

**HISTOPATHOLOGICAL EFFECTS OF THE APHRODISIAC SILDENAFIL (VIAGRA)
ON TESTICULAR AND CARDIAC TISSUES IN ADULT ALBINO RATS**

BY

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BMS2001195



**DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY.**

OCTOBER, 2025

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**THIS PROJECT IS SUBMITTED TO:
THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE
DEGREE**

SUPERVISOR:

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OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **OMORODION ADEGHEOSA MICHAEL (MR)** with matriculation number: **BMS2001195** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DATE

PROF. VICTOR .O. EKUNDINA
(External examiner)

DATE

DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parent **MR AND MRS OMORODION** and my family member for their constant support throughout the process.

ACKNOWLEDGEMENTS

I give thanks to God Almighty for His grace upon my life and for seeing me through this project work. My appreciation goes to my supervisor Dr. N.T. OMORODION for his concern, constructive and supportive ideas which has aided this work. A special thanks goes to the Head of Department, Medical Laboratory Science, Dr (Mrs) Zainab Omoruyi for her support in ensuring smooth conclusion this project work and also the entire staff of the department for investing so much in my academic development. My appreciation also goes to my lecturers in histopathology Dr. Mrs. B. E. Ogeyemhe and Dr. E. B. Odigie for their constant advice and encouragement. God bless them abundantly. My deepest gratitude goes to my family and loved ones. My Dad (ENG Amos Omorodion) and My Mum (Mrs Maureen omorodion) For all the prayers, advices and all round support. I am grateful to my uncle and aunt(MR and MRS EKHOVBIYE IGBINOVIA) for her support and unwinding encouragement towards the success of this project and my Academics in General, and also for their financial assistance. To my friends for always having my back throughout my stay in the university, God bless you all richly. Lastly, a big shout out goes to my friends and colleagues in Medical laboratory science, you made my process through this degree one to cherish and remember for the rest of my life, God bless.

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ABSTRACT

In an effort to enhance sexual performance, some individuals resort to using aphrodisiac substances such as sildenafil (Viagra). This study was conducted to assess the effects of sildenafil on the heart and testicular tissues of adult albino rats. A total of 18 male albino rats, each weighing between 200g and 220g, were obtained from the animal house at Anatomy Department. The rats were randomly assigned into three groups (A, B, and C), with six rats per group. Group A served as the control and received only standard feed and water. Group B rats were administered sildenafil orally at a dose of 5 mg/kg body weight, dissolved in saline, daily for four consecutive weeks. Group C rats received a higher dose of 10 mg/kg body weight for the same duration, followed by an additional four-week withdrawal period without treatment. Body weights were recorded at the beginning of the experiment (week 0) and on the final day prior to sacrifice. At the end of the experimental period, all animals were sacrificed, and tissue samples were processed for histological analysis. Serial tissue sections were cut at 5 μ m thickness using a microtome and stained with hematoxylin and eosin. Selected tissue sections were documented through photomicrography. Results indicated that the 10 mg/kg dose of sildenafil led to a notable increase in monocyte and granulocyte counts, while red blood cell levels decreased across the treated groups. Weight measurements revealed only minor differences between the control and sildenafil-treated groups after the four-week period. Histological examination of testicular tissues from the 10 mg/kg group showed mild necrosis in cardiac muscle fibers, seminiferous tubules, and interstitial tissues. Additional findings included vascular congestion, hypertrophy of Leydig cells, and degeneration of spermatogonial cells. These findings highlight the need for further investigation into the molecular mechanisms triggered by prolonged exposure to PDE5 inhibitors. Such research may guide the safer and more effective therapeutic use of aphrodisiacs, particularly when administered at lower doses and for shorter durations.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

The rise in human life expectancy has created a growing demand for substances that can improve the quality of this prolonged lifespan (Abdullahi & Tukur, 2023). Among these needs is the desire to enhance sexual arousal, pleasure, and potency—a pursuit that dates back to ancient times. Cultures around the world have traditionally used various substances, often passed down through generations, as aphrodisiacs (Dabhadkar *et al.*, 2023; McNay, 2020). These agents are widely regarded as crucial for initiating sexual excitement, maintaining relationships, and ensuring sexual satisfaction (Byers, 2019). Rather than waning, interest in aphrodisiacs has grown over time.

Sexual responses in both men and women are regulated by a complex interplay of excitatory and inhibitory signals from the autonomic nervous system, involving the genital region and central nervous system (Wamoyi *et al.*, 2019). Excitatory signals, often triggered by visual, mental, or physical stimuli, travel via the parasympathetic pathway, while inhibitory ones follow the sympathetic route (Skopek *et al.*, 2022). In men, sexual arousal results from the release of pro-erectile neurotransmitters like nitric oxide and acetylcholine, which relax penile smooth muscles, increasing blood flow and leading to erection (Walster *et al.*, 2018). Aphrodisiacs function by enhancing these pathways to improve either sexual arousal or erectile performance.

An aphrodisiac is any substance that enhances libido, sexual performance, or pleasure (Van *et al.*, 2020). The term originates from “Aphrodite,” the Greek goddess of love. These substances may

be plant-based, animal-derived, or mineral in origin. Based on their mechanism of action, aphrodisiacs are grouped into three categories: those that enhance desire (libido), those that boost potency (erectile function), and those that increase sexual pleasure (Goredema, 2020).

Throughout history, both men and women have pursued sexual fulfillment through various means, including the use of performance-enhancing substances (Bello, 2017). Many of these products aim to treat erectile dysfunction or improve sexual performance. Sexual health is a critical aspect of human identity and emotional well-being. Satisfaction in this area significantly contributes to individual happiness and relationship stability. A healthy sex life supports emotional intimacy, while dysfunction may lead to dissatisfaction, emotional distress, and strained relationships (Aidoo, 2018). Sexual dysfunction is often linked to underlying health conditions such as hypertension, diabetes, or heart disease, which can contribute to anxiety, depression, and lowered self-esteem in affected individuals (Al-Amin *et al.*, 2021; Duyilemi *et al.*, 2018). Healthy intimate relationships heavily depend on sexual compatibility, and when sexual function declines, relationships may deteriorate (Mbah *et al.*, 2015). Studies indicate that approximately 15–41% of men experience sexual dysfunction. In countries such as Nigeria, Egypt, and Pakistan, estimates show that 40–60% of men face some form of sexual dysfunction (Balasubramani *et al.*, 2021). Despite the prevalence of these issues, many men avoid seeking medical advice, often due to cultural taboos or beliefs about discussing sexual health (Akanmode, 2015). As a result, they resort to self-medication using aphrodisiac substances. Common forms of dysfunction in men include premature ejaculation, erectile dysfunction, and hypoactive sexual desire disorder (HSDD), with premature ejaculation reported as the most prevalent globally, affecting 25–40% of men across all age groups (Bancroft, 2020).

