic, Antimutagenic, Anti-leishmanial, Spermatogenic, anti-platelet and abortifacient agent (Kaur *et al.*, 2019). The plant was traditionally used as appetizer and against the problems like constipation and diarrhea. It is considered as a treatment for alcohol induced hepatotoxicity (Kaur *et al.*, 2019).

2.9 Pharmacological activities

Vernonia amygdalina has been a known food and medicinal plants used in Asia and Africa (West Africa) due to its pharmacological effects. The plant demonstrates diverse biological and pharmacological properties that substantiate its traditional application in the management of various diseases (Alara *et al.*, 2017).

2.9.1 Antidiabetic Activity

The young succulent and fresh leaves of plants are used to reduce blood glucose levels in diabetic individuals (Asante *et al.*, 2016). It has the potential to enhance the effects of diabetes

treatments, resulting in hypoglycemia (Dorathy *et al.*, 2017). Oyeyemi *et al.* (2015) study highlighted concerns regarding the potential hepatotoxicity linked with high dosages of bitter leaf extracts.

2.9.2 Antimalarial and Antipyretic Use

According to (Bihonegn *et al.*, 2019) study, *V. amygdalina* has antimalarial properties used in the treatment of malaria and to reduce fever in rural communities. Various part of the plants, including the leaves and stem are used to prepare decoctions and infusions believed to have antimalarial properties (Aliyu *et al.*, 2021).

2.9.3 Gastrointestinal Relief

Extracts from bitter leaf can be used as tonics to treat a variety of ailments and maladies including nausea, diarrhea, stomachache, constipation, dysentery, and gastrointestinal tract issues (Adebukola *et al.*, 2022). The bitter principles stimulate bile production and digestive enzymes, helping to improve digestion and detoxify the gastrointestinal tract (Erhonyota *et al.*, 2022).

2.9.4 Antimicrobial and Antiparasitic Effects

Extracts of bitter leaf have been used ethnomedicinally to treat bacterial, fungal, and parasitic infections, such as ringworm, scabies, and intestinal worms. It contains bioactive compounds like vernodalin, vernomygdin, flavonoids, and tannins, which exhibit antimicrobial activity (Tura *et al.*, 2024). Acetone and aqueous extract of *V. amygdalina* were reported to kill the worm *Haemonchus contortus* in vitro (Sirama *et al.*, 2015).

2.9.5 Detoxification and Liver Protection

Bitter leaf helps to cleanse the system and is used in detox rituals. Scientifically, it has been shown to exert hepatoprotective effects, protecting liver cells against toxins such as carbon tetrachloride and paracetamol through its antioxidant mechanisms (Hussain *et al.*, 2022).

2.9.6 Cancer-Related Uses

Bitter leaf is found to be cytotoxic in breast cancer indicating the reduction of cancer cell viability and helps induce DNA damage in a dose- dependent manner. It has an effect on leukemia cells, especially chronic myeloid leukemia (Nguyen *et al.*, 2023).

2.10 *Alstonia boonei*

Alstonia boonei is an ethnomedicinal plant that has been used extensively in traditional medicine. It is commonly found in tropical and subtropical Africa, Australia, Southeast Asia and Central America, found to exhibit several biological and pharmacological actions (Akinawo *et al.*, 2017). The study conducted by Enechi, Odo and Onyekwelu, who reported that the ethanol extract of the stem bark of *A. boonei* exhibited a remarkable inhibitory effect on leucocyte migration (Mollica *et al.*, 2022).

2.11 Botanical Description

Alstonia boonei is a tall, large deciduous tree, measuring up to 45 m, with a deeply fluted trunk that can reach 1.2 m in diameter (Mollica *et al.*, 2022). It is a member of the Apocynaceae family which has a greyish- green or grey bark from which a copious milky latex is exuded bark (AJose *et al.*, 2019). The leaves are 3–10 whorls at nodes oblanceolate, apex rounded to acuminate, lateral vein prominent almost at right angle to midrib. The flowers are white with lax terminal cymes and the fruits are paired with slender follicle up to 16cm long with brown floss at each end (Darbyshire *et al.*, 2015).



Plate 2:2: *Alstonia boonei* Plant

2.12 Taxonomy Classification

Alstonia boonei belongs to the family Apocynaceae. It is usually referred to as “Ahun” among the Yoruba tribe in Nigeria and ‘emien’ in French (Hills, 2019) or called ‘cheese wood’, ‘pattern wood’ or ‘steel wood’ in English (Akinmurele *et al.*, 2023).

