EVALUATION OF THE APHRODISIAC AND RELAXATION EFFECTS OF THE ETHANOL EXTRACT OF *ALSTONIA BOONEI* LEAVES ON WISTAR RATS AND CORPUS CAVERNOSUM MUSCLE

BY

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FEBRUARY, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN.

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY, (PHARM D.) DEGREE IN THE UNIVERSITY OF BENIN, BENIN CITY.

FEBRUARY, 2025

CERTIFICATION

This is to certify that this project work was carried out by DURU-CHARLES UCHENNA CHARLES, in the department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin,

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DEDICATION

This Project is dedicated to God Almighty, and to my Parents.

ACKNOWLEDGEMENT

First and foremost, I am deeply grateful to God Almighty, the Author and finisher of my faith and who I am today, whose Grace has carried me throughout every season of this journey. In times of strength and in moments of uncertainty, His presence has been my steady guide and unfailing anchor.

My heartfelt appreciation goes to my supervisor, Dr. F. C. Amaechina, whose insightful guidance, patience, and unwavering support have been instrumental to the success of this work. You challenged me with encouragements to think critically, research diligently, and present my best.

To my wonderful parents, Ichie (Pharm) Duru E. C (FPSN) and Lolo Duru I. P and siblings, thank you for believing in me even when the path wasn't clear. Your understanding, Prayers, and sacrifices have laid the foundation upon which I now stand. You were there with me when the wind shifted, and you stayed with me till the storm passed. I will forever be grateful.

To the lecturers and academic staff of the Faculty of Pharmacy, both past and present, who have contributed to my success in this journey one way or the other, your words and actions reached deeper than you know. I say a big thank you.

To the friends I didn't go searching for, but found me and held my hands to the finish line, thank you. You brought light into difficult moments. Your companionship reminded me that even in unfamiliar chapters, one can find family again. To the OTF's (Idojeh Goodluck, Henry-Uzor Oghenefego, Akpovire Great-Nelson, Osakpamwan Courage, Ophori Hale), Simon, Sabrina, it doesn't end here, my people. To Ekpoh Eruore Orobosa, I don't think words would be enough to

describe the impact you had on my life. You gave me more than words; you offered patience, strength, quiet wisdom, and soft places to land. It doesn't end here.

To my colleagues in the Golden set, we made it. Thank you for every shared memory, laugh, and late-night study sessions. The journey has been long, but sharing it with you has made it worthwhile.

Finally, to every unseen hand, every kind word, every prayer whispered on my behalf, I am deeply thankful. This project is not only a culmination of academic effort but a testament to resilience, Grace, and the power of community. I sincerely thank you all!!!

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ABSTRACT

Erectile dysfunction (ED) is a prevalent condition characterized by the persistent inability to achieve or sustain penile erection sufficient for satisfactory sexual performance standard. The condition often arises from impaired relaxation of the corpus cavernosum smooth muscle, a physiological process essential for penile erection, as it permits increased arterial inflow and blood retention within the penile tissue. Disruption of this mechanism is a major contributor to ED, making the corpus cavernosum a primary target for pharmacological intervention. Conventional management typically involves phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil and tadalafil, which enhance nitric oxide–mediated smooth muscle relaxation. However, prolonged use of these agents has been linked to undesirable side effects and contraindications in certain individuals, thereby increasing the interest in natural alternatives derived from medicinal plants with established traditional aphrodisiac uses. The therapeutic potential of plant-derived agents for ED treatment has gained increasing attention because they are derived from natural sources and show reduced toxicity while targeting multiple biological pathways.

This study investigated the aphrodisiac and smooth muscle–relaxant effects of *Alstonia boonei* extract using both *in vivo* and *in vitro* experimental models in male Wistar rats. Sexual behavior parameters like genital sniffing, genital groaning, mounting, intromission, and ejaculation frequencies were evaluated following oral administration of the extract at graded doses (62.5,

125, and 250 mg/kg), with sildenafil (5 mg/kg) serving as the standard and distilled water as a control using a total of 20 male rats divided into 5 groups (n=4 per group). The in vitro assay assessed the extract's effect on isolated corpus cavernosum muscle, including its influence on potassium chloride (KCl)-induced contractions.

The results revealed a dose-dependent increase in sexual activity and relaxation of corpus cavernosum muscle, indicating enhanced erectile function. These findings validate the traditional use of *Alstonia boonei* as a natural aphrodisiac and suggest its potential as a safe, plant-based therapeutic alternative for erectile dysfunction pending further pharmacological and clinical evaluation.

Keywords: *Alstonia boonei*, erectile dysfunction, corpus cavernosum, aphrodisiac, smooth muscle relaxation, Wistar rats, calcium channel blockade.

CHAPTER ONE

INTRODUCTION

Erectile dysfunction (ED) is a common sexual health disorder in men, defined as the consistent inability to achieve or sustain an erection adequate for satisfactory sexual activity. It affects millions of men worldwide, with prevalence rates rising progressively with age. According to the Massachusetts Male Aging Study (Feldman *et al*., 1994), approximately 52% of men between 40 and 70 years' experience some form of erectile difficulty, while complete erectile failure occurs in about 10% of this group. Recent investigations, however, indicate that ED is no longer confined to older men; Burnett *et al*. (2018) reported an increasing occurrence among younger males, largely linked to modifiable lifestyle factors such as obesity, cigarette smoking, excessive alcohol intake, and psychological stress.

Beyond its influence on sexual performance, ED has profound psychological and physiological implications, contributing to low self-esteem, relationship problems, and a higher risk of cardiovascular disease (Jackson *et al*., 2019). The condition stems from a combination of physiological and psychological determinants, including hormonal disturbances, endothelial dysfunction, diabetes mellitus, hypertension, and mental health disorders such as anxiety (Kaya *et al*., 2015). Owing to its multifactorial nature and widespread impact, a clear understanding of the anatomy and physiology of the male reproductive system is fundamental to appreciating the mechanisms underlying normal erectile function and its associated dysfunctions.

1.0 Anatomy and Physiology of the Male Reproductive System

The male reproductive system is made up of internal glands like the testes, prostate, and related ducts, as well as external structures like the scrotum and the penis, which contains the corpus cavernosum, corpus spongiosum, and erectile tissues that work together to produce, maintain, and transport sperm and seminal fluids. These structures play a pivotal role in erection and ejaculation, processes critical for natural reproduction and sexual health. An essential component of erection, the corpora cavernosa fills with blood during arousal to allow for penile rigidity.

External Structures

Penis:

The penis functions as both a copulatory organ and a channel for urine and semen. Composed of two corpora cavernosa (paired erectile tissues responsible for rigidity), one corpus spongiosum (surrounds the urethra, remains softer during erection and prevents its compression during erection, ending in the glans penis, ensuring the passage of semen during ejaculation. The paired corpus cavernosum contains erectile tissues and is surrounded by the tunica albuginea, a dense fibrous sheath of connective tissue with relatively few elastic fibers (Sangiorgi *et al*, 2021)

Scrotum:

The scrotum is a cutaneous sac that houses the testes, epididymides, and the lower portion of the spermatic cords. Its primary physiological function is to regulate the temperature of the testes (at about 2-4°C below core body temperature), which is effective for spermatogenesis and fertility (Setchell, 2006). This regulation is mediated by the scrotal muscles, the cremaster muscles, a

skeletal muscle that contracts to elevate the testes towards the body in cold conditions or during stress and relaxes to lower them in warmth (Chung *et al*., 2011), and the dartos muscle, a smooth muscle layer that wrinkles the scrotal sac to conserve heat in cold environments and relaxes to increase surface area for cooling in warm conditions (Wong *et al*., 2015).

Internal Structures

Testes:

The testes are the main reproductive organs in males. They are paired glands located in the scrotum, enclosed by the tunica albuginea. They contain seminiferous tubules for sperm production, while the Leydig cells in the interstitial tissues for testosterone secretion under the influence of the luteinizing hormone (LH) (Ganong, 2016; Walker, 2017).

Epididymis:

The epididymis is a coiled tube that lies posterior to the testes and serves as a site for sperm maturation, acquiring motility before storage, ensuring that (Cooper, 2011; Sullivan and Mieusset, 2016) ensuring that sperms are functionally competent before ejaculation (Cornwall, 2009).

Vas deferens:

The vas deferens, also called the ductus deferens, is a muscular tube that transports sperm from the epididymis to the ejaculatory duct. It possesses a thick smooth muscle wall that produces peristaltic contractions during ejaculation to propel sperm forward, controlled by the sympathetic nervous system (Guyton and Hall, 2021).

Seminal Vesicles:

The seminal vesicles are paired glands located behind the bladder, responsible for producing a significant portion (60-70%) of the total semen volume (Mann and Lutwak-Mann, 1981). They secrete a fructose-rich alkaline fluid, which provides energy for sperm and prostaglandins that support sperm motility and transport (Pilatz *et al*., 2015).

Prostate Gland:

The prostate is a walnut-sized gland located just below the bladder. It secretes a slightly acidic, milky fluid rich in citrate, zinc, and prostate-specific antigens (PSA) (De Marzo *et al*., 2007). Prostatic secretions make up about 25-30% of semen volume, serving to liquefy semen via PSA, enhance motility, and protect sperm by neutralizing vaginal acidity (McNeal, 1988; Ganong, 2016). However, conditions such as benign prostatic hyperplasia (BPH) or prostatitis can negatively impact urinary and sexual function, including erectile capacity (Roehrborn, 2011).

Corpus Cavernosum and Erectile Function

The corpora cavernosa play an important role in penile erection. During sexual arousal, parasympathetic signals trigger the release of nitric oxide (NO), leading to the relaxation of cavernosal smooth muscle (Burnett, 1997; Andersson & Wagner, 1955). This relaxation leads to the relaxation of the helicine arteries, allowing increased blood flow into the sinusoids of the corpora cavernosa. As these spaces expand, venous outflow is compressed against the tunica albuginea, creating a venous obstruction that maintains penile rigidity (Hsu *et al*, 2005; Lue, 2000).

Detumescence, or the return to a flaccid state, occurs when phosphodiesterase type 5 (PDE5) breaks down cGMP, restoring smooth muscle contraction and allowing venous drainage to resume (Andersson, 2011). Thus, understanding the anatomy and physiology of the male reproductive system,

particularly the role of the corpora cavernosa, is essential for comprehending male sexual function and addressing related disorders.

Corpus Cavernosum Muscle and Its Function

The corpus cavernosum is composed of two cylindrical erectile structures extending along the shaft of the penis. Each corpus cavernosum is encased in a dense fibrous covering known as the tunica albuginea, which is vital for maintaining erectile firmness by compressing venous outflow during erection (Lue, 2000). Internally, the corpora cavernosa consist of a dense network of sinusoidal spaces lined with endothelial cells and surrounded by corpus cavernosum smooth muscle (CCSM) fibers (Souza *et al.*, 2022). In the non-erect or flaccid state, these smooth muscle cells remain contracted, limiting arterial blood entry and maintaining a low intracavernosal pressure that prevents erection (Andersson and Wagner, 1995). During sexual arousal, however, the smooth muscle relaxes, allowing increased blood inflow, expansion of the sinusoids, and subsequent penile erection (Dean and Lue, 2005; Souza *et al.*, 2022).

Role of the Corpus Cavernosum Muscle in Erection and Physiological Processes

Penile erection is a complex neurovascular phenomenon, coordinated by the relaxation of CCSM, increased arterial inflow, and reduced venous drainage. This process is primarily regulated by the autonomic nervous system, particularly through parasympathetic activation, which releases neurotransmitters and endothelial mediators that initiate erection (Andersson, 2003). Among these mediators, nitric oxide (NO) is the key agent driving CCSM relaxation by influencing intracellular calcium regulation (Kaya *et al.*, 2015). Upon sexual stimulation, NO is released from non-adrenergic, non-cholinergic (NANC) neurons and the endothelial lining of the corpora cavernosa (Burnett, 1997;

Andersson, 2011).