To address these concerns, some men turn to aphrodisiacs such as sildenafil (Viagra) to enhance their sexual performance (Shamloul, 2016). This often stems from discomfort with medical consultations and a preference for non-prescription interventions. While these substances are believed to be effective, some may have harmful effects on internal organs, including the testicles, heart, kidneys, brain, and liver. Sildenafil (Viagra), a well-known aphrodisiac, is primarily prescribed for erectile dysfunction and pulmonary arterial hypertension (Smith *et al.*, 2022). It works as a phosphodiesterase-5 (PDE5) inhibitor and was the first of its kind to be FDA-approved. It has also been used off-label for conditions like Raynaud's phenomenon and female sexual arousal disorder. While it is approved for adult use, it is not recommended for children. Although effective, combining sildenafil with other treatments like bosentan has shown limited additional benefits.

1.2 Statement of Problem

The global rise in sexual dysfunction has led to increased use of aphrodisiacs, with projections indicating that over 320 million people may be affected by 2025 (Balasubramani *et al.*, 2021). Drugs originally intended for other medical purposes, such as Viagra, are now commonly misused, particularly among young men, manual laborers, commercial drivers, and students. This trend is particularly concerning in Africa, Asia, and the Middle East. Abuse of such substances has been linked to numerous side effects, including anorexia, drowsiness, weakness, weight changes, testicular damage, and inflammation of heart tissues.

1.3 Justification for the Study

The pursuit of sexual fulfillment is a universal aspect of human life. However, many men suffering from sexual dysfunction avoid medical treatment, opting instead for over-the-counter solutions like Viagra. The misuse of such substances is now a global concern. Although some research has been conducted, the field is still developing, and many studies lack sufficient sample sizes. The histological effects of Viagra, particularly on the testes and heart, are underexplored, making this study necessary.

1.4 Significance of the Study

Aphrodisiac substances, including Viagra, have various effects on male sexual behavior and health. Usage rates vary globally but are notably high in many African communities. Given the widespread use of these substances among Nigerian men, this study aims to investigate their haematological and histological effects, particularly on the heart and testes.

1.5 Aim of the Study

To evaluate the effects of the aphrodisiac drug sildenafil (Viagra) on the testes and heart tissues of adult albino rats.

1.6 Objectives of the Study

1. To assess the impact of sildenafil (Viagra) on body weight.
2. To determine its effect on hematocrit levels.
3. To evaluate histological changes in the heart and testes due to sildenafil use.
4. What is the effect of the aphrodisiac Sildenafil (Viagra) on the body weight of experimental animals?
5. How does the administration of Sildenafil (Viagra) influence the hematocrit value in experimental subjects?
6. What histological changes occur in the heart and testes following the administration of Sildenafil (Viagra)?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The testis

The testes are the primary male sex glands, serving both endocrine and exocrine functions. These oval, bean-shaped reproductive structures are located within the scrotum and are separated by the scrotal septum. Each testis measures approximately 3 to 5 cm in length and 2 to 3 cm in width (Krings, 2016). When palpated through the scrotal sac, the testes feel smooth and soft.

The spermatic cord suspends the superior aspect of each testis, while the inferior end is attached to the scrotum by the scrotal ligament, a remnant of the gubernaculum. Typically, the left testis lies slightly lower than the right. The testes are enclosed by a double-layered tunica vaginalis, which covers all surfaces except the posterior and superior borders where the epididymis and spermatic cord are attached (Mason, 2022). The visceral, or inner, layer of the tunica vaginalis closely invests the testes, epididymis, and vas deferens. On the posterolateral surface, there is a small recess between the testis and the body of the epididymis, known as the sinus of the epididymis.

Beneath the tunica vaginalis lies the tunica albuginea, a tough fibrous capsule that provides structural support to the testes (Schwartz and Young, 2019). The epididymis is a narrow, highly coiled, and elongated structure located along the posterior border of each testis. When uncoiled, it measures approximately 20 feet in length. It consists of three main regions: the head (caput), body (corpus), and tail (cauda). The head lies at the superior pole of the testis and receives sperm from the efferent ducts, allowing their passage into the distal portions of the epididymis. Due to its considerable length, the epididymal duct provides ample space for sperm maturation and storage (Yoo *et al.*, 2019).