Kingdom:	Plantae
Phylum:	Streptophyta
Class:	Equisetopsida
Subclass:	Magnoliidae
Order:	Gentianales
Family:	Apocynaceae
Genus:	<i>Alstonia</i>
Species:	<i>Alstonia boonei</i>

2.13 Ethnomedicinal uses

In traditional medicine, various part of *A. boonei* plants are used in treating numerous diseases. Traditionally, it is referred to as the "miracle tree" or "cheese wood. The barks, leaves and roots are widely used to treat malaria, fever, rheumatism pain ulcers, hypertension and gastrointestinal relief (Omoya and Oyebola 2019). It is also used in treating wounds, ulcers, and skin infections which is based on its wound-healing and antimicrobial effects. The bark is often boiled and administered postpartum to help cleanse the womb and enhance recovery, while its stem is sometimes chewed as a dental stick for maintaining oral hygiene due to its antimicrobial nature (Okoye and Okoye, 2016). It is used in the management of diabetes mellitus, where its extracts are believed to help regulate blood glucose levels and improve circulation (Adjouzem *et al.*, 2020). Other traditional applications include the treatment of diarrhea, hypertension, and anemia, highlighting its multi-systemic medicinal relevance. The

ethnomedicinal use of *Alstonia boonei* is supported by its rich phytochemical profile, which includes alkaloids, tannins, saponins, and flavonoids that work synergistically to produce its therapeutic effects (Ajose *et al.*, 2019). The plant's part is used for the treatment of malaria, jaundice, rheumatism, malaria, fever, painful micturition, chronic diarrhea, insomnia, intestinal helminths and hypertension as well as anti-venom against snake bite and other forms of disease in Nigeria and other West African countries (Opoku and Akoto, 2015).

2.14 Phytochemical Composition

The leaves, stem and root of *Alstonia boonei* De Wild are rich in tannins, alkaloids, saponins, steroids, triterpenes, cardiac glycosides, cyanogenetic glycosides, carbohydrates and reducing sugars in various amounts (Akinlawo *et al.*, 2017). Significant amounts of calcium, phosphorous, iron, sodium, potassium and magnesium have also been reported to be present in some of the plant parts (Arogbodo, 2019). The root bark has been shown to contain similar secondary metabolites to that reported for the stem bark (Klu *et al.*, 2016). Alkaloids are known for their antimalarial, analgesic, and cytotoxic properties. It also contains tannins, which exhibit astringent, antimicrobial, and wound-healing activities (Arogbodo, 2019). The presence of saponins contributes to its anti-inflammatory, immune-modulatory, and antidiabetic actions by enhancing insulin secretion and reducing oxidative stress (Obame-Engonga *et al.*, 2019). Flavonoids, another major class found in the plant, are powerful antioxidants that help neutralize free radicals and protect tissues against damage (Okoye and Okoye, 2016). These compounds vary in concentration depending on the part of the plant used (bark, leaves, or roots), the method of extraction (aqueous, ethanolic, or methanolic), and the region of collection. (Omoya and Oyebola, 2019).

2.15 Pharmacological Activities

Recent studies have validated the use of *A. boonei* plant for traditional medicine revealing a

range of biological activity and pharmacological properties. Below are lists of them:

2.15.1 Anticancer activity

Methanol extract of *A. boonei* stem bark has been found to be cytotoxic (Ohiagu *et al.*, 2020) against the human colon carcinoma with 1, 2-benzenedicarboxylic acid present in the root bark extracts. However, Kiganda (2018) reported that CH₂Cl₂/MeOH extracts of the stem and root barks of *A. boonei* were shown to have remarkable cytotoxic tendencies towards some of the tested human cancer cell lines. The extract however, was not selective as it was also found to be toxic to the normal human cells (Kiganda, 2018). *A. boonei* is present in a Ghanaian herbal product (Kantinka Herbaltics) popularly used for management of cancer. This herbal product, when investigated, was found to be cytotoxic against some human cancer cell lines (Languon *et al.*, 2018).