NO activates soluble guanylate cyclase (sGC) within smooth muscle cells, which converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) (Andersson and Wagner, 1995). Elevated cGMP levels lower intracellular calcium concentrations, causing smooth muscle relaxation and vasodilation of the helicine arteries (Dean and Lue, 2005). Consequently, the sinusoidal spaces expand, promoting a rapid influx of blood into the corpus cavernosum (Lue, 2000).

As blood fills the cavernosal chambers, the tunica albuginea compresses the subtunical venous plexus, restricting venous outflow and maintaining penile rigidity — a mechanism known as the veno-occlusive process, which is essential for sustaining erection (Hatzichristou *et al.*, 2000; Goldstein and Bivalacqua, 2020). The capacity of CCSM to relax and maintain this state determines normal erectile function; thus, impairments in this mechanism often result in erectile dysfunction (ED) (Andersson, 2011).

Detumescence, the process of returning to the flaccid state, occurs when this biochemical cascade reverses. The enzyme phosphodiesterase type 5 (PDE5) breaks down cGMP, leading to elevated intracellular calcium, smooth muscle contraction, decreased arterial inflow, and restoration of venous outflow (Dean and Lue, 2005; Souza *et al.*, 2022). Any disruption in vascular, neurological, or molecular mechanisms that regulate this cascade can compromise CCSM relaxation and contribute to the development of ED (Andersson, 2011).

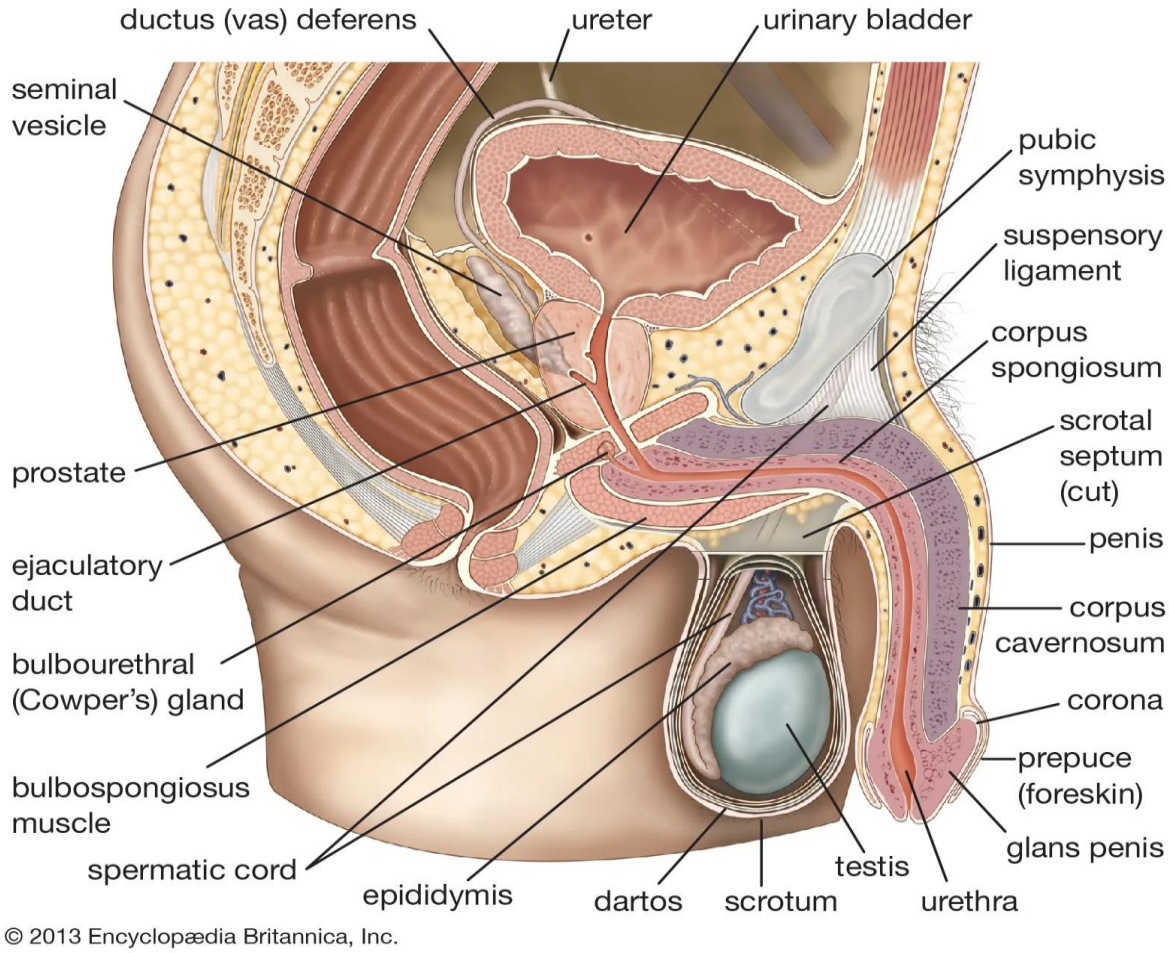

Fig. 1.0; A diagram of the male reproductive organ

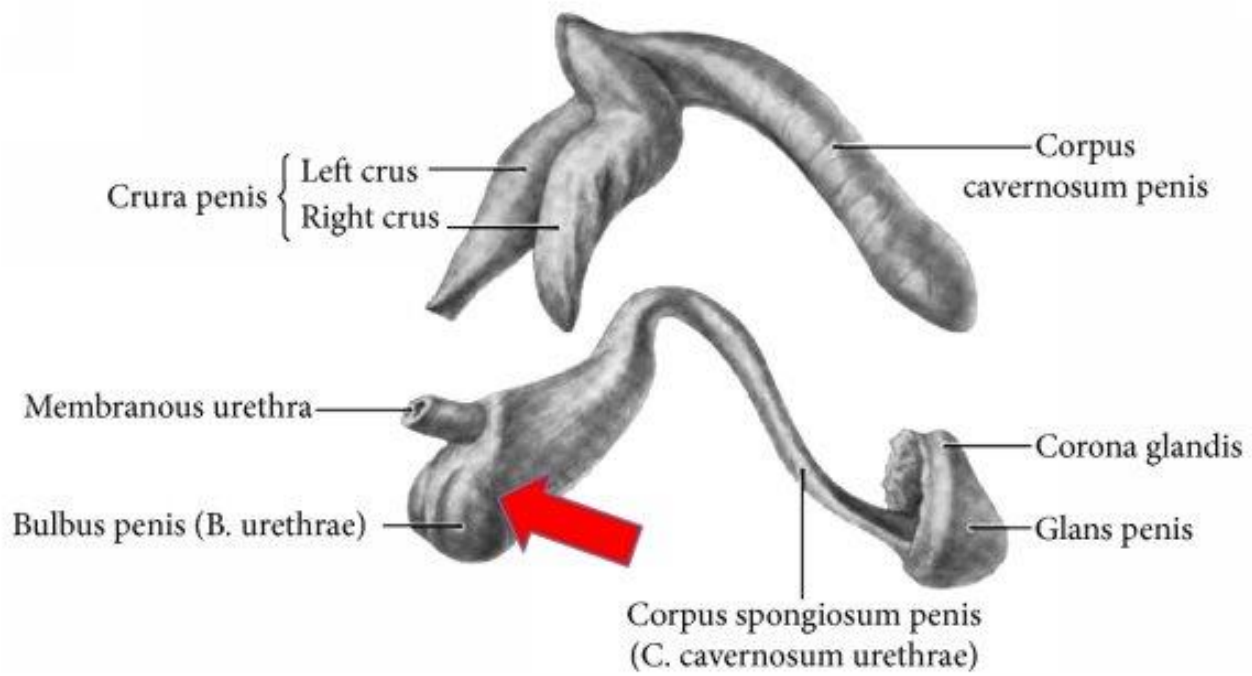

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Fig 1.1: A diagram of the anatomy of the male corpus cavernosa

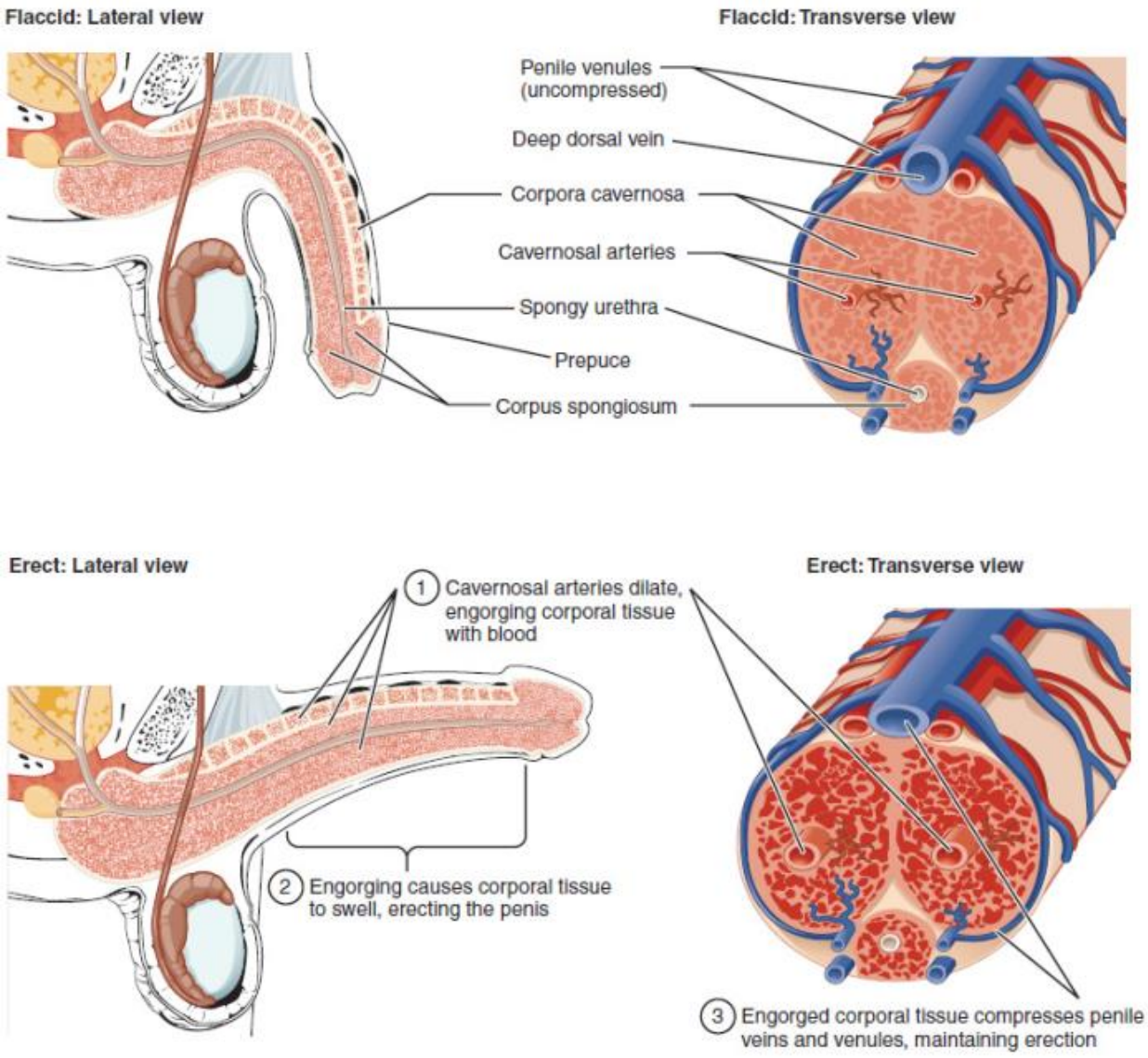

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Fig 1.2; A diagram portraying a functional and dysfunctional corpus carvenosum

1.2 Hormonal Regulation of Erectile Function

Erectile function is not just a neurovascular event; it is also intricately regulated by endocrine function, involving the hypothalamus, pituitary gland, and testes, referred to as the hypothalamic-pituitary-gonadal (HPG) axis. Hormones maintain the structural integrity of the erectile tissues, regulate neurotransmission, and influence libido, making them important for normal male sexual function. Key hormones in this regulatory system include testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

Testosterone: Secreted primarily by Leydig cells of the testes under luteinizing hormone stimulation, testosterone has both central and peripheral influences on erectile function.