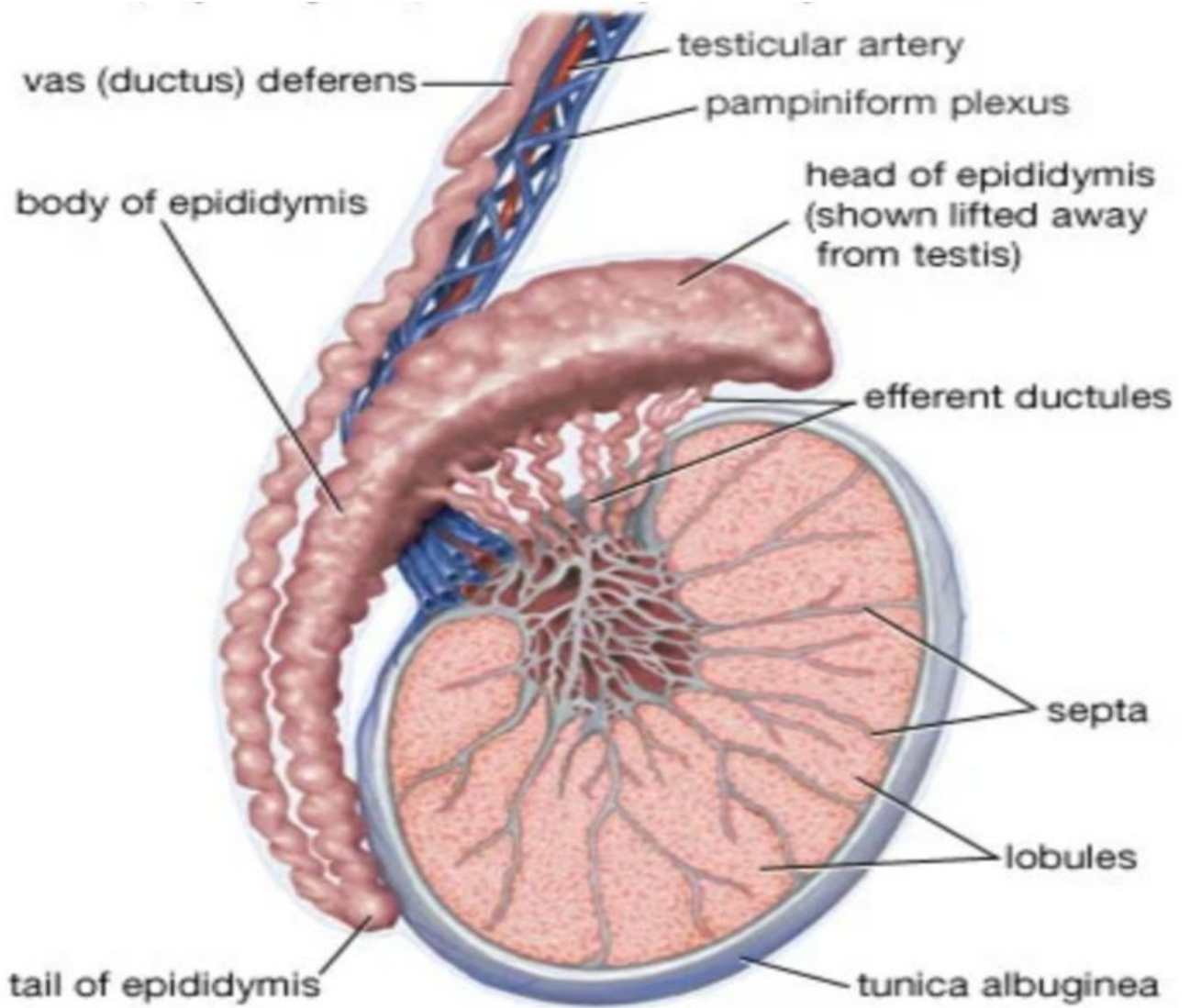


Figure 2.1: a cross sectional section of the the testis (Smith *et al.*, 2022)

2.1.1 Anatomy and Physiology of the testes

2.1.2 Structure and Function

The testis is the male reproductive organ responsible for two main functions: sperm production and the secretion of androgens, predominantly testosterone. Testosterone levels are regulated by luteinizing hormone (LH) released from the anterior pituitary, while sperm production is controlled by follicle-stimulating hormone (FSH) (Idung *et al.*, 2019).

2.1.3 Embryology

Testicular development begins in the retroperitoneal region from an undifferentiated gonadal ridge. The presence of the SRY gene on the Y chromosome directs this primitive structure to differentiate into testes. In the absence of this gene, as in females, the gonad develops into ovaries (Edelstein *et al.*, 2021). As fetal development advances, the testes begin secreting testosterone, which promotes the formation of male internal and external genitalia.

The tunica albuginea, a connective tissue layer, extends inward to separate and support the seminiferous tubules within the testis. Between the eighth and tenth weeks of gestation, Sertoli cells start producing Müllerian-inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), leading to regression of the Müllerian ducts (Govindasamy *et al.*, 2017). In adult males, remnants of these ducts persist as the appendix testis and the prostatic utricle.

During the final trimester of pregnancy, the testes descend from the abdominal cavity into the scrotum. This migration occurs through the peritoneum, abdominal wall, and inguinal canal. The descent is guided by the processus vaginalis, an extension of the peritoneum that later regresses to form the tunica vaginalis surrounding each testis. Failure of the processus vaginalis to close properly can result in conditions such as a communicating hydrocele or an inguinal hernia (Etuk *et al.*, 2017).

2.1.4 Blood Supply and Lymphatics

The testes receive their primary arterial blood supply from the testicular arteries, which arise from the abdominal aorta just below the renal arteries. These vessels travel retroperitoneally, cross the ureters, and enter the spermatic cord through the inguinal canal (Etuk *et al.*, 2017). Additional blood supply is provided by the artery to the vas deferens and the cremasteric artery. Venous drainage occurs via the pampiniform plexus, a network of veins that coalesce to form the testicular veins. The right testicular vein empties directly into the inferior vena cava, whereas the left testicular vein drains into the left renal vein. Lymphatic drainage of the testes follows the course of the testicular arteries and terminates in the preaortic lymph nodes (Etuk *et al.*, 2017).

2.1.5 Nerves

The testes receive both sympathetic and parasympathetic nerve innervation, which contributes to the regulation of testicular function and the cremasteric reflex.

2.1.6

Muscles

During their descent into the scrotum, the testes become enveloped by several muscular and fascial layers, including the internal spermatic fascia, the cremasteric muscle and fascia, and the external spermatic fascia. The extent of testicular descent is influenced by temperature and is primarily controlled by the cremaster muscle (Del Mar Sánchez-Fuentes *et al.*, 2022).

2.1.7 Physiological Variants

Within the testis, there are two embryonic remnants that have no known functional significance. The first is the appendix testis, a small, pear-shaped vestige of the paramesonephric (Müllerian) duct, found in approximately 2% of cases near the head of the epididymis. The second is the appendix of the epididymis, derived from the mesonephric duct, which is present in about 25%

of testes. Additionally, in roughly 7% of men, the epididymis is positioned on the anterior surface of the testis (Del Mar Sánchez-Fuentes *et al.*, 2022).