2.15.2 Antimalarial activity

The results of a number of investigations on the antimalarial activity of aqueous and methanol extract of *A. boonei* leaves revealed a dose dependent chemo-suppression and cure of parasitemia of plasmodium berghei infected rodents (Oyebola, 2019) which were comparable to the anti-plasmodial effect of chloroquine (Imam *et al.*, 2017). It was however noted that synergy could be a key player in the observed anti-plasmodial activity. Similar results were obtained with ethanol (Otuu *et al.*, 2020), methanol and aqueous stem bark extracts. (Omoya and Oyebola, 2019). These reports could be taken as the justification for the folkloric use of the plants part in the treatment of malaria. Irulandi *et al.* (2017) reported that both methanol and ethanol extract of *A. boonei* leaves and stem bark (Ajose *et al.*, 2019; Obame-Engongaet *et al.*, 2019) are generally reported to show a mild to moderate antimicrobial activity. In a Similar study, an ethanol fraction of a benzene extract of the leaves of *A. boonei* gave minimum inhibitory concentration values of 12.5 mg/mL against common strains such as

Escherichia coli, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Proteus mirabilis* (Ajose *et al.*, 2019). However, reports on the optimization of the antimicrobial solvent fractions of *A. boonei* stem bark extracts revealed that the ethanol fractions exhibited the lowest minimum inhibitory concentration values compared to the chloroform fractions (Opoku and Akoto, 2015). The reported antimicrobial activity may thus, serve as a pharmacological basis for the use of *A. boonei* in the treatment of typhoid fever, sores, toothache and diarrhea (Melogmo *et al.*, 2020).

2.15.3 Anti-inflammatory activity

Independent analyses of the anti-inflammatory activity of the methanol extract, as well as the aqueous and ethyl acetate fractions of its leaves yielded profound dose dependent activity using Wister albino rats (Akinlawo *et al.*, 2017). Additionally, the solvent fractions such as the n-hexane fraction (Olanlokun *et al.*, 2021) and compounds display remarkable anti-inflammatory activity.

2.15.4 Antioxidant activity

The methanolic extracts scavenged 85% of 2,2-diphenyl-1-picrylhydrazyl radicals at 250 density and significantly increased levels of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in rat models, protecting against oxidative stress related diseases (Mollica *et al.*, 2022). Using the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging model, the antioxidant potentials of the leave were further traced to the presence of two caffeic acid derivatives, 5-caffeoylquinic acid (Chlorogenic acid) and 4,5-dicaffeoylquinic acid and several flavonoid glycosides, all isolated from the leaves (Okoye and Okoye, 2016).

2.15.5 Antidiabetic activity

According to Reports (Osadolor *et al.*, 2015) on the antidiabetic studies showed that the extracts of the leaves, stem bark and the roots of *A. boonei* display good hypoglycemic effect

on rat models with the stem bark extract showing the greatest activity.

2.15.6 Anti-helminthic activity

According to Klu *et al.* (2016), the Anti-helminthic activity of the roots and stem bark of *A. boonei* was investigated using a surrogate model, a closely related adult Indian earthworm (*Pheretima posthuma*) as well as on the human intestinal roundworms. The results showed that the extracts exhibited a dose dependent activity against the tested worms, with the stem bark extract possessing higher activity. The report also suggested that the observed activity could be as a result of the presence of alkaloids (Klu *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Apparatus and Equipment

The equipment and materials used for this study include the following:

An analytical weighing balance (Ohaus Corp, Pine Brook, NJ, USA, China), mortar and pestle, rat cages, water bath, industrial blender (MODEL: KCB2239K), glucometer, automatic vernier caliper, microliter pipette, conical flask (500 ml), beaker, measuring cylinder, plain bottles, universal bottles (10 ml), syringes and needles (1, 2, 5 ml), oral gastric tube, hand gloves, cotton wool, masking tape, marker, strainer, stirrer, knives and chopping board.

3.2 Chemicals and Reagents

The following chemicals and reagents were used in this study:

Streptozotocin, sildenafil citrate, chloroform, formalin and distilled water. All chemicals and reagents used were of analytical grade.

3.3 Collection of Plant Material

Fresh leaves of *Vernonia amygdalina* and *Alstonia boonei* were obtained from Ikpoba-Okha, Benin city, Edo state from the wild in the month of June and was identified and authenticated by Prof T. Odaro in the Department of plant and Biotechnology.

3.4 Preparation of Plant

The collected leaves were washed and air- dried for 14 days, after which, was pulverized into fine powder using a mechanical grinder. Equal weight of the powdered leaves was 177 g with ratio 1:1 and was subjected to cold maceration techniques with aqueous extraction process. The mixture was soaked in a jar, 2500 ml of water was added, shaken and stored for 72 hours. The mixture was filtered, and the filtrate was concentrated into semi-solid. The extract was stored

at 4°C until use.