Central action: Testosterone regulates sexual desire and arousal by acting on the hypothalamus and the limbic system (Hull & Dominguez, 2007). Decreased libido is associated with reduced levels of testosterone.

Peripheral action: Testosterone upregulates the expression of nitric oxide synthase (NOS) in penile tissues, thereby enhancing the release of nitric oxide (NO) (Traish *et al*., 2011). This is essential for the relaxation of the corpus cavernosum smooth muscle through the NO-cGMP pathway.

Testosterone also maintains the structural architecture of the trabecular smooth muscle, endothelium, and tunica albuginea, as hypogonadism leads to smooth muscle atrophy, fibrosis, and venous leakage, all of which impair erectile function (Yassin & Saad, 2008).

1.3 Factors Affecting Corpus Cavernosum Muscle Function

The proper function of the corpus cavernosa smooth muscle is central to achieving and maintaining erections. This muscle's proper functioning depends on the delicate balance of neural, hormonal, endothelial, and structural factors. Disruption in any of these pathways can impair cavernosal smooth muscle relaxation and contribute to erectile dysfunction (Andersson, 2011).

1.3.1 Endothelial and Vascular Function

The endothelium of cavernosal sinusoids produces Nitric oxide (NO), prostacyclin, and other vasodilators. Endothelial dysfunction reduces NO bioavailability and compromises smooth muscle relaxation i.e, inadequate relaxation of the CCSM. This results in insufficient blood flow to the corpus cavernosum, thereby compromising the ability to achieve or maintain an erection (Saenz de Tejada *et al*., 1989; Burnett and Musicki, 2021).

1.3.2 Oxidative Stress and Structural Changes

Chronic oxidative stress from metabolic syndrome, aging, or diabetes increases oxidative reactive oxygen species (ROS), which degrades nitric oxide (NO) bioavailability and promotes fibrosis in the corpus cavernosum. Fibrotic remodeling reduces smooth muscle elasticity and its ability to relax, contributing to erectile dysfunction Ferrini *et al*., 2002; Musicki & Burnett, 2006).

1.3.3 Hormonal Regulation

Testosterone plays a crucial role in maintaining cavernosal structure and responsiveness. It upregulates nitric oxide (NO) synthase and supports trabecular smooth muscle integrity. Low testosterone levels (hypogonadism) are associated with smooth muscle atrophy, fibrosis, and reduced erectile responsiveness (Traish *et al*., 2011; Yassin & Saad, 2008)

1.4 Erectile dysfunction and pathophysiology

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance (NIH, 1993). It is a complex condition involving vascular, neurological, hormonal, and psychological components that work together to regulate penile erection (Lue, 2000).

1.4.1 Dysfunction in the Corpus Cavernosum Muscle

The dysfunction of the corpus cavernosum muscle represents the central defect in many forms of

erectile dysfunction. The CCSM is responsible for regulating penile blood flow and veno-occlusion, and its coordinated relaxation is essential for erection. Normally, erection is initiated by sexual stimulation, which triggers the release of nitric oxide (NO) from endothelial cells and neuronal sources within the corpus cavernosum, resulting in relaxation of corpus cavernosum smooth muscle (CCSM) (Burnett, 1977; Andersson, 2011). Pathological conditions such as diabetes mellitus, hypertension, and aging cause endothelial damage and oxidative stress that impair NO synthesis and signaling (Musicki & Burnett, 2006)

1.5 Role of Medicinal Plants in Erectile Dysfunction Management

Various medicinal plants have been employed to address sexual dysfunction, including ED. These plants often contain bioactive compounds that enhance sexual function and performance by increasing blood flow, modulating hormone levels, or exerting neuroprotective effects (Gauthaman *et al*., 2022)

Notable Medicinal Plants and Their Active Constituents

Over the years, numerous medicinal plants have been identified for their potential to improve male sexual performance and manage erectile dysfunction (ED), through various mechanisms, primarily by enhancing nitric oxide (NO) synthesis, promoting vasodilation, or inhibiting phosphodiesterase-5 (PDE5). Below are some notable examples.

Panax ginseng (Red Ginseng): Contains ginsenosides, which enhance NO synthesis, promoting vasodilation and improved erectile function (Kim *et al*., 2023)

Tribulus terrestris: contains protodioscin, a compound that increases androgen receptor density and NO release, enhancing erectile function (Gauthaman *et al*., 2022).

Yohimbe (*Pausinystalia johimbe*): Contains yohimbine, an alpha-2 adrenergic receptor antagonist, which promotes vasodilation and increases penile blood flow (Guay *et al*., 2022).

Epimedium (Horny Goat Weed): Contains icariin, a PDE5 inhibitor that enhances cGMP levels,

supporting smooth muscle relaxation and erectile function (He *et al*., 2023). While these medicinal plants have demonstrated promise, their efficacy and safety profiles vary, and they may interact with other medications. Therefore, consultation with a healthcare professional is recommended before incorporating them into treatment plans (Gauthaman *et al*., 2022).

Pharmacological Treatment of Erectile Dysfunction

Erectile dysfunction is commonly managed using various drug classes, each targeting specific mechanisms to enhance penile blood flow and erectile function.

1. Phosphodiesterase Type 5 (PDE5) Inhibitors

These drugs inhibit PDE5, an enzyme that degrades cyclic guanosine monophosphate (cGMP).

By increasing cGMP levels, they promote smooth muscle relaxation in the corpus cavernosum, enhancing blood flow and facilitating erection. PDE5 inhibitors require sexual stimulation to be effective (Krzastek *et al*., 2023).

Examples: Sildenafil (Viagra), Tadalafil (Cialis), Vardenafil (Levitra), Avanafil (Stendra)

Limitations: PDE5 inhibitors may cause headaches, flushing, dizziness, nasal congestion, and visual disturbances. They are contraindicated in patients using nitrates due to the risk of severe hypotension (Burnett and Musicki, 2021).

2. Alpha-2 Adrenergic Receptor Antagonists

These drugs block alpha-2 adrenergic receptors, leading to increased sympathetic outflow, vasodilation, and improved penile blood flow (Guay *et al*., 2022).

Example: Yohimbine (derived from *Pausinystalia johimbe*)

Limitations: Yohimbine can cause anxiety, increased heart rate, and hypertension, limiting its use in patients with cardiovascular diseases (Gauthaman *et al*., 2022).

3. Testosterone Replacement Therapy (TRT)

Used in men with hypogonadism (low testosterone levels), TRT restores normal testosterone levels, enhancing libido and erectile function. However, it is only effective in patients with testosterone deficiency (Krzastek *et al*., 2023).

Examples: Testosterone enanthate, Testosterone undecanoate, Transdermal testosterone patches

Limitations: TRT can lead to polycythemia, fluid retention, acne, and suppression of endogenous testosterone production. It is also linked to an increased risk of cardiovascular events

(Burnett and Musicki, 2021).

4. Prostaglandin E1 (PGE1) Analogues

These drugs act as vasodilators, directly relaxing the smooth muscle of the corpus cavernosum.

They are often used when PDE5 inhibitors are ineffective or contraindicated (Guay *et al*., 2022).

Examples: Alprostadil (administered via intracavernosal injection or intraurethral suppository)

Limitations: Alprostadil can cause penile pain, priapism (prolonged erection), and fibrosis at the injection site, making it less favorable for long-term use (Souza *et al*., 2022).

5. Dopamine Receptor Agonists

By stimulating dopamine receptors in the central nervous system, these drugs enhance sexual desire and erectile function (He *et al*., 2023).

Examples: Apomorphine, Bromocriptine

Limitations: Dopamine agonists can cause nausea, dizziness, and hypotension, limiting their tolerability (Krzastek *et al*., 2023).

6. Rho-Kinase Inhibitors and Soluble Guanylate Cyclase (sGC) Activators

These emerging treatment options targ*et al*ternative pathways to promote smooth muscle relaxation and penile blood flow (Burnett and Musicki, 2021).

Examples: Fasudil (Rho-kinase inhibitor), Riociguat (sGC activator)

Limitations: These drugs are still under investigation, and their long-term safety and efficacy profiles are not well established (Guay *et al*., 2022).

The Role of *Alstonia boonei* in Erectile Dysfunction Management

Given the limitations of conventional ED treatments, there is growing interest in alternative therapies like *Alstonia boonei*, a medicinal plant traditionally used for its anti-inflammatory, analgesic, and vasodilatory properties. Recent studies suggest that *Alstonia boonei* may enhance erectile function through multiple mechanisms:

Smooth Muscle Relaxation: Extracts of *Alstonia boonei* have been shown to induce relaxation in the corpus cavernosum muscle, which is crucial for penile erection.

Nitric Oxide Modulation: The plant may enhance NO synthesis, improving vasodilation and blood flow to the penile tissues, similar to PDE5 inhibitors but with fewer side effects (Souza et al., 2022).

Antioxidant and Anti-Inflammatory Effects: Oxidative stress and chronic inflammation contribute to endothelial dysfunction, a key factor in ED. *Alstonia boonei* possesses strong antioxidant properties, which may help mitigate these effects (Burnett and Musicki, 2021).

Potential Testosterone Modulation: Some phytochemicals in *Alstonia boonei* may support hormonal balance, improving androgen levels that play a role in erectile function (Gauthaman et al., 2022).

While orthodox drugs remain the primary treatment for ED, they have notable limitations, including adverse effects, contraindications, and high costs. Herbal remedies like *Alstonia boonei* present a promising alternative, offering a multi-targeted approach with potentially fewer side effects. However, further clinical studies are needed to fully establish its safety and efficacy in

ED management. A combination of both pharmaceutical and natural therapies may provide a more holistic approach to treating erectile dysfunction.

1.6 *Alstonia boonei* (Family: Apocynaceae)

1.6.1 Botanical Description

Alstonia boonei is a large deciduous tree that can grow up to 45 meters in height, with a cylindrical trunk and prominent buttresses (Adotey *et al*., 2012). The leaves are arranged in whorls, are oblanceolate, and have lateral veins at right angles to the midrib (Sowndhararajan et al., 2013). The tree produces yellowish-white flowers in terminal cymes and paired slender follicles containing tufted seeds for wind dispersal. A milky latex exudes from cuts on the trunk (Adotey *et al*., 2012)

Fig 1.3; A diagram of *Alstonia boonei* leaves

Fig 1.4; A diagram of *Alstonia boonei* leaves

1.6.2 Ethnomedicinal Uses of *Alstonia boonei*

Sexual Health: The bark is traditionally used as an aphrodisiac to enhance sexual performance (Adotey *et al*., 2012).

Fever Management: Decoctions of the bark are employed in treating malaria and reducing fever (Asuzu & Chineme, 2013).

