APHRODISIAC PROPERTIES OF THE BIHERBAL LEAF AQUEOUS EXTRACT IN STREPTOZOTOCIN INDUCED ANAPHRODISIAC EFFECT IN MALE RATS

BY

Uwa Blessed IGUNMWONYI

LSC2009889

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

This is to certify that this project titled **APHRODISIAC PROPERTIES OF THE BIHERBAL LEAF AQUEOUS EXTRACT IN STREPTOZOTOCIN INDUCED ANAPHRODISIAC EFFECT IN MALE RATS** was carried out by **Uwa Blessed IGUNMWONYI** with matriculation number LSC2009889, of the Department of Science Laboratory Technology (Physiology/Pharmacology), Faculty of Life Sciences, University, Benin City, Edo state, Under the supervision of DR. B. O. Gabriel.

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DR. B. O. GABRIEL
PROJECT SUPERVISOR Date

______________________________ ______________________

DR. P.O. ALONGE
PROJECT COORDINATOR Date

______________________________ ______________________

PROF. J. O. OSARUMWENSE
HEAD OF DEPARTMENT Date

______________________________ ______________________

EXTERNAL EXAMINER Date

DEDICATION

This work is dedicated to Almighty God and my parents Barr and Mrs Igunmwonyi

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ABSTRACT

The study aimed to evaluate the effects of biherbal leaf aqueous extract in Streptozotocin induced anaphrodisiac male Wistar rats. Thirty two Wistar rats were divided into six groups: a normal control group received 0.5ml/kg of distilled water, another group received gibenclamide at 10mg/kg, the negative control received 50mg/kg of STZ, the treatment groups were divided into three each receiving 50, 100 and 200 mg/kg of the biherbal extract only for 28days. Mating behaviour and testosterone levels were assessed. The results showed an increase in testosterone levels and mating behaviour of the albino rats when administered 50mg/kg and also showed that higher doses of the biherbal leaf extract had negative effects on the Wistar rats. The findings suggests that the combination of *Alstonia boonei* and *Vernonia amygdalina* has aphrodisiac properties without adverse effects on organ integrity making it a perfect candidate for further research as an aphrodisiac agent.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF THE STUDY

The limitations of synthetic drug therapy have catalyzed a renewed interest in natural, plant based remedies, especially within traditional medicine systems such as Ayurveda, Traditional Chinese Medicine (TCM), and African ethnomedicine. Plants offer a rich reservoir of bioactive compounds alkaloids, saponins, flavonoids, tannins, glycosides, and phenolics. Many of which possess aphrodisiac, antidiabetic, antioxidant, and anti-inflammatory properties. Among these, *Aquilaria malaccensis* has emerged as a promising candidate (Zaida *et al.,* 2023).

Diabetes mellitus (DM) is a chronic, heterogeneous group of metabolic disorders with a global reach, marked by persistent hyperglycemia due to insufficient insulin production, impaired insulin action, or a combination of both (Banday and Sameer (2020)).

Diabetes is primarily classified into Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), each with distinct etiologies but converging on the hallmark of chronic hyperglycemia. While T1DM is autoimmune in nature and involves the destruction of pancreatic β-cells, T2DM is predominantly associated with insulin resistance and is more prevalent globally (American Diabetes Association Professional Practice Committee (2020)). Uncontrolled hyperglycemia leads to a multitude of systemic complications, including retinopathy, nephropathy, neuropathy, cardiovascular disease, immune dysfunction, and less commonly emphasized but equally important, reproductive and sexual dysfunction. These complications impair the patients quality of life, reduce lifespan, and impose immense social and economic costs (American Diabetes Association, 2023). Among male patients, one of the most distressing yet underreported complications of diabetes is sexual dysfunction, particularly anaphrodisia, which refers to a pathological absence or suppression of sexual desire or libido (World Health Organisation 2021). Anaphrodisiac in diabetic males is a multifactorial condition, involving complex interactions among endocrine, vascular, neurogenic, and psychogenic pathways. In diabetes, hyperglycemia-induced oxidative stress and inflammation disrupt normal testicular architecture and function, lower serum testosterone levels, impair nitric oxide-mediated vasodilation of penile arteries, and damage autonomic nerves responsible for arousal and erection. This leads not only to reduced libido but also to poor sexual performance, infertility, and emotional distress (Adeoye and Oladipo 2020).

1.2 AIM AND OBJECTIVES OF THE STUDY

The aim was to evaluate the aphrodisiac properties of *Vernonia amygdalina* and *Alstonia Boonei* acqeous extract on streptozotocin induced diabetes in rats.

The following are the objectives of this study:

1. to watch the impact of the biherbal extract on libido levels in streptozotocin induced diabetic rats.

2. to investigate the potential of the extract in aphrodisiac property in male whisker rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 APHRODISIAC

Aphrodisiacs are agents or substances believed to stimulate sexual desire, arousal, behavior, or performance. Throughout history, different cultures have utilized them to manage sexual disorders, enhance libido, and promote reproductive well-being. The term "aphrodisiac" originates from Aphrodite, the Greek goddess of love, reflecting their long-standing link with sexual vitality. These substances can be derived from natural sources such as plants, animals, and minerals, or produced synthetically, and they act through diverse physiological and psychological mechanisms (Yakubu and Afolayan, 2021).

The pharmacological mechanisms underlying aphrodisiac effects operate through several biological pathways. These include modulation of neurotransmitters in the central nervous system such as dopamine, serotonin, and nitric oxide as well as hormonal regulation, particularly of testosterone. Aphrodisiacs may also enhance genital blood circulation, libido, sexual motivation, and erectile function (Suresh Kumar and Singh, 2021). In recent years, natural plant-based aphrodisiacs have received increased scientific interest because they are more affordable, readily available, and generally safer than synthetic options such as sildenafil (Viagra), which, although effective, may cause adverse effects or interact negatively with other medications (Rowland *et al*., 2020).

2.1.1 Classification of Aphrodisiacs

Aphrodisiacs are generally categorized into three major groups according to their mode of action and primary effects:

1. **Psycho-physiological aphrodisiacs**:

These agents enhance sexual desire through stimulation of the central nervous system or by eliciting emotional and sensory excitement. Examples include aromatic substances like perfumes, visual stimuli, and certain herbal remedies containing psychoactive constituents (Suresh Kumar & Singh, 2021).

2. **Physiological aphrodisiacs**:

This group exerts direct effects on sexual organs or endocrine functions to improve performance. They include herbal extracts capable of promoting penile erection, elevating testosterone secretion, or improving sperm parameters (Suresh Kumar & Singh, 2021).

3. **Mixed aphrodisiacs**:

These compounds act on both psychological and physiological levels simultaneously. A number of medicinal plants traditionally employed for sexual health belong to this category, as they exhibit both mood-enhancing and fertility-boosting activities (Suresh Kumar & Singh, 2021).

Plant-derived aphrodisiacs such as *Vernonia amygdalina* (bitter leaf) and *Alstonia boonei* (pattern wood) are extensively utilized in African traditional medicine. These species are reported to contain bioactive molecules including alkaloids, flavonoids, saponins, and terpenoids that contribute to modulation of sexual behavior and enhancement of reproductive function (Yakubu and Afolayan, 2021).