2.1.8 Surgical Considerations

Early identification of cryptorchidism is vital to preserving fertility and minimizing the risk of testicular cancer (Abubakar *et al.*, 2017). If the testis cannot be surgically positioned within the scrotum for regular monitoring, it should be removed. The unaffected testis is also at increased risk for cancer, making regular self-examination important. Accumulation of fluid between the tunica vaginalis layers may result in hydrocele, which can be caused by inflammation, trauma, or congenital issues. Testicular torsion in young males is a medical emergency; blood flow must be restored within six hours to prevent testicular loss. The contralateral testis is often surgically fixed during this procedure to prevent recurrence. Undescended testes often coexist with inguinal hernias, whereas retractile testicles typically do not (Del Mar Sánchez-Fuentes *et al.*, 2022).

2.1.9 Clinical Significance

Cryptorchidism not only causes infertility but also raises the risk of malignancy. If not surgically corrected, affected testes require vigilant observation. Orchidopexy, the surgical repositioning of undescended testes, is the standard treatment and should be done before the child turns one to reduce cancer risk (Abubakar *et al.*, 2017). Hydroceles, or fluid accumulation around the testis, may be caused by infection, trauma, or incomplete closure of the processus vaginalis.

2.2 Testosterone

Testosterone is the primary male sex hormone responsible for sexual differentiation, the development of male characteristics, sperm production, and fertility. Its influence begins during fetal life. In the first six weeks of development, the reproductive tissues of males and females are identical. Around the seventh week, the SRY gene on the Y chromosome triggers the

differentiation of the gonads into testes. Sertoli cells within the developing testes secrete Müllerian-inhibiting substance (MIS), which causes the regression of female reproductive ducts. Meanwhile, Leydig and endothelial cells produce testosterone, stimulating the transformation of the Wolffian ducts into the male internal reproductive structures.

In peripheral tissues, testosterone is converted into dihydrotestosterone (DHT), which drives the formation of the prostate gland and external genitalia. During the third trimester, testosterone also facilitates the descent of the testes into the scrotum. In the absence of the SRY gene, the gonads develop into ovaries, MIS is not produced, and the female reproductive system forms instead.

2.2.1 Function

Testosterone plays a crucial role in primary sexual development, including testicular descent, sperm production, genital formation, and the regulation of libido. By the seventh month of gestation, testosterone levels are sufficient to initiate testicular descent. If this process has not occurred by four to six months after birth, testosterone therapy may be administered to encourage descent.

The hormone is also essential for the development of secondary sexual characteristics such as body hair distribution, deepening of the voice, increased muscle mass, and bone growth. During puberty, testosterone stimulates bone elongation and later contributes to the closure of the epiphyseal growth plates. It also enhances red blood cell production, accounting for the higher hematocrit levels observed in males.

With advancing age, testosterone levels gradually decline, resulting in decreased libido, muscle mass, bone density, and red blood cell production, along with an increase in body fat accumulation.

2.2.2 Mechanism

During puberty, testosterone secretion is regulated by the hypothalamic-pituitary-gonadal (HPG) axis. The hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH acts on the Leydig cells in the testes to produce testosterone. This process is controlled by a negative feedback mechanism, in which elevated testosterone levels suppress GnRH release and reduce pituitary sensitivity to it.

GnRH is secreted in rhythmic pulses every one to three hours. Although this pulsatile pattern persists throughout life, testosterone levels reach their peak during the third decade and gradually decline thereafter. Before puberty, low GnRH activity results in minimal testosterone production, but increased hypothalamic stimulation during puberty triggers higher GnRH release and, consequently, elevated testosterone synthesis.

In Leydig cells, testosterone synthesis begins with cholesterol and proceeds through intermediates such as dehydroepiandrosterone (DHEA) and androstenedione. The final conversion to testosterone is catalyzed by the enzyme 17β -hydroxysteroid dehydrogenase. Most circulating testosterone is bound to plasma proteins, primarily sex hormone-binding globulin (SHBG) and albumin. Only the unbound or free fraction is biologically active and acts on target tissues including bone, muscle, the prostate, and seminal vesicles. Within these cells, testosterone is further converted into dihydrotestosterone (DHT) by 5α -reductase. Both testosterone and DHT bind to androgen receptors to regulate gene expression and maintain male physiological functions. The adrenal cortex also contributes by producing weaker androgens, such as DHEA and androstenedione, which can be converted into testosterone in peripheral tissues.

2.3 The Heart

The heart is a muscular organ situated centrally within the chest cavity, positioned behind the sternum. It consists of four chambers: the right and left atria located superiorly, and the right and left ventricles positioned inferiorly (Abubakar, 2019). The right atrium and right ventricle together comprise the right heart, while the left atrium and left ventricle functionally form the left heart.