Inflammation: The plant is commonly used for rheumatic and arthritic pains, demonstrating its Anti-inflammatory effects (Oladipupo *et al*., 2021).

Other Uses: The bark is used to treat jaundice, aid lactation, and counteract snake bites (Adotey *et al*., 2012)

1.6.3 Phytochemical Constituents

Alstonia boonei contains several bioactive compounds (Sowndhararajan *et al*., 2013; Oladipupo *et al*., 2021):

Alkaloids: Echitamine, echitamidine, voacangine, akuammidine (Adotey *et al*., 2012).

Iridoids: Boonein, loganin (Asuzu & Chineme, 2013).

Triterpenoids: Lupeol, ursolic acid, ß-amyrin (Sowndhararajan *et al*., 2013).

Flavonoids: Rutin, isoquercetin (Oladipupo *et al*., 2021).

1.6.4 Pharmacological Properties

Research highlights the following pharmacological effects of *Alstonia boonei* (Adotey *et al*., 2012; Sowndhararajan *et al*., 2013):

Anti-inflammatory: The bark extract shows significant anti-inflammatory properties, supporting its traditional use for treating arthritis (Oladipupo *et al*., 2021).

Antioxidant: The plant's antioxidant activity may contribute to its therapeutic effects

(Sowndhararajan *et al*., 2013).

Antipyretic: The bark's antipyretic properties align with its traditional use in fever management (Asuzu & Chineme, 2013).

Antimalarial: The plant has been proven effective against malaria parasites (Adotey *et al*., 2012).

Aphrodisiac: Traditional use for enhancing sexual health suggests potential benefits in sexual function (Oladipupo *et al*., 2021).

1.7 Aims and Objectives of the study

1.7.1 Aims:

The study aimed to investigate the effect of *Alstonia boonei* extract on the corpus cavernosum muscle in Wistar rats.

1.7.2 Objectives:

The objectives of the study were to :

1 To evaluate the effect of *Alstonia boonei* extract on the contractility of the corpus cavernosum muscle.

2. To assess the histological changes in the corpus cavernosum after treatment.
3. To determine the antioxidant activity of *Alstonia boonei* in the corpus cavernosum muscle tissue.
4. To compare the effects of *Alstonia boonei* extract with a standard erectile dysfunction drug (e.g, Sildenafil)

1.8 Experimental Use of Wistar Rats in Erectile Physiology Research

Wistar rats are widely used as model organisms in biomedical research due to their genetic consistency, well-documented physiological traits, and ease of maintenance. They play a crucial role in investigating various physiological functions, including erectile function and smooth muscle activity in the corpus cavernosum. Their use facilitates the exploration of underlying mechanisms of erectile

dysfunction and the evaluation of potential therapeutic agents before progressing to human trials (Jetir, 2023).

1.8.1 Justification for the Study (Statement of Purpose)

An estimated 15% to 30% of men over 40 worldwide suffer from erectile dysfunction (ED), a prevalent sexual condition marked by the inability to obtain or sustain an erection suitable for sexual activity (Souza *et al*., 2022). Impaired corpus cavernosum smooth muscle relaxation, which lowers blood flow and impairs erectile function, is a major mechanism in ED (Souza *et al*., 2022). In African traditional medicine, *Alstonia boonei*, a member of the Apocynaceae family, has been used extensively to treat fever, malaria, and rheumatic pain. It has also been reported to have aphrodisiac qualities (Adotey *et al*., 2012).

Its antispasmodic benefits are highlighted by recent pharmacological studies, which may indicate a mechanism for smooth muscle relaxation, which is essential for erectile function (Owolabi *et al*., 2022).

I. **Development of Alternative Therapies for ED**

Development of Alternative Therapies for ED: Phosphodiesterase type 5 (PDE5) inhibitors, like sildenafil, are efficient ED treatments, but they are frequently restricted by side effects and contraindications, including headache, dizziness, and cardiovascular issues (Shamloul & Ghanem, 2013). Examining *Alstonia boonei*'s potential may help find plant-based treatments that are more accessible and have fewer adverse effects, particularly in environments with low resources (Owolabi *et al*., 2022).

II. **Connecting Traditional Knowledge with Modern Medicin**

Alstonia boonei bark decoctions have long been used for sexual enhancement by traditional healers in West Africa (Adotey *et al*., 2012). There is, however, little scientific support for

these applications. In order to encourage the incorporation of traditional medicine into modern healthcare, this study will offer scientific evidence to support or contradict these traditional beliefs.

III. Comprehending Mechanisms of Action

Through smooth muscle relaxation mediated by nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) pathways, the corpus cavernosum plays a crucial role in erectile function (Souza *et al*., 2022). *Alstonia boonei*'s possible modes of action, such as increased NO release, antioxidant effects, or inhibition of smooth muscle contraction, may be revealed by examining its effects on these pathways (Owolabi *et al*., 2022; Oladipupo *et al*., 2021).

IV. Contribution to phytomedicine research

African medical plants like *Alstonia boonei* have received less attention than medicinal plants with aphrodisiac qualities like Panax ginseng and Tribulus terrestris, which have been the subject of numerous studies (Shamloul and Ghanem, 2013). This study may increase the number of plant-based ED treatments available and stimulate more research into African phytomedicine. In summary, the purpose of this research is to investigate scientifically how *Alstonia boonei* may enhance erectile function by relaxing the corpus cavernosum muscle. The results validate traditional African medical practices and may lead to new plant-based treatment options for ED.

1.8.2 Justification for the Use of Wistar Rats in Scientific Research

The Wistar rat (Rattus norvegicus) is one of the most widely used laboratory animals in biomedical and pharmacological research due to its well-characterized physiology, manageable size, and adaptability to experimental manipulation. Its use is justified by ethical, biological, and scientific considerations that make it a reliable model for investigating human physiology and pathological

processes, including studies on erectile dysfunction and pharmacological interventions (Harkness *et al*., 2010).

1. **Physiological and Anatomical Similarity to Humans**
    - Wistar rats share genetic, anatomical, and physiological similarities with humans, making them suitable for extrapolating research findings (Charles River Laboratories, 2016).
    - Their reproductive, cardiovascular, endocrine, and nervous **systems** function in ways comparable to humans, including nitric oxide (NO)-mediated vasodilation and hormonal regulation (Andersson, 2011).
    - The NO–cGMP pathway, essential for corpus cavernosum relaxation and penile erection, is conserved between rats and humans.
2. **Ease of Handling and Experimental Sustainability**

- Wistar rats are **docile, easy to handle**, and adapt well to laboratory environments (Harkness *et al*., 2010).
- They have a short gestation period, large litter size, and rapid growth rates, facilitating adequate sample sizes and reproducible results.
- Their relatively large penile size allows for direct in-vitro assays on the corpus cavernosum, making them ideal for erectile physiology studies.

3. **Availability of Baseline Physiological Data**

- Comprehensive data exist on their heart rate, blood pressure, hormone levels, and reproductive cycle, ensuring standardized reference values for comparison (Sengupta, 2013).
- Their use in countless pharmacological and toxicological studies provides a rich historical

database, improving research consistency (Suckow *et al*., 2005).

4. **Ethical Acceptibility and the 3Rs Principle**

- The use of Wistar rats complies with the 3Rs principle [Replacement, Reduction, and Refinement], which promotes ethical animal research (Russell & Burch, 1959).
- Their widespread use reduces the number of new animal species required for validation, minimizing experimental variability and suffering.
- They are the most validated mammalian models for testing safety, efficacy, and pharmacodynamics before human trials (Festing & Altman, 2002).

5. **Relevance to Erectile Function studies**

- Wistar rats exhibit reproductive physiology and penile responses similar to those in humans (Traish *et al*., 2011).
- Their corpus cavernosum smooth responds predictably to nitric oxide (NO) donors, PDE% inhibitors, and hormonal modulation, making them suitable for pharmacological studies of erectile dysfunction.
- They provide a reliable in-vivo and in-vitro model for testing plant extracts such as *Alstonia boonei* in smooth muscle relaxation and erectile physiology research.

1.8.3 Common Experimental Models for Erectile Function Studies

A variety of experimental models, from in vitro studies to in vivo animal models, are used to study erectile function, smooth muscle dynamics, and possible treatments. Each model has its own advantages and disadvantages, and the selection of a suitable model is contingent upon the goals of the study and the requirement for physiological relevance.

In-Vitro Models

Endothelial cell assays and primary cultures of corpus cavernosum smooth muscle cells are two

examples of cell culture techniques used to investigate the cellular and molecular mechanisms underlying erectile function. These models provide fine control of experimental conditions and assist high-throughput screening of natural substances. However, they lack the systemic interactions essential to study whole-organ responses (Labtoo, 2023).

Small Animal Models

Because of their rapid breeding cycles, well-characterized vascular responses, and genetic resemblance to humans, rodents (especially Wistar rats) are frequently employed in erectile physiology research. They are commonly used in research on nitric oxide signaling, smooth muscle relaxation, and the effects of natural aphrodisiacs. However, when applying findings to clinical settings, one must take into account variations in vascular structure and metabolic rate in comparison to humans. (Frontiers in Cardiovascular Medicine, 2023).

Large Animals Models

Because of their anatomical and physiological resemblance to humans, larger animals like rabbits and monkeys are sometimes employed in studies on erectile function. For researching drug efficacy and penile hemodynamics, these models are especially helpful. However, its broad use is restricted by logistical difficulties, high costs, and ethical issues (Oxford Academic, 2023).

Ex Vivo Corpus Cavernosum Models

Ex vivo preparations, like isolated corpus cavernosum strip investigations, make it possible to measure smooth muscle relaxation and contractility directly. These techniques shed light on the pharmacological effects of extracts from plants, such as *Alstonia boonei*, without affecting the body. Nevertheless, isolated tissue preparations' lack of neurohumoral control restricts their use in in vivo settings (Revista Española de Cardiologia, 2023).

1.8.4 Ethical Considerations in Animal Research

Ethical considerations are a fundamental aspect of animal experimentation, ensuring that research is conducted responsibly, humanely, and in accordance with international standards. The goal is to protect animal welfare while maintaining scientific integrity. Studies involving **Wistar rats** or other animal models must comply with ethical guidelines that emphasize humane treatment, justification of use, and minimization of suffering (Russell & Burch, 1959).

The 3Rs Principle (Replacement, Reduction, and Refinement)

Replacement: Encourages the use of alternative models, such as in vitro assays and computational simulations, when feasible.

Reduction: Aims to minimize the number of animals used without compromising research quality.

Refinement: Involves optimizing experimental procedures to minimize pain and distress (Fredowsian and Beck, 2011)

Regulatory and Institutional Oversight

All animal studies must be approved by an Institutional Animal Care and Use Committee (IACUC) or an equivalent Ethical Review Board before commencement (Suckow *et al*., 2005)

Researchers are required to submit detailed experimental protocols describing the purpose, number of animals, procedures, and justification for animal use.

Compliance with national and international laws, such as the Guide for the Care and Use of Laboratory Animals by the National Council (NRC, 2011), is mandatory.

Humane Treatment and Welfare Standards

Animals must be housed in environments that meet standards for temperature, humidity, lightening, ventilation, and social interaction (Harkness *et al*., 2010).

They should have free access to food and clean water and be handled by trained personnel to reduce

stress.

Pain management must be incorporated into all procedures (including appropriate use of anesthetics and analgesics), and animals showing signs of severe pain or distress must be humanely euthanized according to approved guidelines (Flecknell, 2002).

Scientific Justification and Societal Benefit

The use of animals must be scientifically justified, ensuring that the expected benefits of the research outweighs any potential harm to animals.