2.1.2 Mechanisms of Aphrodisiac Action

Aphrodisiacs exert their effects on sexual performance through multiple physiological and biochemical pathways:

Hormonal Regulation:

Certain aphrodisiacs act by modulating the hypothalamic–pituitary–gonadal (HPG) axis, resulting in elevated secretion of sex hormones, particularly testosterone, which plays a key role in libido enhancement and sperm production (Suresh Kumar and Singh, 2021).

Vasodilatory Effect:

Several plant-based aphrodisiacs promote the synthesis of nitric oxide (NO), a signaling molecule responsible for relaxing vascular smooth muscles. This process enhances blood circulation within the penile tissues, thereby facilitating erection (Suresh Kumar and Singh, 2021).

Neurotransmitter Modulation:

Some bioactive compounds influence neurotransmitters such as dopamine and serotonin, both of which are vital for sexual motivation, arousal, and mood balance (Suresh Kumar and Singh, 2021).

Stress and Anxiety Reduction:

A number of adaptogenic herbs used as aphrodisiacs help to alleviate psychological stress and anxiety, factors that often contribute to diminished sexual performance (Suresh Kumar and Singh, 2021).

Understanding these underlying mechanisms provides scientific support for the traditional use of natural aphrodisiac plants. For example, the flavonoids and saponins found in *Vernonia amygdalina* have been shown to elevate testosterone levels and enhance sexual behavior in experimental animal studies (Suresh Kumar and Singh, 2021).

2.1.3 Traditional and Modern Uses of Aphrodisiacs

In African traditional medicine, numerous plant species have long been utilized to treat conditions such as low libido, infertility, and sexual debility. These herbal remedies are typically prepared as infusions or decoctions and administered orally. Their perceived effectiveness is based on generations of empirical use, though recent pharmacological investigations are providing scientific support for many of these traditional claims (Akinmoladun et al., 2021).

In modern medical practice, the demand for herbal aphrodisiacs has increased, largely due to the side effects commonly linked to synthetic pharmaceuticals. There is a growing global inclination toward natural or plant-derived therapies for managing sexual dysfunction, especially among older adults and patients with underlying conditions like diabetes and cardiovascular disorders that restrict the use of conventional drugs such as phosphodiesterase-5 inhibitors (Ahmed et al., 2022).

Within this context, exploring the aphrodisiac efficacy of biherbal combinations like *Vernonia amygdalina* and *Alstonia boonei* is particularly relevant. Both plants have significant ethnomedicinal importance and contain bioactive constituents with potential to alleviate chemically induced sexual impairments, including those triggered by streptozotocin in diabetic animal models (Ezeonwumelu et al., 2018).

2.2 Male Sexual Dysfunction and Diabetes

Male sexual dysfunction is a complex and multifactorial condition characterized by one or more abnormalities in the sexual response cycle, including decreased libido, erectile dysfunction (ED), ejaculation disorders, and infertility. These dysfunctions can significantly affect the psychological and emotional well-being of men, as well as their interpersonal relationships. The prevalence of male sexual dysfunction has been on the rise globally, particularly among individuals with chronic health conditions such as diabetes mellitus (Gettie *et al*., 2021)

Diabetes mellitus, especially type 1 and type 2 diabetes, is one of the most significant risk factors for sexual dysfunction in males. Multiple studies have documented that men with diabetes are more prone to develop ED and related disorders due to the systemic complications of the disease. The development of diabetes-induced sexual dysfunction is associated with vascular insufficiency, hormonal imbalances, oxidative stress, and neuropathy, all of which contribute to impaired sexual performance (Corona *et al*., 2021).

2.2.1 Pathophysiology of Diabetes-Induced Sexual Dysfunction

The pathophysiological mechanisms linking diabetes to male sexual dysfunction are multifaceted and involve endocrine, neurological, vascular, and psychological factors. Diabetes affects sexual function primarily through three major pathways: endothelial dysfunction, autonomic neuropathy, and hormonal dysregulation(Chitaley and Webb, 2022).

a. Endothelial Dysfunction and Vascular Impairment

Erectile function is highly dependent on adequate blood flow to the corpora cavernosa. Diabetes impairs endothelial function by reducing nitric oxide (NO) bioavailability, a critical vasodilator necessary for penile erection. Hyperglycemia-induced oxidative stress leads to the generation of free radicals, which further impair no synthesis and promote vascular stiffness and atherosclerosis. The reduction in penile blood flow results in decreased rigidity and sustainability of erections (Chitaley and Webb, 2022)

b. Diabetic Neuropathy

Peripheral and autonomic neuropathy are common complications of long-standing diabetes. These neuropathies impair the transmission of nerve impulses necessary for initiating and maintaining an erection. Damage to the parasympathetic nervous system, which is responsible for penile vasodilation, can significantly impair reflexogenic and psychogenic erections. Moreover, sensory neuropathy may reduce penile sensitivity, contributing to decreased sexual arousal and satisfaction (Tesfaya and Feldman, 2020).

c. Hormonal Dysregulation

Testosterone plays a critical role in libido, erectile capacity, and spermatogenesis. Diabetes, particularly type 2 diabetes, is often associated with hypogonadism due to impaired hypothalamic-pituitary-gonadal axis regulation. Insulin resistance and obesity in diabetic patients contribute to decreased testosterone levels, exacerbating sexual dysfunction. Low testosterone levels can also impair nitric oxide production, further worsening erectile issues (Triash *et al*., 2022)

2.2.2 Psychological Impacts and Quality of Life

Beyond the physiological effects, diabetes-related sexual dysfunction can have profound psychological consequences. The inability to perform sexually may lead to feelings of inadequacy, low self-esteem, depression, anxiety, and strained relationships. These psychological factors can independently or synergistically worsen sexual dysfunction, creating a vicious cycle. Studies have shown that men with diabetes and and report lower quality of life and increased rates of marital dissatisfaction (Bahlhara and Sakar, 2020).

2.2.3 Therapeutic Approaches to Diabetes-Related Sexual Dysfunction

Management of sexual dysfunction in diabetic men typically requires a holistic approach, involving glycemic control, lifestyle modifications, psychotherapy, and pharmacological interventions. Common drugs include phosphodiesterase type 5 inhibitors (e.g., sildenafil), testosterone replacement therapy (in hypogonadal individuals), and intracavernosal injections. However, these pharmacological agents are not without limitations; adverse effects, contraindications in cardiovascular patients, and high costs often limit their use (Hackett *et al*., 2020). This has led to a growing interest in natural alternatives, especially plant-based aphrodisiacs. Herbal formulations are being increasingly explored for their efficacy in improving sexual function by enhancing NO production, increasing testosterone levels, reducing oxidative stress, and promoting blood flow. In particular, the use of *Vernonia amygdalina* and *Alstonia boonei* plants known for their antioxidant, antidiabetic, and reproductive enhancing properties holds promise as a potential natural remedy for diabetes-induced sexual dysfunction. These plants are traditionally employed in African ethnomedicine and have shown various bioactivities relevant to reproductive health (Ahmed *et al*., 2022)

2.3 Sreptozotocin-Induced Sexual and Reproductive Dysfunction

Several studies have demonstrated the adverse effects of STZ-induced diabetes on male reproductive health. These include a marked reduction in libido, mounting frequency, intromission, ejaculation latency, and testosterone levels. Additionally, testicular histological examinations reveal degeneration of seminiferous tubules, reduction in Leydig and Sertoli cell populations, and impaired spermatogenesis (Wang *et al*., 2023).The mechanism by which STZ impairs reproductive function is multifactorial. Chronic hyperglycemia promotes the generation of reactive oxygen species (ROS), which damage testicular tissues and inhibit steroidogenic enzymes responsible for testosterone synthesis. Moreover, the suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion due to hypothalamic and pituitary dysfunction further contributes to sexual impairment (Wang *et al*., 2023).