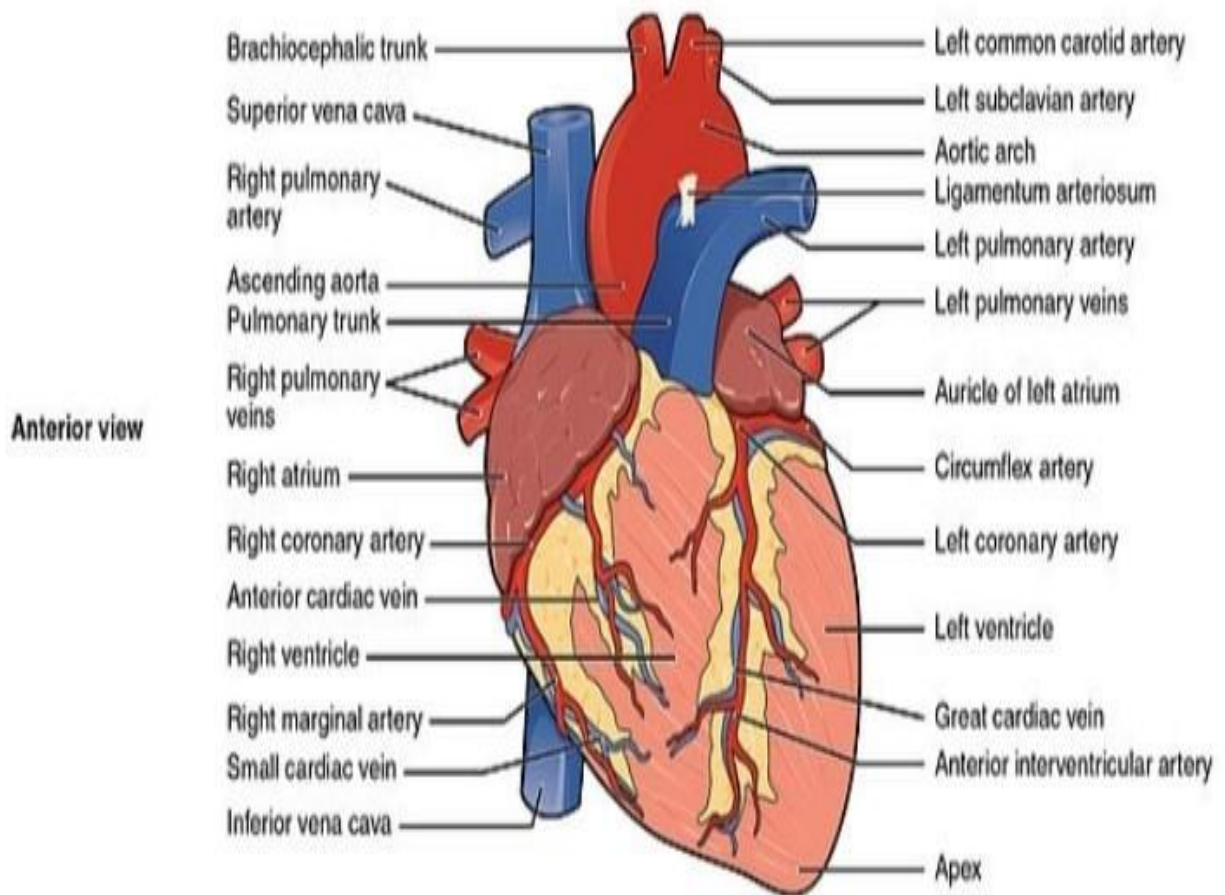


Figure 2. 2: The anterior section of the heart (Aderinto, 2022)

2.3.1 Anatomy and Physiology of the Heart

2.3.2 Structure and Function

The heart is composed of four chambers, arranged into two functional pumps—the right and left hearts—which maintain blood flow through the pulmonary and systemic circulations, respectively (Abubakar, 2019). The right atrium receives deoxygenated blood from the systemic circulation via the superior and inferior vena cavae, as well as from the coronary sinus, which drains blood from the myocardium itself. Acting as a reservoir, the right atrium collects this deoxygenated blood and directs it through the tricuspid valve into the right ventricle, the main pumping chamber of the right heart (Aderinto, 2022).

The right ventricle then propels the blood through the right ventricular outflow tract, across the pulmonic valve, and into the pulmonary artery, which carries it to the lungs for oxygenation. Within the pulmonary capillaries, gas exchange occurs as blood takes up oxygen from the alveoli (Aderinto, 2022). The now oxygenated blood returns to the heart via four pulmonary veins—two from each lung—which empty into the left atrium. Similar to the right atrium, the left atrium serves as a collection chamber and transfers blood into the left ventricle through both passive filling and active contraction (Bloch *et al.*, 2017).

The oxygenated blood passes through the mitral valve to fill the left ventricle, the primary pumping chamber of the left heart. The left ventricle then ejects blood through the aortic valve into the systemic circulation, delivering oxygen and nutrients to tissues throughout the body. This process repeats with every heartbeat, ensuring continuous circulation. The four cardiac valves—tricuspid, pulmonic, mitral, and aortic—function to maintain unidirectional blood flow, permitting forward movement while preventing backflow (Bloch *et al.*, 2017).

2.3.3 Conduction System

The rhythmic pumping action of the heart is controlled by an intricate electrical conduction system that coordinates the timing of contractions across its chambers. Cardiac muscle fibers contract in response to electrical impulses generated and transmitted through this system. The sinoatrial (SA) node, the primary pacemaker of the heart, is located at the junction of the superior vena cava and the right atrium (Bloch *et al.*, 2017). It spontaneously generates electrical impulses at a rate of about 70 beats per minute.

From the SA node, the electrical signal spreads across the right atrium and reaches the left atrium via Bachmann's bundle, ensuring coordinated atrial contraction. The impulse then travels through the atrial myocardium to the atrioventricular (AV) node, which is located within the triangle of Koch—a region bordered by the tricuspid valve, the tendon of Todaro, and the lip of the coronary sinus ostium. The AV node transmits the electrical signal to the bundle of His but introduces a brief delay, allowing the atria to fully empty their blood into the ventricles before ventricular contraction begins.

The bundle of His then divides into right and left bundle branches, which further subdivide into numerous Purkinje fibers (Brotto and Smith, 2019). This His-Purkinje network rapidly conducts the electrical signal throughout both ventricles, ensuring that they contract nearly simultaneously. The result is a coordinated and efficient ventricular contraction that maintains effective blood flow throughout the body (Aderinto, 2022).

2.3.4 Embryology

The heart originates from two endocardial tubes that fuse, loop, and undergo septation to form the mature cardiac structure. During fetal development, an opening between the atria, known as

the foramen ovale, and a duct connecting the pulmonary artery to the aorta—the ductus arteriosus—allow blood to bypass the nonfunctional fetal lungs (Brotto and Smith, 2019). Shortly after birth, both the foramen ovale and the ductus arteriosus close, establishing distinct pulmonary and systemic circulations.

2.3.5 Blood Supply and Lymphatics

The heart receives its blood supply from two main coronary arteries: the left main coronary artery and the right coronary artery. The left main coronary artery provides approximately 80% of the total blood flow to the myocardium. It is a short vessel that quickly divides into two major branches: the left anterior descending (LAD) artery, which supplies the anterior two-thirds of the interventricular septum and the adjacent anterior wall of the left ventricle, and the circumflex artery, which delivers blood to the lateral and posterior walls of the left ventricle (Ezumah, 2023). The right coronary artery, along with its branches, supplies blood to the right atrium, right ventricle, and the inferior portion of the left ventricle. The coronary arteries and veins course over the surface of the heart, forming an intricate vascular network. Most of the coronary veins converge into the coronary sinus, which runs along the left posterior atrioventricular groove and drains into the right atrium. Additionally, small veins known as the Thebesian veins open directly into all four cardiac chambers (Ezumah, 2023).