Research should aim to generate valuable knowledge in areas such as medicine, pharmacology, or toxicology that cannot be obtained through non-animal alternatives (Olsson *et al*., 2012)

In studies such as the evaluation of the relaxation effects of *Alstonia boonei* on the corpus cavernosum muscle, animal use is justified by the need to understand complex in-vivo physiological mechanisms before clinical translation.

Recognizing Animal Sentience

Recent conversations have focused on recognizing the sensibility of lab animals. Ethical research methods can be promoted by observing behavioral markers of suffering and adjusting experimental techniques accordingly (Fenton, 2019).

Research on how *Alstonia boonei* affects corpus cavernosum muscle function can yield important insights while upholding ethical standards for animal experimentation by combining scientific rigor with ethical considerations.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

This study made use of a range of materials, grouped into biological specimens, laboratory glassware, and general laboratory supplies. Each of these materials played an important role in ensuring accuracy, consistency, and reliability during the experiments carried out to examine the effects of *Alstonia boonei* extract on the corpus cavernosum muscle of Wistar rats.

2.1.1 Biological Materials: The main biological specimen employed in this study was the corpus cavernosum muscle obtained from adult male Wistar rats. The tissue was carefully isolated and prepared to assess its contractile and relaxation responses under controlled laboratory conditions. To preserve a stable physiological environment, the physiological salt solution was continuously aerated with a Carbogen gas mixture (95% oxygen and 5% carbondioxide), which ensured adequate oxygenation and maintained the appropriate pH balance throughout the experiment.

2.1.2 Laboratory Glassware: A variety of glassware was employed for solution preparation, sample handling, and experimental procedures.

Beakers (50-500mL) were utilized for mixing and temporary storage of liquid solutions, while conical and volumetric flasks (100-500mL) ensured precise preparation of standard solutions.

Petri dishes served as containers for holding tissue samples before mounting in the organ bath.

Measuring cylinders (10-500mL) facilitated accurate measurement of liquid volumes.

Glass funnels in combination with filter paper were used for the filtration of plant extracts and other laboratory solutions.

2.1.3 General Laboratory Supplies: To ensure sterility, precision, and safety throughout the experimental procedures, several essential laboratory materials were utilized.

Disposable gloves were worn to prevent contamination during the handling of samples and chemical reagents, while lab coats and safety goggles provided protection against chemical splashes and potential biological exposure.

A digital weighing balance was employed for accurate measurement of plant extracts and other experimental substances.

Additionally, paraffin was used to seal containers and prevent contamination or evaporation of prepared solutions.

2.1.4 APPARATUS

Various laboratory instruments were employed to facilitate tissue preparation, experimental setup, and data acquisition. These apparatus were essential for ensuring precision, maintaining controlled experimental conditions, and obtaining accurate and reproducible measurements throughout the study.

2.1.5 Organ Bath System

The organ bath (25-50mL capacity) was used to immerse the isolated corpus cavernosum muscle in a physiological solution, thereby simulating in vivo conditions.

A water bath maintained at 37°C ensured the organ bath system remained at physiological body temperature throughout the experiment. Additionally, an aerator (Carbogen gas delivery system)

supplied a continuous flow of 95% oxygen and 5% carbon dioxide, which helped sustain tissue viability and maintain the appropriate physiological pH balance during the experimental procedures.

2.1.6 Data Recording and Measurement Instruments

An isometric force transducer was employed to detect and convert mechanical tension changes within the corpus cavernosum muscle into electric signals for precise analysis.

A data acquisition system integrated with specialized computer software was used to record and analyze the contractile and relaxation responses generated during the experiments.

A physiography provided a real-time graphical representation of muscle contractions and relaxations, enabling continuous monitoring and accurate interpretation of the tissue's functional activity.

2.1.7 Tissue Dissection and Handling Instruments

Fine and blunt-tipped forceps were used for gripping and positioning the corpus cavernosum tissue during dissection and mounting.

Scapel and sterile blades facilitated the precise excision of the corpus cavernosum muscle.

Straight and curved scissors were utilized to trim excess connective tissue, ensuring clean and well-defined tissue segments for experimentation.

2.1.8 Precision Measurement Equipment

Micropipettes (0.1-1000 µL) were used for the accurate measurement and transfer of small liquid volumes, including plant extracts and drug solutions.

Graduated pipettes facilitated the precise measurement and dilution of test solutions, ensuring uniformity in reagent concentration and experimental reproducibility.

2.1.9 CHEMICALS AND REAGENTS

Various chemicals and reagents were used to prepare physiological solutions, induce smooth muscle contractions, and evaluate the effects of *Alstonia boonei* extract on the corpus cavernosum muscle.

2.1.10 Chemicals for Physiological Salt Solution Preparation

The physiological salt solution mimicked the extracellular fluid environment to support muscle viability. Key components included

Sodium chloride (NaCI): Maintained osmotic balance and electrolyte stability.

Potassium chloride (KCI): Regulated smooth muscle activity and membrane potential. Calcium chloride dihydrate (CaCh-$2H_2O$): Provided calcium ions essential for muscle contraction.

Magnesium sulfate heptahydrate (MgSO.-$7H_2O$): Facilitated enzymatic activity and promoted muscle relaxation.

Sodium bicarbonate (NaHCO,): Acted as a pH buffer to maintain physiological stability. Dextrose (Glucose): Served as an energy source for tissue metabolism.

Potassium dihydrogen phosphate (KH.PO4): Assisted in pH regulation and cellular functions.

2.1.11 Vasoactive Agent

Potassium Chloride (KCI): Induced smooth muscle contraction by depolarizing the cell membrane.

2.1.12 Plant Extract

The ethanol extract of *Alstonia boonei* was tested for its potential effects on corpus cavernosum muscle function.

Extract Concentrations: The plant extract was prepared at six different concentrations: 1.5625 mg/mL, 3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, and 50 mg/mL.

Administration Volumes: Each concentration was tested using measured volumes of 25 µL, 62.5µL, 125µL, 250 µL, and 500 µL to ensure precise dosing.

2.1.13 PREPARATION OF PHYSIOLOGICAL SALT SOLUTION

The physiological salt solution was prepared to maintain tissue viability and function throughout the experiment.

2.1.14 Preparation Procedure

Dissolution of Main Components

Distilled water was measured into a clean beaker.

The following chemicals were added sequentially, ensuring complete dissolution before adding the next component:

Sodium chloride (NaCl) -6.9 g/L

Sodium bicarbonate (NaHCO)-2.1 g/L

D-glucose-2.0 g/L

Potassium dihydrogen phosphate (KH2PO4)-0.16 g/L.

Potassium chloride (KCI) 0.36 g/l.

Magnesium sulfate monohydrate (MgSO4 H2O)-0.29 g/l.

Preparation of Calcium Chloride Solution

Calcium chloride (CaCl) -0.37 g/L was dissolved separately in a small volume of distilled water to prevent precipitation.

The solution was slowly added to the main mixture while stirring continuously.

Aeration of the Solution

Carbogen gas (95% O2, 5% CO2) was continuously bubbled through the solution to maintain oxygenation and pH balance.

Storage and Usage

The freshly prepared solution was stored at room temperature and used immediately.

For extended use, continuous aeration was maintained to maintain its stability.

2.1.15 PLANT EXTRACT PREPARATION

The extraction of *Alstonia boonei* was carried out using an ethanol method to obtain its bioactive compounds.

2.1.16 Collection and Authentication

Fresh leaves of *Alstonia boonei* were collected and authenticated by a botanist, from the University of Benin Ugbowo Campus, Edo State, Nigeria, during the rainy season in June 2025. The geographical coordinates of the collection site were approximately 6°23’38” N 5°36’53” E.

The leaves were thoroughly cleaned to remove dirt and debris.

2.1.17 Drying and Grinding

Leaves were air-dried in a well-ventilated area, avoiding direct sunlight to preserve active compounds.

The dried leaves were ground into a fine powder using a mechanical grinder and stored in an airtight container.

2.1.18 Extraction Procedure

A Soxhlet apparatus was used to extract 571g of pulverized plant material with 1.5 L of ethanol at 70°C. This extraction process was performed thrice, and the combined extracts were then concentrated using a rotary evaporator until a solid, dark-black mass was obtained. The obtained extract was securely sealed in an airtight container and stored at room temperature until required for experimental use.

2.2 METHOD

2.2.1 Aphrodisiac/Invivo assay

Experimental Animals

A total of 25 Wistar rats (20 adult males and 10 adult females) were used for the in vivo study.

The male rats were housed under standard laboratory conditions, with a 12-hour light/dark cycle and free access to food and water. They were acclimatized for seven days before the commencement of the experiment.

Experimental Design

The 20 adult male Wistar rats were randomly assigned into five groups (n = 4 per group):

Group 1 (Control): Received distilled water.

Group 2 (Standard): Received a reference aphrodisiac drug.

Group 3: Received *Alstonia boonei* extract at 62.5 mg/kg.

Group 4: Received *Alstonia boonei* extract at 125 mg/kg.

Group 5: Received *Alstonia boonei* extract at 250 mg/kg.

All treatments were administered orally. The 10 adult female Wistar rats were alternated with the male rats as part of the study protocol.

Sexual Behavior Assessment

The male rats were individually introduced to a receptive female (previously primed with estrogen and progesterone) in a dimly lit observation cage. The following parameters were recorded over a 30-minute period:

Genital Sniffing (number of times the male rat sniffed the female's genital region)

Genital Groaning (vocal expressions associated with sexual arousal and genital itching)

Mounting Frequency (number of mounting attempts)

Intromission Frequency (number of penetrative thrusts)

Ejaculation Frequency (number of ejaculations within the observation period)

2.2.2 In vitro assay for corpus cavernosum muscle contractility

Male Wistar rats were used for the in vitro assay. The animals were humanely sacrificed by cervical dislocation, after which the penis was carefully dissected and the corpus cavernosum was isolated. The tissue was gently freed from surrounding connective and vascular structures and cut into segments of approximately 2 cm in length. Each segment was mounted in **a** 10 mL organ bath containing Tyrode’s physiological salt solution (composition: NaCl 136.9 mM, KCl

2.68 mM, $MgCl_2$ 1.05 mM, $NaHCO_3$ 11.9 mM, NaH_2PO_4 0.42 mM, glucose 5.55 mM, and $CaCl_2$ 1.8 mM). The solution was continuously aerated with Carbogen gas (95% O_2 and 5% CO_2) and maintained at 37°C to simulate physiological conditions (Jeremy *et al.*, 2001).

The lower end of the tissue was securely tied to a tissue holder using silk thread, while the upper end was connected to a Ugo Basile isometric force-displacement transducer (Type 82145**)** attached to a Ugo Basile data capsule for the recording of contractile responses. The preparations were allowed to equilibrate for 30 minutes under a resting tension of 1.0 g, with the Tyrode's solution replaced every 10 minutes during the equilibration period to maintain tissue viability (Thompson *et al.*, 2001).

Following equilibration, the baseline contractile activity of the corpus cavernosum muscle was recorded. Thereafter, cumulative concentrations of *Alstonia boonei* extract (ranging from 1.5625 to 50 mg/mL) were administered to the organ bath. The resulting changes in muscle tension were recorded and analyzed to determine the relaxation effects of the extract on the corpus cavernosum muscle.

2.2.3 Effect of *Alstonia boonei* Extract on Spontaneous Contractions of Corpus Cavernosum Muscle

The isolated corpus cavernosum muscle segments were allowed to exhibit spontaneous contractions in Tyrode's physiological salt solution for a period of 30 minutes to establish a stable baseline. Once stability was achieved, cumulative concentrations of *Alstonia boonei* extract (1.56, 3.125, 6.25, 12.5, 25, and 50 mg/mL) were administered in corresponding volumes of 25 µL, 62.5 µL, 125 µL, 250 µL, and 500 µL, respectively. Each concentration was allowed a

contact period of four minutes to observe its effect on the spontaneous contractile activity of the corpus cavernosum muscle.