These pathologies make STZ-induced diabetic rats an ideal model for testing the restorative effects of natural aphrodisiac compounds. Research has shown that antioxidants and phytochemicals with androgenic or anti-inflammatory properties can significantly mitigate these effects and restore normal sexual behavior and reproductive function (Wang *et al*., 2023).

2.4.1 Botanical Description

Vernonia amygdalina Delile, commonly known as bitter leaf, is a tropical shrub or small tree widely distributed throughout West and Central Africa. It grows typically in savannah and forest zones and is extensively cultivated around homes and gardens due to its medicinal and culinary importance. It belongs to the Asteraceae (Compositae) family and is characterized by soft-wooded stems and elliptic to lanceolate leaves that possess a distinctly bitter taste (Egedigwe *et al*., 2023). The plant can grow up to 3 meters in height and bears green leaves with hairy surfaces and rough textures. It produces small tubular purple flowers arranged in clusters and a characteristic pungent aroma. *Vernonia amygdalina* thrives in loamy soils with adequate sunlight and rainfall and is adaptable to a wide range of tropical climatic conditions. Due to its ethnomedicinal versatility, the leaves, roots, and bark of the plant are used in traditional African medicine for treating various diseases including malaria, gastrointestinal disorders, diabetes, and reproductive dysfunctions (Egedigwe *et al*., 2023).

2.4.2 Taxonomy classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: Vernonia

Species: *Vernonia amygdalina* Delile

Plate 2.1: Whole plant parts of *Vernonia amygdalina*

The genus *Vernonia* is one of the largest in the Asteraceae family, comprising over 1,000 species distributed mainly in tropical and subtropical regions. Vernonia amygdalina is distinguishable from other species by its characteristic bitter taste and widespread use in ethnobotanical practices across sub-Saharan Africa (Egedigwe *et al*., 2023).

2.4.3 Phytochemistry of *Vernonia amygdalina*

Phytochemical investigations of the plant have revealed that it contains a diverse range of bioactive compounds responsible for its pharmacological activities. The main phytochemical constituents include: Alkaloids, Saponins, Tannins, Flavonoids, Terpenoids, Sesquiterpene lactones, Phenolic compounds

Flavonoids and phenolic acids contribute to the plants strong antioxidant activity. Sesquiterpene lactones such as vernodalin, vernolide, and vernomygdin are largely responsible for the plants bitter taste and are known for their anti-inflammatory and anticancer properties (Egedigwe *et al*., 2023). Alkaloids and saponins present in the plant have been linked to improved male reproductive function through modulation of steroidogenic enzymes and hormonal regulation (Egedigwe *et al*., 2023). Moreover, the presence of edotides (small bioactive peptides) and glycosides enhances the plant's pharmacological potential, particularly in combating oxidative stress and improving fertility parameters. These compounds may synergize to exhibit aphrodisiac effects, which makes *Vernonia amygdalina* a strong candidate for biherbal formulations aimed at reversing diabetes-induced sexual dysfunction

2.4.4 Pharmacological Properties.

Vernonia amygdalina has been extensively studied for its broad-spectrum pharmacological activities. Its therapeutic versatility is attributed to its rich phytochemical content. The major pharmacological properties include:

a. Antidiabetic Activity

Vernonia amygdalina has shown significant hypoglycemic activity in both animal models and clinical studies. Aqueous extracts of the leaves have been reported to reduce blood glucose levels, improve insulin sensitivity, and restore pancreatic β-cell integrity (Egedigwe *et al*., 2023). The mechanisms involve antioxidant-mediated protection against oxidative stress-induced β-cell damage and upregulation of glucose transporter activity. In the context of STZ-induced diabetes models, the plant mitigates hyperglycemia and improves lipid profiles, which indirectly helps restore endocrine function and reproductive health (Egedigwe *et al*., 2023).

b. Antioxidant and Anti-inflammatory Effects

Oxidative stress is a central feature of diabetes and male reproductive dysfunction. *Vernonia amygdalina* demonstrates potent antioxidant activity through its flavonoid and polyphenolic content, which scavenge free radicals and enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Egedigwe *et al*., 2023). Additionally, its anti-inflammatory activity has been documented in several models, with notable suppression of pro-inflammatory cytokines and inhibition of inflammatory mediators such as COX-2 and nitric oxide (Egedigwe *et al*., 2023). This anti-inflammatory property is beneficial in alleviating testicular inflammation commonly associated with diabetic complications

c. Aphrodisiac and Fertility-Enhancing Properties

Research has demonstrated that *Vernonia amygdalina* possesses notable aphrodisiac effects, particularly in male rodents. Aqueous leaf extracts have been shown to enhance sexual behavior indices such as mounting frequency, intromission, and ejaculation latency. These effects are often accompanied by elevated serum testosterone and improved sperm count and motility (Egedigwe *et al*., 2023). The phytochemicals responsible for these effects include saponins and alkaloids, which stimulate the hypothalamic-pituitary-gonadal axis, and antioxidants that protect testicular tissues from oxidative damage. In diabetic models, the plant also helps restore normal histological architecture of the testes and improves serum reproductive hormone levels.

d. Antimicrobial and Antimalarial Activity

Extracts of *Vernonia amygdalina* have shown activity against a wide range of bacteria and parasites. The plant is traditionally used for treating malaria and gastrointestinal infections. Its antimicrobial effects are attributed to sesquiterpene lactones and alkaloids, which interfere with microbial DNA replication and cell wall synthesis (Egedigwe *et al*., 2023).

e. Hepatoprotective and Cardioprotective Activities

The hepatoprotective effects of *Vernonia amygdalina* have been validated in studies involving chemically induced liver damage. The plant enhances liver function enzymes and prevents lipid peroxidation, making it useful in managing complications of diabetes such as non-alcoholic fatty liver disease (Egedigwe *et al*., 2023). Additionally, its lipid-lowering properties help reduce cardiovascular risks associated with diabetes by lowering LDL-cholesterol and improving HDL levels (Egedigwe *et al*., 2023).