A network of small lymphatic vessels lies beneath the epicardium and endocardium of the ventricles, draining into lymphatic ducts located in the atrioventricular groove. However, the precise lymphatic anatomy of the human heart remains incompletely defined.

2.3.6 Nerves

Both the sinoatrial (SA) node and the atrioventricular (AV) node receive dual autonomic innervation. Sympathetic nerve fibers arise from the sympathetic ganglia, while parasympathetic

angina pectoris or myocardial infarction and often requires revascularization procedures to restore adequate perfusion.

2.4 Clinical Significance

The heart is an essential organ whose continuous function is critical for life. If the heart stops beating, blood flow and oxygen delivery to the body cease, resulting in irreversible brain damage within approximately 4 to 5 minutes (Schmitt, 2021). Cardiac function can be impaired or halted by various conditions, including reduced blood supply to the myocardium (coronary artery disease), narrowing or incompetence of the heart valves (valvular heart disease), weakening of the cardiac muscle (cardiomyopathy), or abnormal and ineffective heart rhythms (Schmitt, 2021).

2.5 The global origin of pre-modern aphrodisiacs

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces veneral desire and increases pleasure and performance. This word is derived from Ἐφροδίται the Greek Goddess of love and these substances are derived from plants, animals or minerals and since time immemorial they have been the passion of man (Patrick *et al.*, 2017). A lot of natural substances have historically been known as aphrodisiacs in Africa and Europe, like yohimbine and the mandrake plant, as well as ground rhinoceros horn in the Chinese culture and “Spanish fly” which is actually toxic. Even in today's culture, there are certain foods that are used as aphrodisiacs, including strawberries and raw oysters (Scorgie *et al.*, 2021). Chocolate, coffee, and honey are also believed to have aphrodisiac potential. Although these natural items are claimed as aphrodisiacs, there is no or little scientific confirmation supporting those assertions.

In a recent study conducted in the Boston area, 52% of men between the ages of 40 and 70 reported some degree of erectile dysfunction (ED). Enhanced sexual behavior may provide increased relationship satisfaction and self-esteem in humans. The hunt for an effective

aphrodisiac has been a constant pursuit throughout history (Kontula, 2018). The role of various dopaminergic, adrenergic, and serotonergic agents has been intensively examined in both human and animal studies. Some of these drugs have been considered for their potential role for the treatment of sexual dysfunction, while some others have contributed to the basic neurophysiological processes in sexual arousal (Scorgie *et al.*, 2021).

Aphrodisiacs can be classified by their mode of action into three types: Those that increase libido, potency, or sexual pleasure. Various substances of animal and plant origin have been used in folk medicines of different cultures to energize, vitalize and improve sexual function, and physical performance in men, out of these very few have been identified pharmacologically (Scorgie *et al.*, 2021). For increasing libido, ambrein, a major constituent of *Ambra grisea*, is used in Arab countries. It contains a tricyclic triterpene alcohol which increases the concentration of several anterior pituitary hormones and serum testosterone. Bufo toad skin and glands contain bufotenine (and other bufadienolides), a hallucinogenic congener of serotonin. It is the active ingredient in West Indian “love stone” and the Chinese medication chan su. In traditional Chinese medicine, *Panax ginseng* is used as a sex stimulant. It works as an antioxidant by enhancing nitric oxide (NO) synthesis in the endothelium of corpora cavernosa (CC); ginsenosides also cause transmural nerve stimulation-activated relaxation associated with increased tissue cyclic guanosine monophosphate. For increasing sexual pleasure, cantharidin (“Spanish fly”) from blister beetles, which have been used for millennia as a sexual stimulant (Ojewole, 2017).

Most existing scholarship has assumed that ancient Greece was the source of early modern English, French, Latin, and Dutch uses of aphrodisiacs. Early modern Christianate scholars, like medieval Islamicate ones, referred frequently to ancient medical traditions of Galenic and

Hippocratic medicine (Ojewole, 2017). Yet later scholars elaborated significantly on the ancient Greek knowledge they cited, and little existing scholarship has considered the importance of medieval Islamicate pharmacology as a source of much European knowledge about ancient Greek aphrodisiac usage (Ojo, 2017).

2.6 Causes of impotence

Sexual dysfunction is a significant medical and social issue, affecting approximately 10–52% of men and 25–63% of women. Erectile dysfunction (ED), the leading cause of male impotence, is regarded as one of the most prevalent public health concerns due to its widespread impact on men worldwide. ED is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance (Meana, 2020). It is estimated that 20–30 million men experience some degree of sexual dysfunction, with the condition being particularly common among middle-aged and elderly men.

Impotence affects nearly 50% of men with diabetes mellitus, while atherosclerosis accounts for about 40% of ED cases in men over 50 years of age. Commonly associated risk factors include hypertension, dyslipidemia (elevated cholesterol and triglycerides), diabetes, and cigarette smoking. Endocrine disorders such as low testosterone and thyroid dysfunction also contribute to the development of ED. Additionally, pelvic trauma, major pelvic surgeries (including prostate, bladder, and bowel operations), and pelvic radiation therapy are recognized causes of erectile difficulties (Ojo, 2017). Direct trauma to the perineal region can damage penile blood vessels, potentially leading to ED that may be corrected through penile artery bypass surgery.

Psychological and neurological conditions also play a major role in sexual dysfunction. Anxiety, depression, stress, and fear of sexual activity are prominent psychological factors. Neurological disorders such as stroke, cerebral trauma, Parkinson's disease, and penile abnormalities like

phimosis and Peyronie's disease are further contributors. Other organic causes include chronic renal and hepatic failure, multiple sclerosis, Alzheimer's disease, sleep apnea, and chronic obstructive pulmonary disease. Moreover, chronic alcohol consumption and cigarette smoking negatively affect sexual potency (Meana, 2020).