The procedure was repeated three times for reproducibility, and the mean response was calculated. The changes in muscle tension were analyzed to evaluate the relaxant or contractile effects of *Alstonia boonei* extract on the corpus cavernosum smooth muscle.

2.3 Effect of *Alstonia boonei* Extract on Potassium Chloride (KCl)-Induced Contractions

Potassium chloride (KCI) was used to induce sustained contractions, to evaluate the effect of *Alstonia boonei* extract on depolarization-induced contraction in the corpus cavernosum muscle. High potassium (80 mM KCI) was added into the organ bath to induce membrane depolarization, leading to sustained smooth muscle contraction through the activation of voltage-gated calcium channels

Once a stable contraction was achieved, cumulative concentrations of *Alstonia boonei* extract (1.5625, 3.125, 6.25, 12.5, 25, and 50 mg/mL) were administered in corresponding volumes of 25–500 µL, with each dose allowed a contact period of four minutes to observe its effect on the pre-contracted tissue.

The procedure was repeated three times, and the inhibitory effect of the extract on KCl-induced contractions was measured. The observed reduction in muscle tension following extract administration indicated the relaxant effect of *Alstonia boonei* on KCl-induced contractions in the corpus cavernosum, suggesting a possible mechanism involving calcium channel modulation**.**

2.4 Significance of these Tests

Spontaneous contractions: These were used to evaluate the direct influence of *Alstonia boonei* on the intrinsic tone of the corpus cavernosum smooth muscle in the absence of any external stimulation.

KCl-induced contractions: These experiments assessed whether the extract's relaxant effect involved voltage-gated calcium channels blockade, a well-recognized mechanism underlying smooth muscle relaxation and enhancement of erectile function.

CHAPTER THREE

RESULTS

Statement of Results for the Aphrodisiac Experiment (Genital Sniffing Behavior)

As shown in Figure, genital sniffing behavior varied across the experimental groups. The response rates, arranged from the least to the highest, are as follows

Control (Distilled water)

Dose 1 (62.5mg/kg)

Dose 3 (250mg/kg)

Standard drug (Sildenafil, 5mg/kg):

Dose 2 (125mg/kg)

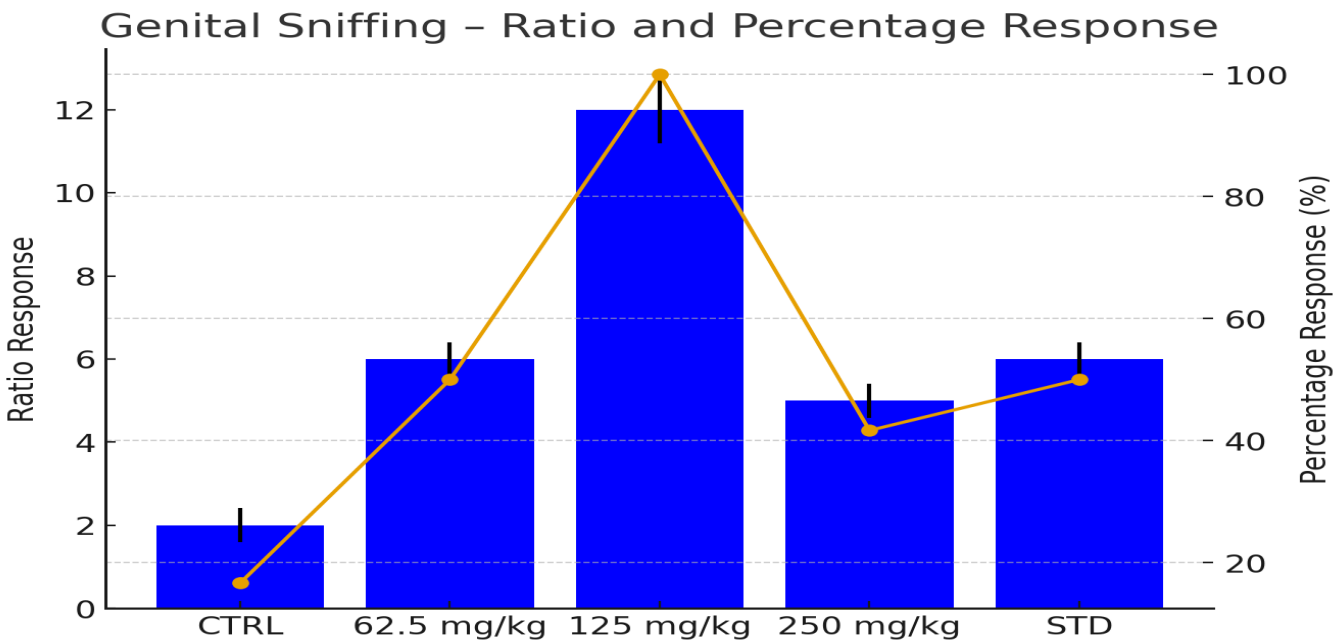

Fig 3.1; Graphical representation of the effect of *Alstonia boonei* extract on genital sniffing behavior in Wistar rats across different doses, including the standard and control group.

Statement of Results for the Aphrodisiac Experiment (Genital Groaning Behavior) as shown in **Figure 1.5** genital groaning behavior varied across the experimental groups. The response rates, arranged from least to highest, are as follows:

Standard drug (Sildenafil, 5 mg/kg):

Dose 2 (125 mg/kg)

Dose 3 (250mg/kg)

Dose 1 (62.55mg/kg)

Control group

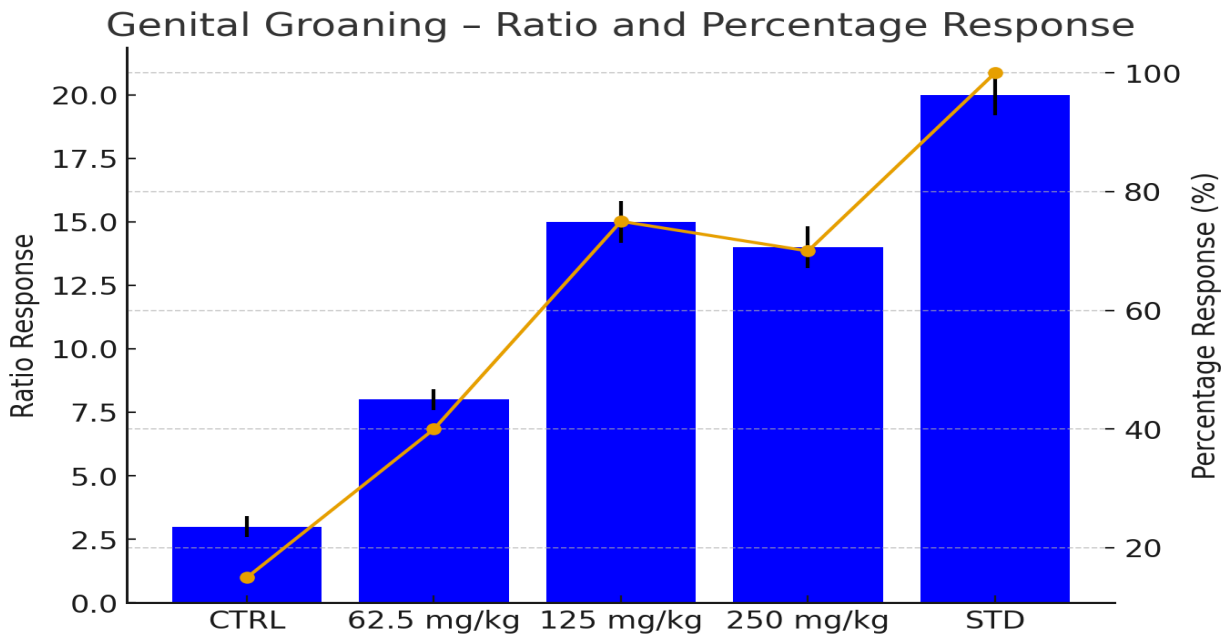

Fig 3.2; Graphical representation of the effect of *Alstonia boonei* extract on genital groaning behavior in Wistar rats across different doses, including the standard and control group.

Statement of Result for the Aphrodisiac Experiment (Mounting Frequency)

As presented in **Figure 1.6**, mounting frequency increased progressively across the treatment groups compared to the control. The response rates, arranged from the least to the highest, are as follows:

Control group:

Dose 1 (62.5 mg/kg):

Dose 2 (125 mg/kg):

Dose 3 (250 mg/kg):

Standard drug (Sildenafil, 5 mg/kg):

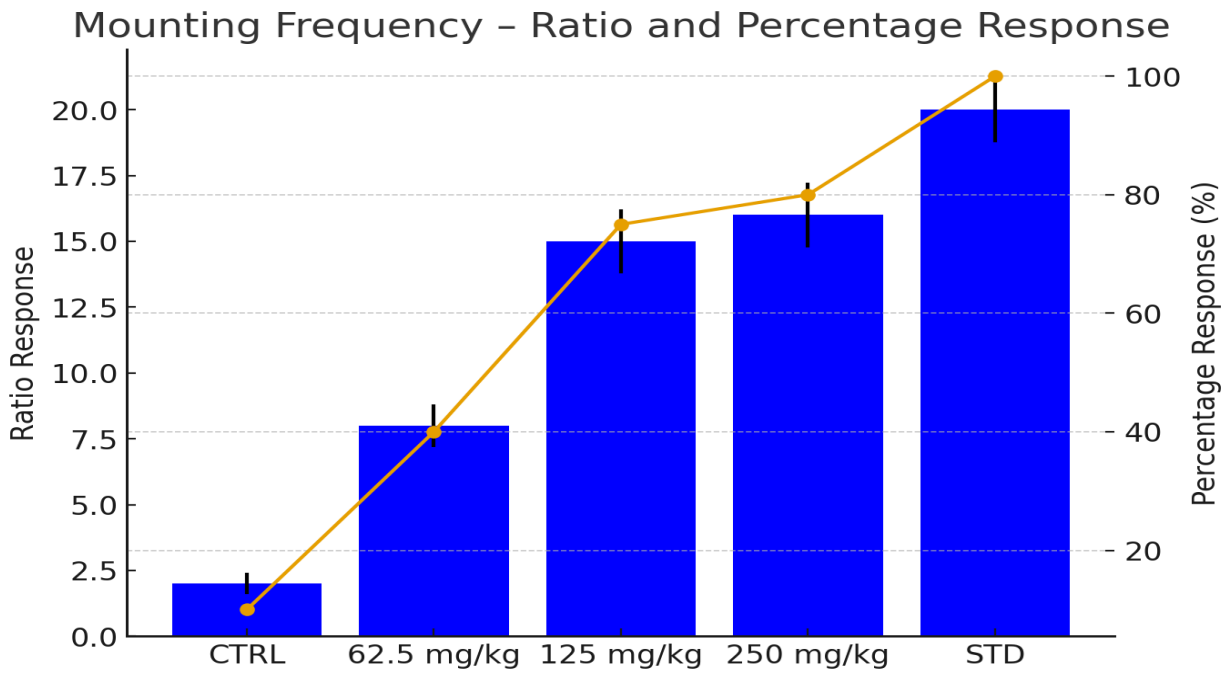

Fig 3.3; Graphical representation of the effect of *Alstonia bonnei* extract on mounting frequency behavior in Wistar rats across different doses, including the standard and control group.