2.5.1 Botanical Discription

Alstonia boonei is a tall, deciduous forest tree belonging to the family Apocynaceae. It is widely distributed across tropical Africa, from Senegal in the west to Uganda and Tanzania in the east. Commonly referred to as "Pattern wood" or "Cheese wood," it can reach heights of 45 meters with a straight trunk and horizontal branches arranged in whorls. The bark is grey to pale brown and is characteristically fissured, exuding a white latex when incised (Okoye *et al*., 2022)

The leaves of *Alstonia boonei* are arranged in whorls of 47 and are obovate or oblanceolate in shape. They are glabrous, with smooth margins and acuminate apices. The tree produces small greenish-white flowers in dense cymose inflorescences and long cylindrical follicles containing seeds with tufts of silky hairs for wind dispersal (Okoye *et al*., 2022) The plant thrives in moist, loamy soils and is often found in secondary forests and open woodlands. Traditionally, the bark and leaves are widely used in African ethnomedicine to manage various ailments such as fever, malaria, inflammation, ulcers, and reproductive disorders, highlighting its broad medicinal application (Okoye *et al*., 2022).

2.5.2 Taxonomy Classification of *Alstonia boonei*

The botanical classification of Alstonia boonei is as follows:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Alstonia

Species: Alstonia boonei De Wild

Plate 2.2: Growing branch and flowers of *Alstonia boonei*

The genus *Alstonia* comprises several tropical trees and shrubs distributed across Asia, Africa, and Australia. *Alstonia boonei* is the principal species used in African herbal medicine, distinguished from other species by its distinct bark, leaf arrangement, and floral morphology (Okoye *et al*., 2022).

2.5.3 Phytochemistry of *Alstonia boonei*

Phytochemical screening of *Alstonia boonei* has revealed the presence of a wide variety of secondary metabolites that are responsible for its therapeutic properties. These include:

Alkaloids (e.g., echitamine, boonein), Saponins, Tannins, Flavonoids, Triterpenoids, Phenolic compounds, Cardiac glycosides, Steroids (Okoye *et al*., 2022).

Alkaloids are the most prominent phytochemicals in *boonei*, with echitamine recognized as the major active compound possessing anti-inflammatory, antimalarial, and antimicrobial activities (Okoye *et al*., 2022). Triterpenoids and flavonoids contribute significantly to the antioxidant and anti-inflammatory actions of the plant, while saponins are implicated in its aphrodisiac potential and hormonal modulation (Izum and Olubodum, 2023).

Phenolic compounds present in the plant extract also scavenge free radicals and protect tissues from oxidative damage, particularly in diabetes-induced stress conditions. The synergy of these compounds makes *Alstonia boonei* a valuable herbal agent for managing metabolic and reproductive dysfunctions (Osagie *et al.,*2023)

2.5.4 Pharmacological Properties

A. Antidiabetic Activity

Alstonia boonei exhibits antidiabetic properties through multiple mechanisms, including inhibition of α-glucosidase and aldose reductase enzymes, enhancement of insulin sensitivity, and protection of pancreatic β-cells. Studies have shown that both bark and leaf extracts significantly reduce blood glucose levels in streptozotocin-induced diabetic rats, demonstrating their hypoglycemic potential (Fonkoua *et al*., 2021). These effects are often attributed to alkaloids and flavonoids that modulate carbohydrate metabolism, reduce oxidative stress, and restore hepatic and pancreatic function. This supports its inclusion in the biherbal formulation designed to manage diabetes-induced sexual dysfunction (Fonkoua *et al*., 2021).

B. Antioxidant and Anti-inflammatory Properties

Oxidative stress is a key contributor to tissue damage in diabetes and reproductive dysfunction. The flavonoid and triterpenoid components of Alstonia boonei confer strong antioxidant activities, increasing the activity of endogenous enzymes such as catalase, glutathione peroxidase, and SOD (Olalokun *et al*., 2021). In addition, the plant exhibits anti-inflammatory effects by inhibiting the synthesis of pro-inflammatory cytokines and prostaglandins. This anti-inflammatory effect is particularly relevant in preserving testicular integrity and mitigating diabetic orchitis, a common complication in STZ-induced diabetic models (Adjuozem *et al*., 2020).

C. Antimicrobial and Antiparasitic Effects

Traditionally used to treat infections, *Alstonia boonei* has demonstrated antimicrobial activity against gram-positive and gram-negative bacteria. Its bark extracts show significant zones of inhibition against Staphylococcus aureus, *E. coli,* and *Pseudomonas aeruginosa.* Alkaloids such as echitamine interfere with microbial DNA synthesis and membrane function (Akinmoladun *et al*., 2014). Additionally, the plant is effective against malaria-causing parasites and is widely used in traditional fever remedies. This broad antimicrobial activity ensures its usefulness in treating reproductive tract infections that may contribute to infertility or sexual dysfunction.

D. Analgesic and Antipyretic Activities

Extracts from the bark and leaves of *Alstonia boonei* possess analgesic and antipyretic properties, comparable to standard NSAIDs. These effects are beneficial in managing pain and systemic inflammation associated with chronic illnesses such as diabetes, thereby contributing to improved quality of life and sexual health (Olanlokun *et al*., 2021).

2.6 Combined Biherbal Formulation of *Alstonia boonei* and *Vernonia amygdalina*

2.6.1 Ethnomedicinal Background and Justification for Combination

Traditional African medicine often utilizes plant combinations to enhance therapeutic efficacy through synergistic interactions. The combination of *Alstonia boonei* (Pattern wood) and *Vernonia amygdalina* (Bitter leaf) is a well-known practice among traditional healers in West Africa for managing fever, infections, inflammation, and sexual dysfunction (Enyi and Ajah, 2024). Both plants individually possess a wide range of pharmacological activities, including antidiabetic, antioxidant, anti-inflammatory, and aphrodisiac effects. When formulated together, they offer a multi-faceted approach to managing complex disorders such as diabetes-induced sexual dysfunction (Alamoladun *et al*., 2025).

Alstonia boonei is rich in alkaloids, triterpenoids, and flavonoids, while Vernonia amygdalina contains sesquiterpene lactones, phenolics, and saponins. These phytochemical constituents exhibit overlapping and complementary therapeutic properties, making the biherbal formulation a promising intervention in diseases with multifactorial etiologies like diabetes and reproductive impairment (Kaur *et al*., 2019)).

2.7 Mechanism of Action of Natural Aphrodisiacs

Natural aphrodisiacs are substances derived from plants, animals, or minerals that stimulate or increase sexual desire, performance, or pleasure (Alamoladun *et al*., 2025). In traditional and modern medicine, many natural aphrodisiacs are derived from medicinal plants, which exert their effects through a variety of mechanisms acting on the neuroendocrine and reproductive systems. Understanding the underlying mechanisms of these substances is vital for developing effective therapies for sexual dysfunction, particularly in conditions like diabetes mellitus, which impair libido, erectile function, and spermatogenesis (Jaiswal *et al*., 2020).