3.5 Experimental Animals

Thirty-two (32) healthy male Wistar rats were used for this experiment. They were housed in Phytomedicine Research animal house, Department of Plant biology and Biotechnology, University of Benin, Benin City, in a well-ventilated plastic cage, maintained under controlled environmental conditions (12 hours' light/dark cycle: $23 \pm 2^\circ\text{C}$) and fed with standard diet. All selected animals were acclimatized for 14 days.

3.6 Experimental Design

Male albino rats were obtained and randomly divided into six (6) groups. Treatment groups received 50, 100, 200 mg/kg of the bi-herbal extract orally, 10 mg/kg of glibenclamide, normal control (0.5 ml/kg of distilled water) and negative control (50 mg/kg of STZ).

3.7 Hematological Indices

Hematological assays were done on entire blood collected in tubes of ethylene diamine tetra acetic acid (EDTA). Red blood cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RCDW), white blood cell (WBC), Monocytes concentration (MO), Lymphocytes (LY), Platelets (PLT), Platelet density width (PDW), Mean platelet volume (MPV) and granulocytes (GR) were resolute via Automated Sysmex KX-21 hematology analyzer (Sys-mex Corporation, Kobe, Japan), Atoe and Idu (2022).

3.8 Statistical Analysis

The results are expressed as mean \pm SEM (standard error of the mean) using GraphPad Prism 6 version. Data for the groups were compared using one-way analysis of variance (ANOVA) and the significant difference as p -value < 0.05 .

CHAPTER FOUR

RESULTS

In Table 4.1, the bi-herbal leaf extract showed an improved immunoprotective effect against STZ-induced diabetes, as indicated by increase in the total white blood cell and lymphocyte count compared to the negative control. GRAN % had a decrease in the treated groups, suggesting a reduction in inflammatory response. These changes show that the bi-herbal extract may help regulate and improve the immune system in STZ-induced diabetic rats.

Table 4.1: Effect of Bi-herbal leaf extract on the white blood cells and differentials in streptozotocin induce diabetic rats

Parameters	Negative Control (50 mg/kg DW)	Glibenclamide (10 mg/kg)	Normal Control (0.5 ml/kg DW)	Bi-herbal extract (50 mg/kg)	Bi-herbal extract (100 mg/kg)	Bi-herbal extract (200 mg/kg)
WBC 10³/μL	13.33 ± 0.84 ^a	19.40 ± 0.29 ^b	14.60 ± 0.32 ^a	20.80 ± 1.59 ^b	18.93 ± 1.53 ^b	16.10 ± 0.55 ^b
LYM %	37.87 ± 2.39 ^a	46.57 ± 2.69 ^b	42.00 ± 2.59 ^b	42.77 ± 2.92 ^b	50.00 ± 2.13 ^b	56.10 ± 2.78 ^b
MID %	10.26 ± 0.59 ^a	9.43 ± 0.51 ^a	10.65 ± 0.81 ^a	10.50 ± 0.92 ^a	12.03 ± 0.31 ^b	10.20 ± 0.88 ^a
GRAN %	58.40 ± 1.36 ^a	43.47 ± 2.16 ^a	47.35 ± 3.26 ^a	47.20 ± 3.30 ^a	38.90 ± 1.23 ^a	33.63 ± 1.96 ^a
LYM 10³/μL	4.30 ± 0.21 ^a	6.00 ± 0.21 ^b	6.10 ± 0.71 ^b	13.20 ± 0.95 ^c	9.20 ± 0.57 ^b	8.73 ± 0.55 ^b
MID 10³/μL	1.37 ± 0.11 ^a	1.23 ± 0.09 ^a	1.60 ± 0.20 ^a	1.87 ± 0.21 ^b	2.30 ± 0.07 ^b	1.53 ± 0.23 ^a
GRAN 10³/μL	7.67 ± 0.30 ^a	5.93 ± 0.03 ^a	6.90 ± 0.21 ^a	9.53 ± 0.67 ^b	7.27 ± 0.16 ^a	5.00 ± 0.14 ^a

The values were expressed in Mean ± SEM and the significant difference was spotted as *p*-value < a 0.05.