Statement of Results for the Aphrodisiac Experiment (Intromission Frequency)

The intromission frequency followed a similar trend to mounting frequency. As shown in Figure, the response increased across doses, with the order of response being:

Control group:

Dose 1 (62.5 mg/kg):

Dose 2 (125 mg/kg):

Dose 3 (250 mg/kg):

Standard drug (Sildenafil, 5 mg/kg):

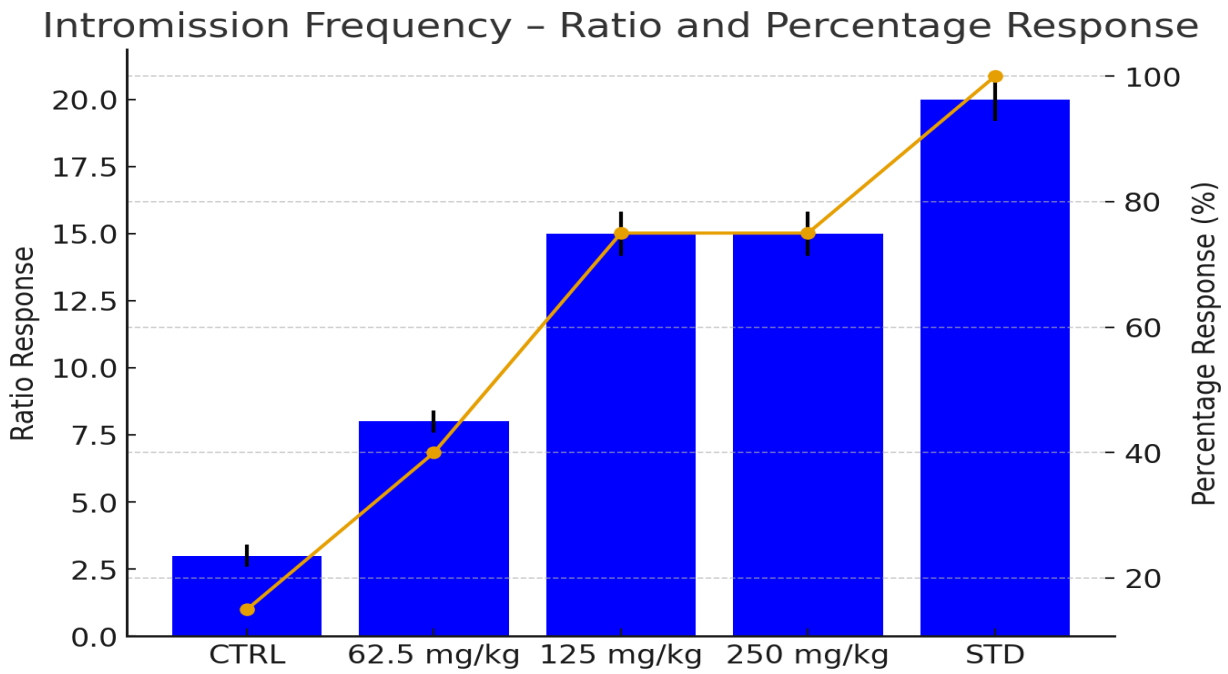

Fig 3.4; Graphical representation of the effect of *Alstonia bonnei* extract on intromission frequency behavior in Wistar rats across different doses, including the standard and control group.

Statement of Results for the Aphrodisiac Experiment (Ejaculation Frequency)

As illustrated in Figure, the ejaculation frequency also increased slightly in the treated groups compared to the control. The order of response was:

Control group:

Dose 1 (62.5 mg/kg):

Dose 3 (250 mg/kg):

Dose 2 (125 mg/kg):

Standard drug (Sildenafil, 5 mg/kg):

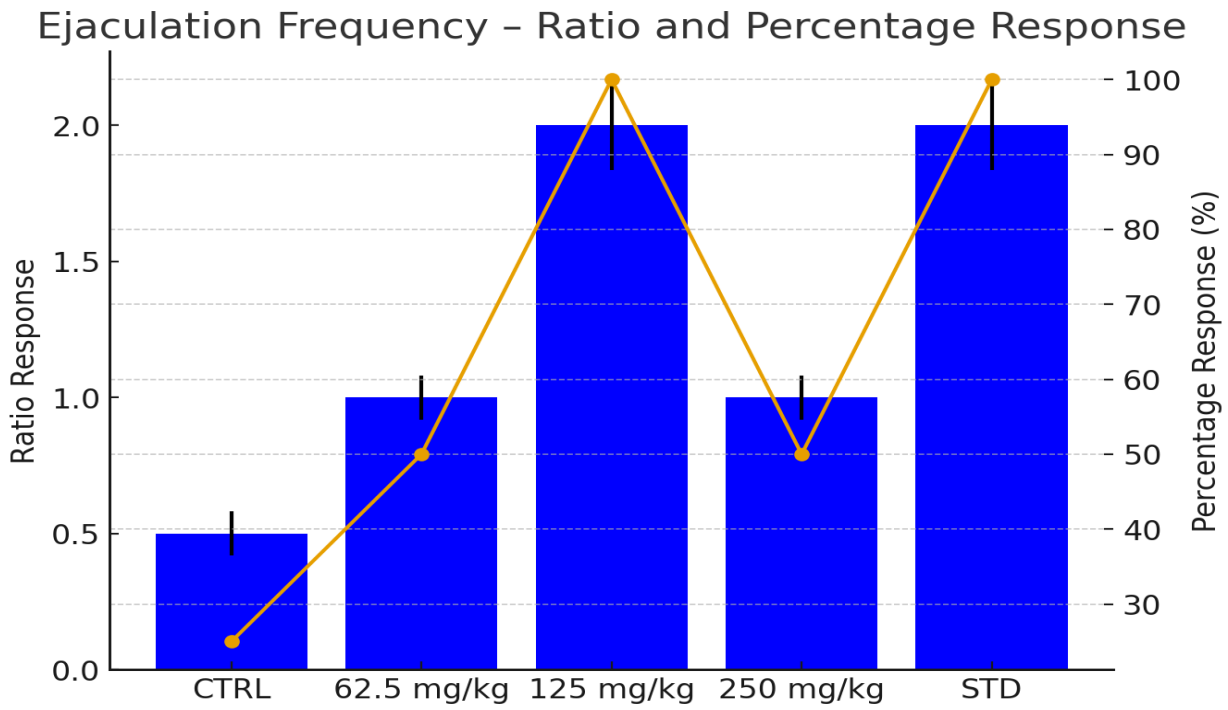

Fig 3.5 Graphical representation of the effect of *Alstonia bonnei* extract on Ejaculation frequency behavior in Wistar rats across different doses, including the standard and control group.

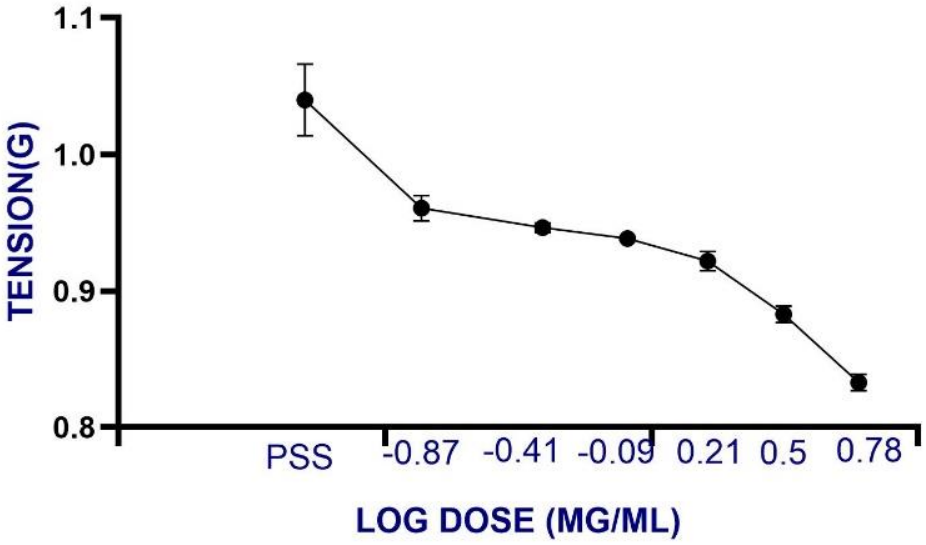

Fig 3.6; Graphical representation of the log Dose effect of *Alstonia bonnei* extract on the intrinsic contraction of the corpus carvenosum muscle

This study reveals a distinct concentration-dependent relaxation of the corpus cavernosum muscle in Wistar rats following cumulative administration of *Alstonia boonei* extract. As shown in Figure, increasing the logarithmic doses of the extract produced a progressive decline in muscle tension, reflecting enhanced smooth muscle relaxation.

At lower concentrations, the extract elicited a mild relaxant response, which became progressively stronger with higher doses, culminating in a significant relaxation effect at the maximum concentration.

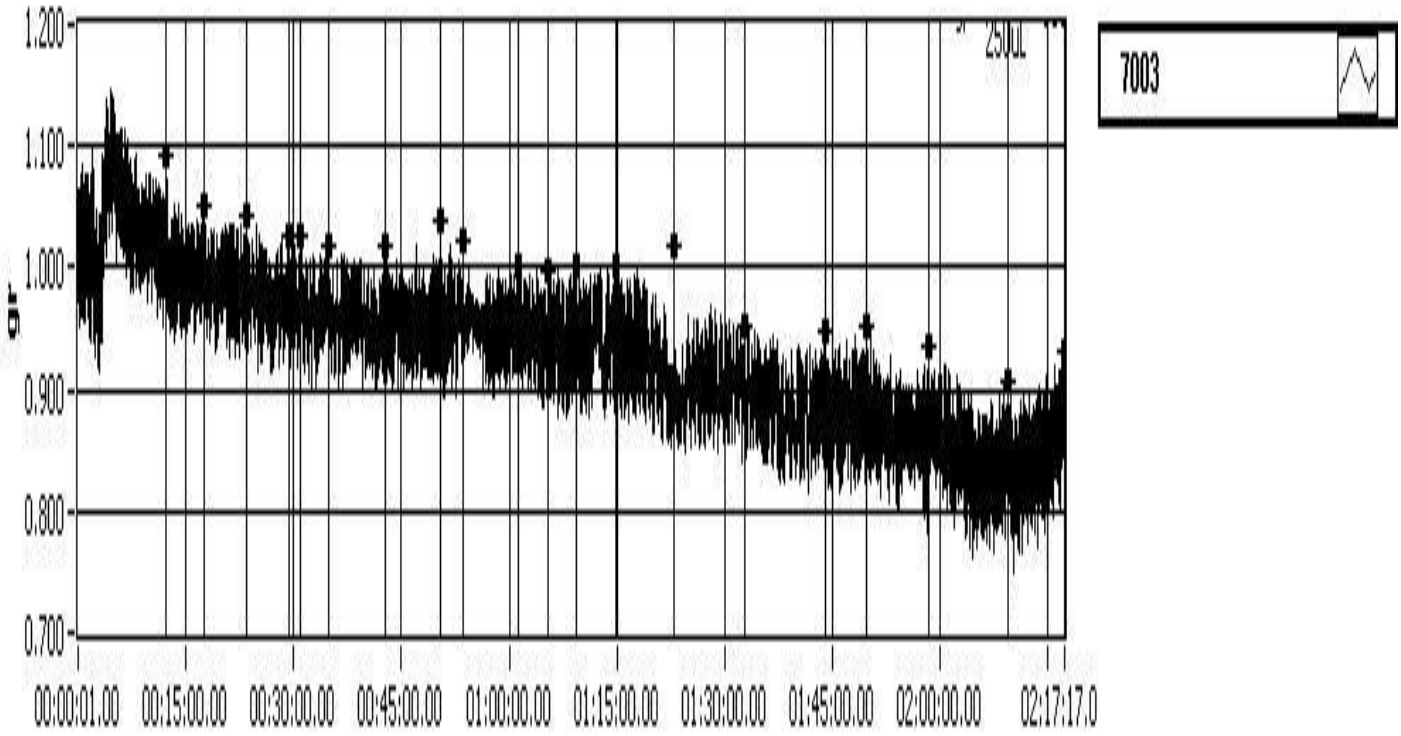

Figure 3.7; Representative chart of the effect of *Alstonia boonei* extract on spontaneous contraction of an isolated corpus cavernosum muscle