2.7.1 Hormonal Modulation

One of the most prominent mechanisms by which natural aphrodisiacs function is through the regulation of reproductive hormones. Many aphrodisiac plants enhance the secretion of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which are central to sexual function in males (Leisegang *et al*., 2022). Testosterone is critical for libido, erectile capacity, and sperm production. Natural products such as flavonoids, alkaloids, and saponins found in plants like Vernonia amygdalina and Alstonia boonei have been shown to upregulate the hypothalamic-pituitary-gonadal (HPG) axis, thereby enhancing testosterone production (Adeyemi *et al*., 2022). Furthermore, increased LH levels stimulate Leydig cells in the testes to secrete testosterone, while FSH supports Sertoli cell function necessary for spermatogenesis. The hormonal balance achieved through the administration of natural aphrodisiacs contributes significantly to improved libido, fertility, and general sexual behavior (Monangeng *et al*., 2023).

2.8 Studies on Herbal Effects on Sexual Hormones and Behavior

Various studies have examined the influence of plant extracts on hormonal levels, which directly correlate with sexual behavior (Ara *et al*., 2023). For example, the aqueous extract of Tribulus terrestris was shown to enhance serum testosterone and luteinizing hormone levels, leading to improved sexual activity in rats. The mechanism was attributed to the stimulation of the hypothalamic-pituitary-gonadal axis (Ara *et al*., 2023).

Another study by Bako *et al*. (2015) explored the role of Zingiber officinale in boosting nitric oxide synthesis, leading to enhanced penile erection and improved mating behavior in male rats. The results underscore the relevance of phytoconstituents like flavonoids and alkaloids in modulating sexual performance.

Given that both V. *amygdalina* and A. *boonei* are rich in similar bioactive compounds, the biherbal extract used in the present study may exert comparable endocrine and vasodilatory effects, justifying its evaluation in a diabetic-anaphrodisiac rat model (Omojokiun *et al*., 2019).

2.8.1 Summary of Comparative Insights

From the reviewed studies, it is evident that both *Vernonia amygdalina* and *Alstonia boonei* possess individual properties that support their roles in enhancing male reproductive function. Moreover, existing evidence shows that: Combined herbal extracts often have synergistic effects superior to those of single plants, STZ-induced diabetic rat models are standard tools for assessing reproductive impairments and treatment outcomes, bioactive compounds like alkaloids, saponins, and flavonoids play key roles in sexual stimulation, hormone regulation, and spermatogenesis. The use of traditional medicinal plants continues to be validated by experimental models for male sexual dysfunction. These comparative insights lend strong support to the current investigation into the aphrodisiac properties of the aqueous extract of V. *amygdalina* and A. *boonei* in STZ-induced anaphrodisiac male rats (Alahmadi, B.A,(2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant collection and identification

Fresh leaves of *Vernonina 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. Osaro in the Department of Plant Biology and Biotechnology.

3.2 Preparation of Plant

The collected leaves were washed and air dried for 14 days, after which was pulverised

into fine powder using a mechanical grinder. Equal weight of the powdered leaves was

177h with ratio 1:1 and was subjected to cold maceration techniques with aqueous extraction process.

The mixture was soaked in a jar, 2500ml 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.

3.3 Experimental Animals

Thirty two (32) healthy male Wistar rats were used for this equipment. 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±2°C) and fed with standard diet. All selected animals were acclimated for 14 days.

3.4 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 gilbenclamide, normal control (0.5ml/kg of distilled water) and negative control (50 mg/kg of STZ).

3.5 Experimental protocol

The test was experimented using a described method. The sexually experienced and healthy male Wistar rats weighing (120-190 g) were chosen for the investigation because they demonstrated a quick sexual impact. Female animals were chosen at random. During the trial, they were randomly divided into 5 groups (n=5) and housed seperately.

Group 1: 0.2 ml/kg of water o.p was administered.

Group 2: 50 mg/kg sidenafil citrate o.p was administered.

Group 2-4: Received *Vernonina amygdalina* and *Alstonia Boonei* extract at graded doses of (25, 50 and 100 mg/kg), respectively for 3 days.

3.6 Male sexual Behaviour test procedure

The experiment was carried out on the third day after the male animal has been treated. The experiment was carried out at a late hour of the night. In the cage, receptive female animals were matched with male animals in a 1:1 female to male ratio. Evaluations of mating performance were immediately noted and sustained for the first two (2) mating successions. The trial lasted around 30 minutes. If the female animals do not show signs of receptivity, they will be replaced. The existence of actions and stages of mating were documented on video camera. The following parameters such as, Mounting afore ejaculation or Mounting Frequency (MF), intromission by the male or Intromission Latency(IL), Intromission of series ejaculation or Ejaculatory Latency (EL), Ejaculation and first Intromission of the subsequent sequences or post-ejaculatory interlude and average time between intermissions.

3.7 Determination of Serum Testosterone Concentration

Serum testosterone level of the tested animals plasma was investigated using an established protocol from the manufacturer's manual. This assay method involved the dispensing of 10üL of testosterone reference standards at (0, 0.1, 0.5, 2.0, 6.0 and 18.0ng/mL), serum(diluted x5) and the controls of testosterone 1 and 2 into a Goat Anti- Rabbit IgG-coated microliter wells (96 wells), 100 ûL of testosterone -HRP conjugate reagent(blue colour) and 50ûL of rabbit anti testosterone reagent were distinctly distributed into each well. This was established on the standard competitive requisite between testosterone in the test plasma specimen (serum). The resultant solution was systematically mixed for 30 seconds and allowed for incubation at 37°C for 90 minutes. The microwells were washed and skinned 5 times in distilled water (to eliminate unbound testosterone peroxidase conjugate) previously dispensing 100 ûL of TMB reagent into the well. The resultant solution was properly mixed mildly for 5 seconds. This was later incubated at room temperature for about 20 minutes to achieve blue colouration(Sakar, 2020).

3.8 Statistical analysis

The results obtained are presented as Mean ± SEM. Data were analysed via one way analysis of variance (ANOVA) and means were compared using the Dunnete test. Significant differences were measured at $p<0.05$. SPSS version 19 was the statistical tool used.

CHAPTER FOUR

RESULTS

The physical methods of mounting and mating behaviour were used to test for the aphrodisiac potential of the extract. Apparently healthy and sexually experienced female rats weighing were artificially brought to estrous 48 and 6 hours respectively before mating. This is because female rats are receptive to the males for mating only during estrous. The receptivity of the females was tested by using male rats other than the ones used in the experiment. Apparently healthy and sexually experienced male rats that showed brisk sexual activity were used to mate the female rats. Before commencing the experiment, the rats were brought to the testing laboratory under dim light at 1800 hours to 2200 hours daily for 4 days to make them familiar with the testing conditions.