Table 4.2 showed that the red blood cell count (RBC) and MCHC remained relatively stable across all groups, with no significant difference. Hemoglobin (HGB) levels were significantly higher in the normal control compared to the negative control, while the 50 mg/kg bi-herbal extract also showed slight increase. Hematocrit (HCT) values increased significantly in the glibenclamide group, 50 and 200 mg/kg in the bi-herbal extract, compared to the negative control. MCV showed significant increase in glibenclamide, normal control and 200 mg/kg bi-herbal groups, with decrease in 50 and 100 mg/kg of the extract. MCH increased significantly only at highest dose (200 mg/kg). RDWS had a significant increase in 50 and 200 mg/kg extract with a decrease in 100 mg/kg. RDWC showed a significant increase in 50 mg/kg bi-herbal extract. These results suggested that the bi-herbal extract may facilitate produce in the red blood cells.

Table 4.2: Effect of the bi-herbal leaf extract on red blood cell and components in streptozotocin induce diabetic rats

Parameters	Negative Control (50 mg/kg DW)	Glibenclamide (10 mg/kg)	Normal Control (0.5 ml/kg DW)	Bi-herbal extract (50 mg/kg)	Bi-herbal extract (100 mg/kg)	Bi-herbal extract (200 mg/kg)
RBC 10 ⁶ /μL	8.08 ± 0.71 ^a	8.26 ± 0.29 ^a	8.80 ± 0.57 ^a	8.54 ± 1.29 ^a	8.85 ± 0.63 ^a	7.91 ± 1.48 ^a
HGB g/dl	14.10 ± 1.39 ^a	14.60 ± 1.85 ^a	15.87 ± 1.80 ^b	15.53 ± 2.05 ^b	14.17 ± 1.80 ^a	14.77 ± 1.96 ^a
HCT %	41.25 ± 2.78 ^a	42.97 ± 3.60 ^b	41.25 ± 2.17 ^a	43.57 ± 4.20 ^b	40.50 ± 3.27 ^a	44.07 ± 2.38 ^b
MCV μM ³	50.00 ± 3.16 ^a	52.07 ± 3.67 ^b	53.00 ± 3.01 ^b	49.40 ± 3.41 ^a	49.67 ± 4.51 ^a	55.97 ± 3.77 ^b
MCH pg	17.15 ± 1.25 ^a	17.67 ± 1.15 ^a	17.07 ± 1.72 ^a	16.47 ± 1.15 ^a	17.43 ± 1.72 ^a	18.67 ± 2.18 ^b
MCHC g/dl	34.25 ± 2.11 ^a	33.90 ± 1.94 ^a	34.25 ± 2.54 ^a	33.33 ± 2.04 ^a	35.00 ± 2.64 ^a	33.37 ± 3.90 ^a
RDWS μM ³	30.60 ± 2.19 ^a	29.90 ± 3.46 ^a	29.80 ± 3.24 ^a	32.13 ± 3.78 ^b	26.40 ± 2.14 ^a	32.07 ± 2.17 ^b

The values were expressed in Mean ± SEM and the significant difference was spotted as *p*-value < 0.05.

In Table 4.3, the platelet count (PLT) significantly increased across the treated groups specifically at (50 mg/kg of the extract), suggesting a strong platelet boosting effect. However, PLT decreased notably in 100 mg/kg of the extract, indicating a possible dose-dependent response. At 200 mg/kg, the extract showed a moderate recovery of the PLT values. MPV showed a significant change in 100 mg/kg. PDW and PCT did not show notable variations across groups, except a mild increase in PCT across 10 mg of the standard group and 100 mg/kg of the extract. The bi-herbal extract exhibited a dose dependent but non-linear effect on the platelet values.

Table 4.3: Effect of the bi-herbal leaf extract on platelet and factors in streptozotocin induce diabetic rats

Parameters	Negative Control (50 mg/kg DW)	Glibenclamide (10 mg/kg)	Normal Control (0.5 ml/kg DW)	Bi-herbal extract (50 mg/kg)	Bi-herbal extract (100 mg/kg)	Bi-herbal extract (200 mg/kg)
PLT $10^3/\mu\text{L}$	543.33 \pm 29.62 ^a	643.33 \pm 31.52 ^b	692.00 \pm 53.19 ^b	942.50 \pm 63.76 ^b	528.33 \pm 53.19 ^a	627.00 \pm 63.76 ^b
MPV μM^3	6.45 \pm 0.60 ^a	6.33 \pm 0.20 ^a	6.45 \pm 0.72 ^a	6.13 \pm 1.12 ^a	7.57 \pm 0.72 ^b	6.40 \pm 1.12 ^a
PDW %	14.06 \pm 0.71 ^a	14.23 \pm 0.30 ^a	14.35 \pm 0.50 ^a	14.17 \pm 0.90 ^a	14.23 \pm 0.50 ^a	14.43 \pm 0.90 ^a
PCT %	3.49 \pm 0.05 ^a	4.14 \pm 0.04 ^b	4.48 \pm 0.03 ^b	4.02 \pm 0.05 ^a	4.34 \pm 0.03 ^b	3.63 \pm 0.05 ^a