Age-related hormonal decline, systemic diseases such as cancer, and the use of certain therapeutic agents—including antihypertensives, antipsychotics, antidepressants, and antidiabetic drugs—can also impair sexual function. In a study conducted by Sell (2017), administration of *Curculigo orchioides* (CB) root extract at doses of 125 mg/kg and 250 mg/kg significantly improved sexual behavior in a dose-dependent manner. The researchers concluded that the roots of CB may have potential therapeutic benefits in treating certain forms of sexual inadequacies, such as premature ejaculation and oligospermia.

2.7 Synthetic aphrodisiacs substances

2.7.1 Phosphodiesterase V inhibitors

In recent years, synthetic substances such as sildenafil and other phosphodiesterase type 5 (PDE-5) inhibitors have been widely used for the treatment of erectile dysfunction (ED). These medications are effective only when the individual is sexually stimulated; however, they do not directly enhance libido (Zare, 2021). Consequently, their therapeutic benefits are limited to male users.

In Germany, PDE-5 inhibitors are available in several formulations, including sildenafil (Viagra® 25 mg, 50 mg, 100 mg), tadalafil (Cialis® 5 mg, 10 mg, 20 mg), and vardenafil (Levitra® 5 mg, 10 mg, 20 mg). To date, more than 50 structural analogues of PDE-5 inhibitors have been identified. Barta *et al.* (2018) analyzed 150 herbal dietary supplements marketed for improving sexual performance and found concerning results (Zare, 2021). Although these

products were advertised as containing only natural compounds, plant extracts, and vitamins, 61% were adulterated with PDE-5 inhibitors 27% with sildenafil, tadalafil, or vardenafil, and 34% with structurally modified analogues. Among the adulterated samples, 64% contained a single PDE-5 inhibitor, while 36% contained mixtures of two, three, or even four such substances. Furthermore, in 25% of the contaminated supplements, the concentration of these drugs exceeded the maximum recommended pharmaceutical dose (Adams, 2015).

2.7.2 Yohimbine

Yohimbine is an indole alkaloid derived from the leaves and bark of the yohimbe tree (*Pausinystalia johimbe*), with notable forensic and pharmacological significance due to its diverse applications. A study by Alkali *et al.* (2019) on dietary supplements containing yohimbe extracts revealed that the yohimbine content in these products can vary widely, ranging from less than 0.1 ppm to 489 ppm, compared to 7,089 ppm in authentic plant material. This discrepancy has been attributed to the significant dilution of commercial formulations. Modern extraction and purification of yohimbine for pharmaceutical use typically involve ethanol or chloroform extraction, wherein the powdered bark is immersed in the solvent, followed by filtration and solvent evaporation to obtain the purified compound.

Pharmacologically, yohimbine acts as a potent antagonist at both presynaptic and postsynaptic α_2 -adrenoreceptors located in smooth muscle and vascular tissues (Alkali *et al.*, 2015). Its ability to enhance sexual drive is believed to result from the blockade of α_2 -adrenoreceptors in the locus coeruleus of the brain, thereby inhibiting erection-suppressing neural signals within the central nervous system. Beyond its therapeutic use, yohimbine has been misused recreationally as both an aphrodisiac and a hallucinogen. More recently, it has gained popularity in bodybuilding communities for its lipolytic and sympathomimetic properties, promoting rapid weight loss and

enhanced physical performance. Owing to its vasodilatory effects, yohimbine has been approved for medical use in the United States and Germany (Yocon Glenwood tablets® 5 mg) for the treatment of erectile dysfunction (Beres, 2019). The recommended oral dose for ED treatment ranges from 5–15 mg (1–3 tablets).

However, the same central and libido-enhancing effects that make yohimbine therapeutically useful also raise concerns regarding its potential misuse in drug-facilitated sexual assaults, where both men and women may be victimized. Adverse effects of yohimbine include hypertension, tachycardia, manic episodes, bronchospasm, palpitations, insomnia, anxiety, irritability, tremors, sweating, nausea, flushing, and headaches—symptoms largely attributed to its central adrenergic stimulation (Bradbury-Jones *et al.*, 2020). Data from the California Poison Control System reported 238 cases of adverse reactions to yohimbine over a seven-year period (2000–2006), with the most frequent symptoms being gastrointestinal distress, tachycardia, anxiety, agitation, and hypertension. Neurotoxic manifestations have been observed following overdoses of 200–500 mg, producing symptoms such as anxiety, drowsiness, disorientation, tremors, and seizures. Ingestion on an empty stomach may also trigger panic attacks (Bradbury-Jones *et al.*, 2020).

Several clinical case reports further highlight the potential toxicity of yohimbine. Documented adverse reactions include anxiety, tremors, hypertension, nausea, headache, skin rashes, dissociative reactions, confusion, atrial fibrillation, seizures, and loss of consciousness. Butzer and Campbell (2018) described a case of retrograde amnesia in a 38-year-old man following ingestion of 350 mg of yohimbine. Sandler and Aronson reported a case involving progressive renal failure, cutaneous eruptions, and a lupus-like syndrome in a 42-year-old African American man after yohimbine use. Myers and Barrueto documented a case of refractory priapism in a 42-year-old man who self-administered a yohimbine-containing product to treat ED and

subsequently presented with a 20-hour erection requiring emergency intervention. Similarly, Chatterji *et al.* (2015) reported a case involving a 16-year-old girl who experienced an acute dissociative reaction characterized by weakness, paresthesia, incoordination, anxiety, headache, and chest pain after ingesting yohimbine.

2.7.3 Flibanserin

Flibanserin (marketed as Addyi®) is a drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of hypoactive sexual desire disorder (HSDD) in women. It was detected in 2 out of 150 supplements purchased online and marketed for enhancing female libido. Additionally, trace amounts (approximately 11 mg per capsule) were identified in an herbal dietary supplement sold in pharmacies for the treatment of female HSDD. Clinical trials have demonstrated that flibanserin is effective in treating HSDD when administered at a daily dose of 100 mg over a 24-week period (Eatough and Smith, 2018).

Pharmacologically, flibanserin acts as an agonist at 5-HT_{1A} receptors, an antagonist at 5-HT_{2A} receptors, and a partial agonist at dopamine D₄ receptors. Its therapeutic effect is attributed to the modulation of serotonin and dopamine neurotransmission, leading to increased sexual desire in women. However, adverse effects such as dizziness, somnolence, nausea, fatigue, and hypotension have been reported in clinical trials.