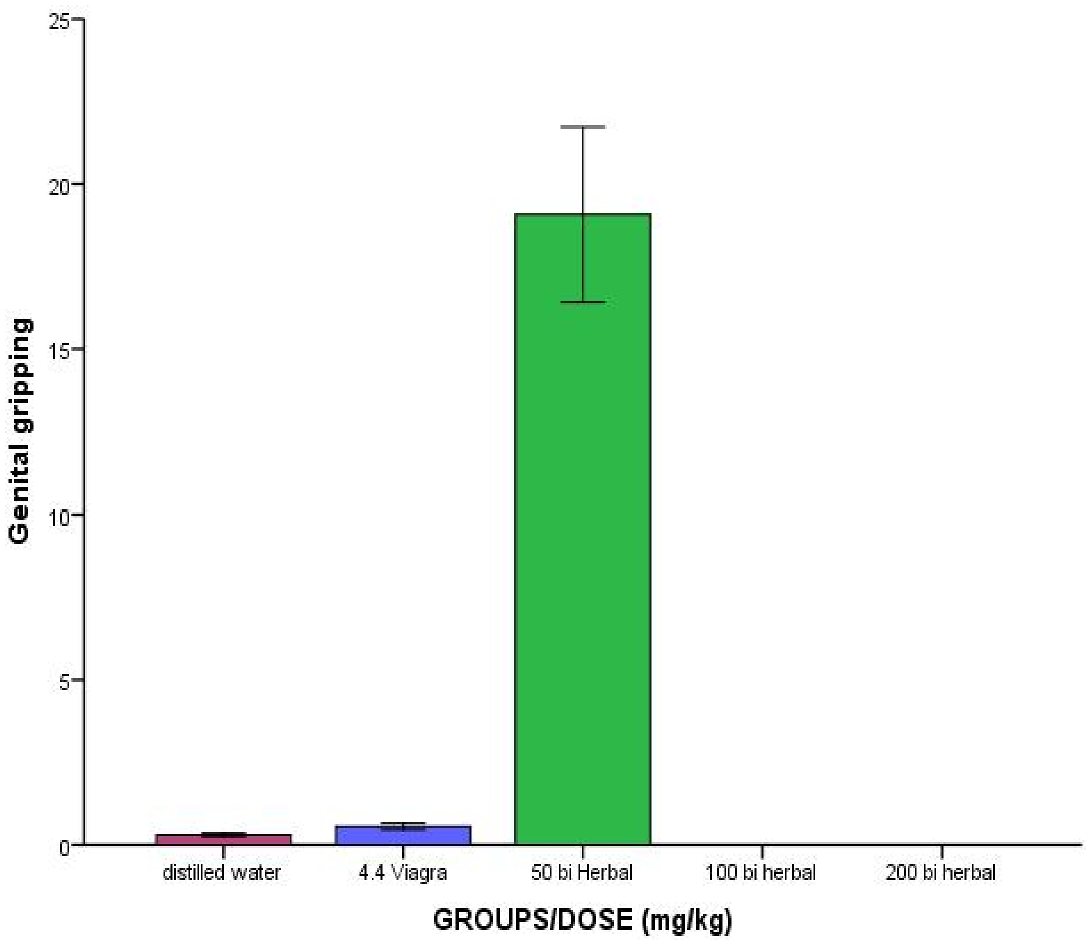

Figure 4.1 Effects of bi herbal extract on Genital gripping of rats. Results were expressed as mean ± SEM. n = 3.

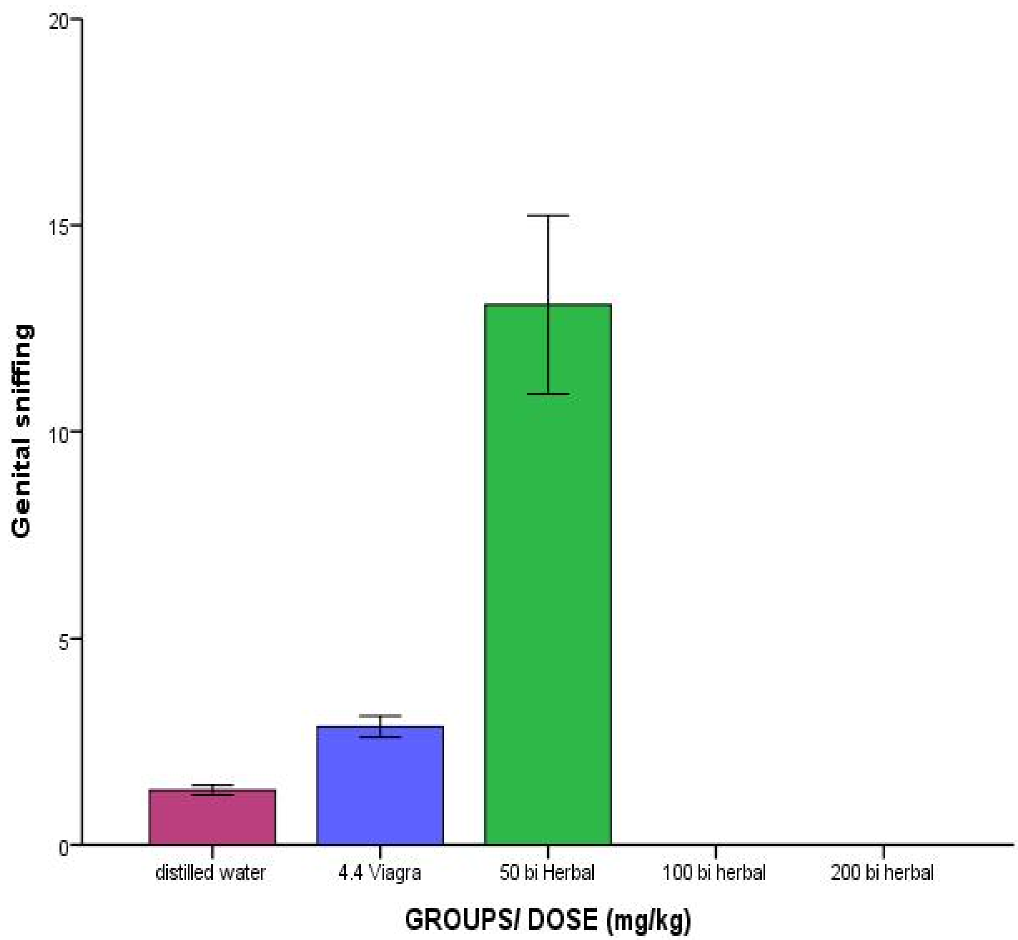

Figure 4.2: Effects of bi herbal extract on Genital sniffing of rats. Results were expressed as mean ± SEM. n = 3.