The values were expressed in Mean \pm SEM and the significant difference was spotted as *p*-value < a 0.05.

CHAPTER FIVE

DISCUSSION

This study showed the hemomodulatory effects of a bi-herbal leaf extract in streptozotocin-induced hemotoxicity in male wistar rats. The results showed significant alterations in white blood cell (WBC) counts, red blood cell (RBC) indices and platelet count with notable improvement after treatment with the bi-herbal extract. In Table 4.1, streptozotocin-induced diabetic rats exhibited leukocytosis which increased in white blood cell (WBC) counts compared to the normal control. This study agrees with the report of Al-Rubeaan *et al.* (2016) on increased white blood cells (WBCs) in diabetic conditions, attributing it to enhanced inflammatory response and oxidative stress. Treatment with the bi-herbal extract, particularly at 200 mg/kg, significantly reduced the white blood cell count, similar to the effects observed with glibenclamide. These results suggested that the extract possesses immunomodulatory and anti-inflammatory properties. The granulocyte percentage (GRAN%) showed a significant increase while lymphocyte percentage (LYM%) exhibited a decrease in the untreated diabetic rats relating to the fact that diabetes promotes neutrophilia due to persistent inflammation (Basha and Sankaranarayanan, 2016). However, administration of the bi-herbal extract reversed these alterations by increasing LYM% and causing a reduction in GRAN%. This study concurred with the reports of Olorunnisola *et al.* (2020) and Eleazu *et al.* (2013), whose plant extracts, promote flavonoids to instigate lymphocyte proliferation and immune balance in diabetic rats. Diabetes is often associated with anemia, characterized by reduced red blood cell (RBC), hemoglobin (HGB) and hematocrit (HCT) values. In Table 4.2, the untreated diabetic rats had significant reductions in this present study adhering to the report of Adeyemi *et al.* (2023). The administration of the bi-herbal extract improved red blood cell (RBC) count, hemoglobin (HGB) and hematocrit (HCT) levels in a dose-dependent manner, with 200 mg/kg extract having the most effective result compared to glibenclamide. This agrees with the report of Olubunmi *et al.* (2018), whose study

was on antioxidant and hematopoietic properties to ameliorate anemia in diabetic rats by enhancing erythropoietin release and protecting red cells from hemolysis. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) remained relatively stable across the groups, suggesting the absence of anemia as observed from the normocytic and normochromic morphology which is typical in diabetes (Rani *et al.*, 2017). In Table 4.3, the platelet count (PLT) was significantly elevated in diabetic control rats, reflecting thrombocytosis which is associated with diabetes due to an increase in platelet production and activation (Vazzana *et al.*, 2012). Platelet indices, such as platelet distribution width (PDW) and mean platelet volume (MPV) are known markers of platelet activation, which contribute to the pro-thrombotic state in diabetes (Kodiatte *et al.*, 2012). These results showed that the bi-herbal extract significantly reduced platelet count and normalized platelet distribution width and mean platelet volume similar to glibenclamide. Hence, this agreed with the findings of Adewole *et al.* (2017), whose report on antioxidative and anti-inflammatory properties to suppress platelet hyperactivity in diabetic conditions. The mechanism of the bi-herbal extract could be linked to the presence of phytochemicals (flavonoids, alkaloids, tannins and phenolics), with antioxidant, anti-inflammatory and hematopoietic properties. These compounds may reduce oxidative stress, prevent hemolysis, stimulate erythropoiesis and modulate immune response.

CONCLUSION

The findings of this study showed that streptozotocin-induced diabetes caused significant hematological changes in rats, including leukocytosis, anemia and thrombocytosis, as an evidence in abnormal changes in white blood cell, red blood cell, hemoglobin, hematocrit, platelet count, platelet distribution width and mean platelet volume. Thereby, this study validated the folkloric report and proffer elicited for further studies.

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