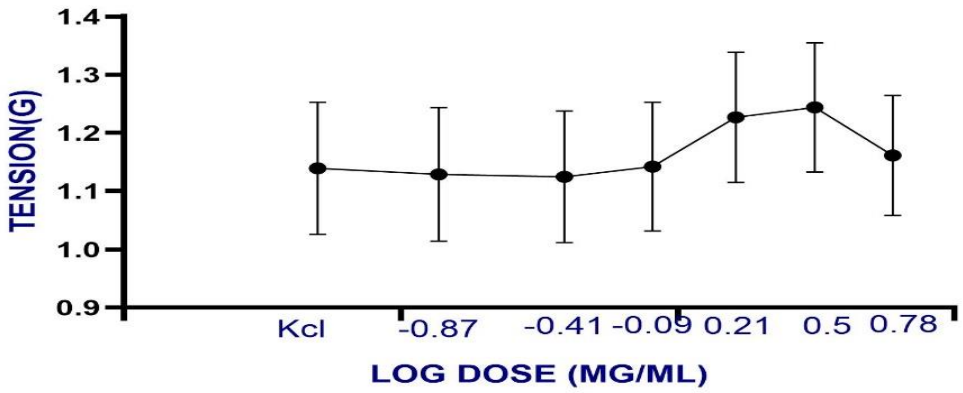

Fig 3.8; Graphical representation of the log Dose effect of *Alstonia bonnei* extract on the KCl-induced contraction of the corpus carvenosum muscle.

Administration of 80mM potassium chloride (KCl) produced a rapid and sustained contraction of the corpus cavernosum muscle, confirming effective membrane depolarization. With the administration of cumulative concentrations of Alstonia boonei extract (1.5625-50mg/ml), a dose-dependent reduction in muscle contraction was observed, with notable relaxation observed only at the highest concentration (50mg/ml). This suggests the extract inhibits KCl-induced depolarization in a dose-dependent manner, possibly through a competitive inhibition of voltage-gated calcium channels responsible for calcium influx and smooth muscle contraction.

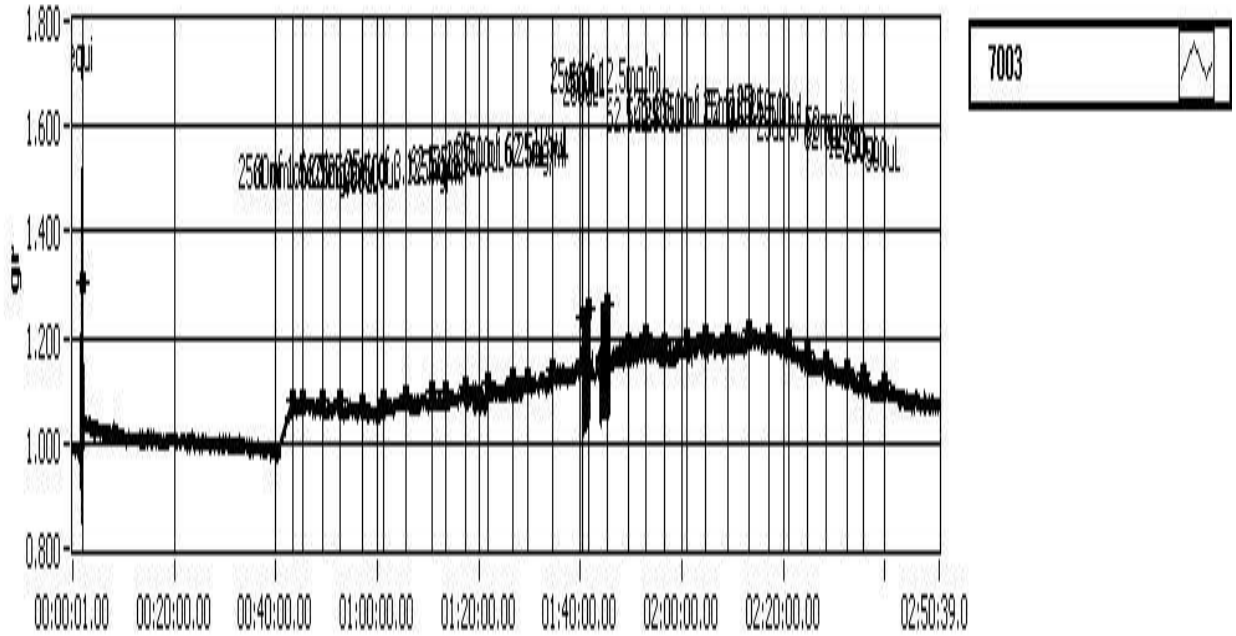

Fig 3.9; A representative chart of the effects of Alstonia boonei extract on KCl-induced contraction of an isolated corpus cavernosum mucle

CHAPTER FOUR

DISCUSSION

The study investigated the aphrodisiac potential of *Alstonia boonei* by assessing a range of sexual behavior parameters and its relaxation effects on the corpus cavernosum muscle in male Wistar rats. The results of the aphrodisiac behavioral experiments revealed that the extract produced varying but generally enhanced sexual motivation and performance across the measured parameters, genital sniffing, groaning, mounting, intromission, and ejaculation frequencies when compared to the control group. Additionally, the extract produced a dose-dependent relaxation of the corpus cavernosum muscle, indicating that its effect may be mediated through the nitric oxide (NO) signaling pathway and dopaminergic stimulation mechanism.

Genital Sniffing

The increase in genital sniffing behavior reflects enhanced sexual curiosity and motivation toward the female rat, which represents an early indicator of libido enhancement. This behavioral change implies that the extract may stimulate the central dopaminergic pathways associated with sexual arousal, consistent with reports that dopaminergic activation increases exploratory and courtship behaviors in male rats (Musicki and Burnett, 2006; Shamloul, 2010).

Genital Groaning

The dose-dependent increase in genital groaning observed among the extract-treated rats provides further evidence of the aphrodisiac potential of *Alstonia boonei*. This form of vocalization is closely linked with heightened sexual excitement and increased readiness to mate. Such behavioral expression is likely mediated through neuroendocrine mechanisms involving

key modulators such as dopamine, testosterone, and nitric oxide (NO), all of which play pivotal roles in stimulating sexual arousal and performance (Andersson, 2011; Shamloul, 2010).

Mounting Frequency

The increase in mounting frequency observed among extract-treated rats indicates enhanced sexual motivation and initiation of copulatory behavior. Mounting represents the male's attempt to mate and serves as an important indicator of libido and arousal. The dose-dependent increase in this parameter suggests that *Alstonia boonei* may stimulate central nervous system pathway, particularly those involving dopamine and testosterone, that are known to influence sexual desire and performance (Musicki and Burnett, 2006; Shamloul, 2010).

Intromission Frequency

In contrast, the rise in intromission frequency reflects improved penile erection and successful vaginal penetration, signifying enhanced erectile function and sexual performance. This effect may be attributed to the extract's ability to promote smooth muscle relaxation within the corpus cavernosum, possibly through modulation of calcium influx or activation of the nitric oxide-cGMP signaling pathway. Such mechanisms are consistent with the physiological processes underlying penile erection (Dean and Lue, 2005; Adebayo *et al*., 2019).

Ejaculation Frequency

The significant increase in ejaculation frequency at higher doses of *Alstonia boonei* reinforces its aphrodisiac potential. This effect may involve increased androgenic activity, neurohormonal modulation, and improved smooth muscle relaxation, facilitating better erectile function and endurance. Comparable findings have been observed with combined extracts of Lepidium

meyenii and Allium tuberosum, which produced synergistic effects on male sexual function, including increased ejaculation frequency (Shin *et al*., 2010).

Corpus Cavernosum Muscle Relaxation

The dose-dependent relaxation of the corpus cavernosum muscle observed with *Alstonia boonei* extract implies that its action may involve the NO–cGMP signaling pathway, a fundamental mechanism responsible for smooth muscle relaxation and penile erection. This pathway is similar to the pharmacodynamic action of phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil, which are clinically employed in the management of erectile dysfunction. The observed relaxant effect could also be linked to the phytochemical constituents of *Alstonia boonei,* notably alkaloids, flavonoids, and saponins that are recognized for their ability to promote vascular smooth muscle relaxation and enhance endothelial function (Shamloul, 2010).

KCl-Induced Contraction of the Corpus Cavernosum Muscle

The gradual reduction in tension observed following the cumulative administration of *Alstonia boonei* extract after KCl-induced contraction suggests a competitive blockade of voltage-gated calcium channels in the corpus cavernosum smooth muscle. Since KCl depolarization causes the opening of L-type calcium channels and a rapid influx of extracellular calcium, the delayed onset of relaxation suggests that the extract competes with calcium entry sites in a concentration-dependent manner. At lower concentrations, the extract was insufficient to counteract the strong depolarizing effect of KCl, but at higher concentrations (notably 50mg/ml), the extract overcame this competition, producing a marked relaxation. This pattern aligns with a reversible, competitive antagonism at the calcium channels, where inhibition depends on relative concentrations of the extract and extracellular calcium. Similar competitive calcium blockage has

been reported for plant-derived alkaloid and flavonoids (Adebayo *et al*., 2019 Oluwole *et al*., 2019).

CHAPTER 5

CONCLUSION, RECOMMENDATION AND CONTRIBUTION

5.1: CONCLUSION

The present study evaluated the aphrodisiac and relaxant effects of *Alstonia boonei* extract using both in vivo behavioral models and in vitro corpus cavernosum assays in male Wistar rats. The findings revealed a clear dose-dependent enhancement of sexual activity, as shown by increased genital sniffing, genital groaning, mounting, intromission, and ejaculation frequencies. These behavioral responses suggest that the extract positively influences both sexual motivation and performance through central neurohormonal stimulation and peripheral mechanisms that enhance erectile function.

5.2: RECOMMENDATION

Additionally, Complementary in vitro experiments demonstrated that *Alstonia boonei* extract induced concentration-dependent relaxation of the corpus cavernosum muscle and inhibited KCl-induced depolarization, indicating possible modulation or competitive blockade of voltage-gated calcium channels. Together, these findings suggest that *Alstonia boonei* exerts its aphrodisiac and erectile-enhancing effects through both central and peripheral pathways, involving neuroendocrine stimulation, calcium channel modulation, and vascular smooth muscle relaxation.

5.3: CONTRIBUTION TO KNOWLEDGE

Overall, this study provides scientific evidence supporting the ethnomedicinal use of *Alstonia boonei* in the management of male sexual dysfunction. The dual behavioral and physiological

outcomes highlight its potential as a natural alternative or adjunct to conventional therapies for erectile dysfunction.

Although this study provides strong evidence supporting the aphrodisiac and smooth muscle relaxant properties of *Alstonia boonei*, it is recommended that further research is necessary to elucidate its precise pharmacological mechanism, isolate, characterize, and identify the specific bioactive constituents, and determine its long-term safety and efficacy in humans.

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