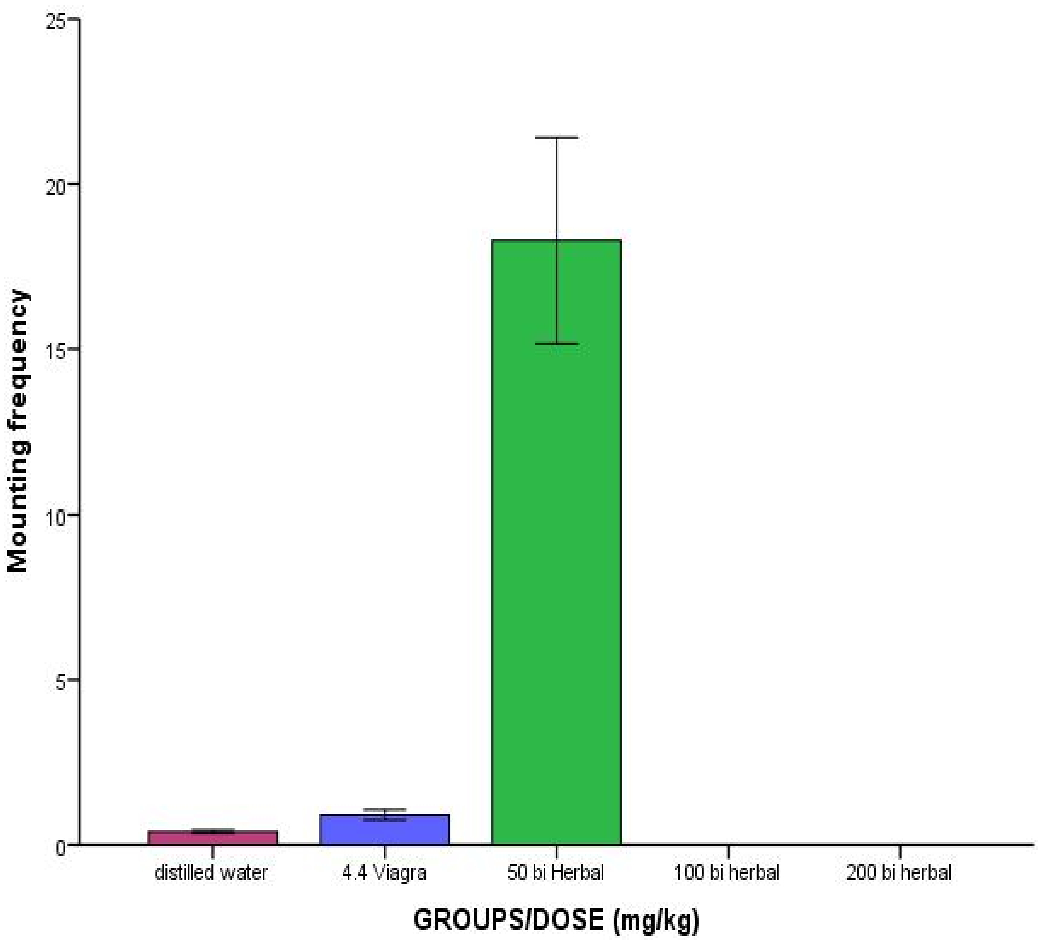

Figure 4.3: Effects of bi herbal extract on Mounting frequency of rats. Results were expressed as mean ± SEM. n = 3.

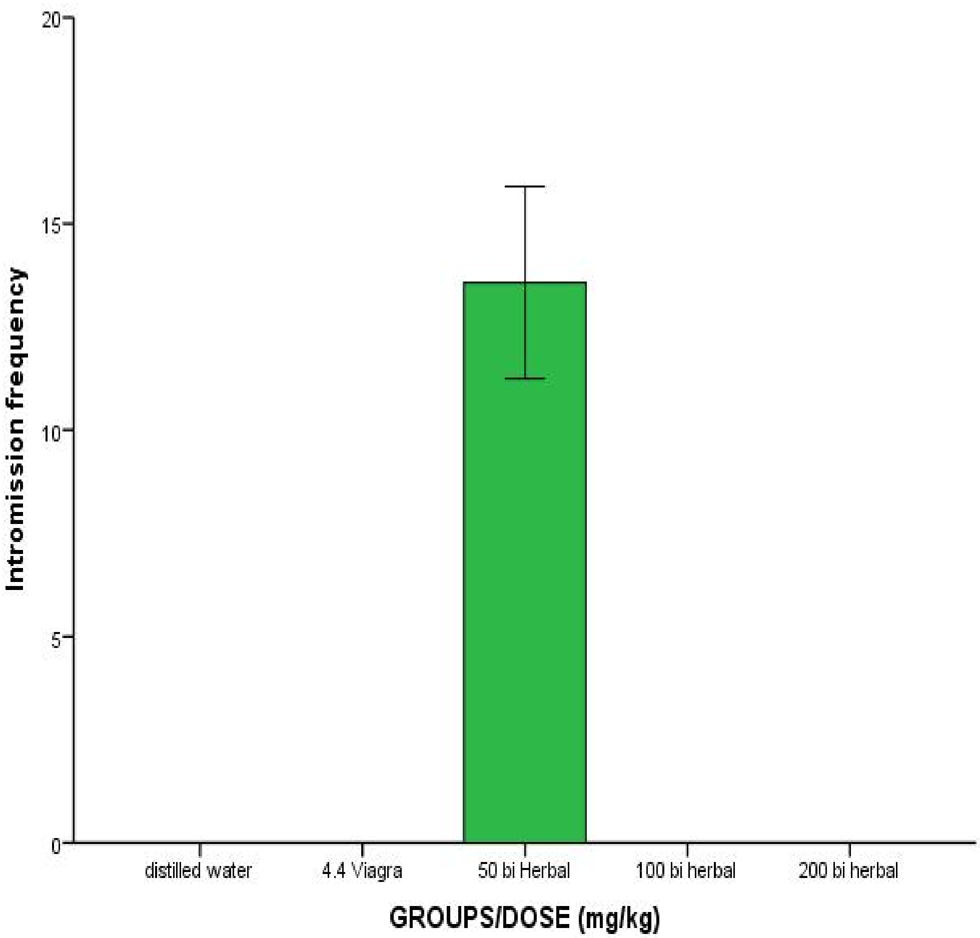

Figure 4.4: Effects of bi herbal extract on intromission frequency of rats. Results were expressed as mean ± SEM. n = 3

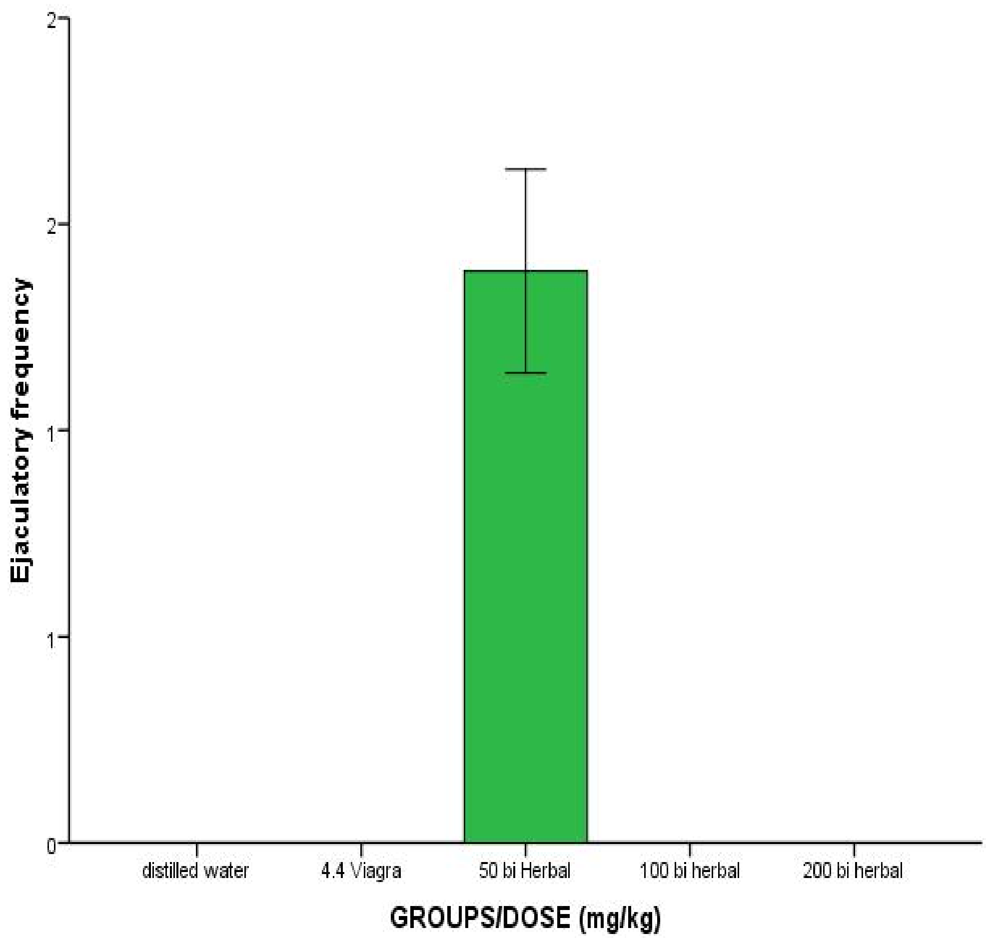

Figure 4.5: Effects of bi herbal extract on Ejaculatory frequency of rats. Results were expressed as mean ± SEM. n = 3

Table 4.1: Effect of the bi herbal extract on the testosterone level of wistar rats

Treatment	DOSE(mg/kg)	TESTOSTERONE
50 bi herbal	50	3.51 ± 0.12^{a}
100 bi herbal	100	3.63 ± 0.13^{a}
200 bi herbal	200	3.91 ± 0.10^{a}
Viagra	4.4	3.21 ± 0.09^{a}
Untreated control		2.70 ± 0.10^{b}

Key: $P < 0.05$ was considered statistically significant Key: $P < 0.05$ was considered statistically significant *values were expressed as Mean* ± SEM; values with same alphabetical superscript are non-significant across the column.. The data were analyzed using Mean±SEM and analysis of variance; where *p-value* > ^{a} = 0.05.

CHAPTER FIVE

5.0 DISCUSSION

This study was designed to evaluate the aphrodisiac properties of a biherbal aqueous extract of *Vernonia amygdalina* and *Alstonia boonei* in a male rat model. The assessment of mating behavior provides a direct and functional measure of aphrodisiac activity, including libido (sexual desire), arousal, and performance (Nwafor *et al*., 2020). The parameters measured Genital Grooming (GG), Genital Sniffing (GS), Mounting Frequency (MF), Intromission Frequency (IF), and Ejaculatory Frequency (EF) are well-established indices in ethnopharmacological research (Nwafor *et al*., 2020).

The observed increase in MF and IF in the treatment groups, particularly at the medium and high doses (50 and 100 mg/kg), is a strong indicator of enhanced sexual motivation and performance. Mounting is a primary indicator of libido, while intromission reflects the ability to achieve and maintain an erection sufficient for penetration (Kondracki *et al*., 2025). The results suggest that the biherbal extract successfully reversed the anaphrodisiac state induced by the experimental conditions. This aligns with the findings of Olalede *et al*. (2020), who reported that aqueous extracts of *Vernonia amygdalina* significantly increased mounting and intromission frequencies in male rats. The phytochemicals in both plants, particularly saponins and alkaloids, are known to stimulate the central nervous system and modulate neurotransmitters like dopamine, which is necessary for sexual motivation and arousal (Babalola *et al*., 2024).

The increase in EF in the treated groups points towards an improvement in the overall sexual performance and the integrity of the ejaculatory reflex. Diabetes-induced neuropathy can impair the autonomic nerves controlling emission and ejaculation (Mostafa and Abdel-Hamid, 2021). The restoration of this function suggests that the extract may have neuroprotective properties or may improve the neural conduction necessary for a successful ejaculatory sequence.

The significant increase in Genital Grooming (GG) and Genital Sniffing (GS) in the treated groups is highly significant. These are appetitive (pre-copulatory) behaviors that shows sexual interest and arousal (Giuliano and Majorino, 2021). The suppression of these behaviors in the negative control group is characteristic of anaphrodisia, where there is a pathological lack of sexual desire. The reversal by the biherbal extract indicates a restoration of libido. This can be linked to the potential of the extract's phytoconstituents to modulate the hypothalamic-pituitary-gonadal (HPG) axis, leading to increased testosterone production, a hormone fundamental for sexual desire (Giuliano and Maiorino, 2021). An important biochemical finding supporting the behavioral data is the significant, dose-dependent increase in serum testosterone levels (Figure 4.2). Testosterone is the primary androgen responsible for male libido, erectile function, and spermatogenesis (Corona *et al*., 2006). Streptozotocin (STZ)-induced diabetes is known to cause hypogonadism by disrupting the HPG axis, leading to reduced luteinizing hormone (LH) and consequently, low testosterone (Nazaruddin *et al*., 2025).

The biherbal extract's ability to elevate testosterone levels can be directly attributed to its rich composition of saponins and alkaloids. Saponins, present in both plants, are known to have gonadotropin-like activity, potentially stimulating the release of LH from the pituitary gland, which in turn drives testosterone synthesis in the Leydig cells of the testes (Akinmoladun *et al*., 2014). This is reported by the work of Adesina *et al*. (2025) on *Alstonia boonei* and Oyeyemi *et al*. (2018) on *Vernonia amygdalina*, both of whom documented significant increases in serum testosterone following treatment with extracts of these plants. The combination in this study likely produced a synergistic effect, making the biherbal formulation more efficacious in restoring normative hormonal balance, thereby directly combating the anaphrodisia central to diabetes-induced sexual dysfunction.

Both *Vernonia amygdalina* and *Alstonia boonei* are renowned for their antioxidant properties, due by their flavonoids, phenolics, and triterpenoids (Adesina *et al*., 2025). By scavenging free radicals and enhancing endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase, the extract would have mitigated oxidative damage to the testicular tissue and the neurovascular structures essential for sexual function. This protective effect would preserve the histological architecture of the testes, and maintain the health of the endothelium and nerves, thereby supporting normal erectile and ejaculatory function.

5.5 CONCLUSION

This study successfully demonstrated the effect of the aqueous biherbal extract of *Vernonia amygdalina* and *Alstonia boonei* possesses with aphrodisiac properties, effectively countering sexual dysfunction in the experimental model. The observed dose-dependent enhancement of key mating behaviors such as mounting frequency, intromission frequency, and ejaculatory frequency alongside an increase in serum testosterone levels, provides evidence of the extract's efficacy in improving sexual performance. These effects are by the rich phytochemical profile of the plants, which likely acted to modulate the hypothalamic-pituitary-gonadal axis, ameliorate diabetes-induced oxidative stress, and improve nitric oxide-mediated vasodilation.

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