Gamma-hydroxybutyric acid (GHB) is another compound with dual significance as both a central nervous system depressant and a substance of abuse. It is a metabolite of gamma-aminobutyric acid (GABA) and is sometimes misused as a knockout drug in drug-facilitated sexual assaults due to its potent narcotic and sedative effects. Nevertheless, GHB has also been reported to exhibit aphrodisiac properties in men. It is known to produce four primary sexually

enhancing effects: disinhibition and relaxation, heightened tactile sensitivity, enhanced erectile capacity, and intensified orgasmic experience (Glass, 2017).

The effects of GHB are dose-dependent. At doses up to 1.5 g, it induces euphoria, relaxation, and disinhibition, acting as an anxiolytic with social effects similar to mild alcohol intoxication. Doses up to 2.5 g further elevate mood and impulse control, enhancing libido and sexual arousal. However, higher doses exceeding 3–4 g can produce strong narcotic and sedative effects. The initial psycho-stimulant effects are followed by deep sedation as the drug's action progresses. Adverse effects typically appear within 15 minutes of ingestion and include headache, somnolence, psychomotor impairment, nausea, vomiting, dizziness, hypertension, speech disturbances, seizures, and disorientation. Severe toxicity can result in drowsiness, deep sleep, unconsciousness, or even death due to respiratory arrest. The duration of GHB's effects ranges from 1 to 7 hours depending on the dose.

Precursors of GHB, such as gamma-butyrolactone (GBL) and 1,4-butanediol (BD), can be easily converted into GHB within the body and are sometimes misused as aphrodisiacs due to their similar psychoactive and sedative properties (Humphreys and Herold, 2017).

2.8 Products with herbal or animal origin

2.8.1 Tribulus terrestris and other protodioscin-containing species (fenugreek and Dioscorea)

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2.8.2 Ambrein

Ambergris is a physiological secretion produced in the intestines of the sperm whale (*Physeter macrocephalus* L., family *Physeteridae*) and is typically expelled into the sea as waste, either through regurgitation or defecation. It appears as gray or brown waxy masses composed primarily of undigested squid and cuttlefish beaks—the sperm whale's principal prey—combined with fatty digestive secretions (Ajuwon, 2015).

The major chemical constituent of ambergris is the triterpenoid alcohol **ambrein**, which has been isolated and identified as its principal active compound. Ambrein is commercially available, with capsules sold online. Historically, ambergris has been utilized in perfumery as a fixative and fragrance enhancer, as well as in traditional medicine for various therapeutic purposes. Notably, it has also been employed to enhance sexual performance, and experimental studies have supported its aphrodisiac potential in male rats (Ajuwon, 2015).

Toxicological studies have shown that ambrein intake induces general sedation and reduced activity in mice, with a reported LD₅₀ of approximately 7,500 mg/kg body weight following intraperitoneal administration. A subcutaneous dose of 250 mg/kg significantly reduced locomotor activity and decreased cerebral levels of norepinephrine, dopamine, and their metabolites in mice. However, adverse effects in humans have not been documented in either scientific literature or online reports (Ajuwon, 2015).

2.8.3 Bufo Toad (love stone)

The skin and glandular secretions of several *Bufo* species have been traditionally used for medicinal and aphrodisiac purposes. The ancient Chinese medication *Chan su* and the Indian aphrodisiac known as “love stone” are both derived from these secretions. When employed as an aphrodisiac, *Bufo* toad preparations are typically administered topically (Ekeh, 2020).

The aphrodisiac and psychoactive properties of *Bufo* secretions are primarily attributed to the presence of bufotenine and its O-methylated derivative, 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT). Both compounds are potent hallucinogens with stimulatory effects (Ekane, 2021). Interestingly, 5-MeO-DMT is also found in certain *Ayahuasca* preparations. These substances are structurally related to serotonin and psilocin and exhibit strong affinity for the 5-HT_{1A} receptor. Because of their intense hallucinogenic activity, *Bufo* toad secretions are rarely used as aphrodisiacs in contemporary practice.

5-MeO-DMT is a fast-acting hallucinogen with a short duration of action in humans. Self-experimentation studies have shown that it induces vivid visual and auditory hallucinations, as well as distortions in time perception. The effects typically begin within 3–4 minutes of administration, peak at around 35–40 minutes, and subside after approximately 60–70 minutes when insufflated. Animal studies involving mice, rats, sheep, and monkeys have demonstrated symptoms such as ataxia, mydriasis, head nodding, tremors, convulsions, and shivering following exposure to 5-MeO-DMT. Among these, sheep were found to be the most sensitive, exhibiting profuse salivation, tachycardia, and respiratory failure at doses as low as 1 mg/kg. The LD₅₀ values in mice ranged from 48 mg/kg to 278 mg/kg depending on the route of administration.

In humans, intoxication with 5-MeO-DMT or related compounds can cause optical hallucinations, dizziness, nausea, hypertension, and disorientation. Between 1993 and 1995, the New York City Poison Control Center (NYCPCC) reported cases involving five previously healthy men who developed severe illness after ingesting a substance marketed as a topical aphrodisiac; four of them subsequently died. The preparation they had consumed was identified as *Chan su*, which was being used as a topical anesthetic and cardiac medication and was found to contain bufotenine.

2.8.4 Spanishfly (Cantharides)

The Spanish fly is the most renowned among a group of more than 2,000 beetle species that have been traditionally used as aphrodisiacs. All members of this group, known as blister beetles, secrete a vesicant liquid that causes blistering upon contact. These beetles are commonly found in regions of southern Europe, Africa, and Asia (Ekane, 2021). The Mediterranean species *Cantharis vesicatoria* is the best known and is specifically referred to as the “Spanish fly,” while *Epicauta vittata* is the most prevalent of the blister beetles identified in the United States.

Commercially available preparations marketed as Spanish fly typically contain only trace amounts of cantharidin, the active compound responsible for the beetle’s pharmacological and toxic effects. However, illicit formulations with significantly higher concentrations of cantharidin are also available and can cause severe poisoning. Despite its known toxicity, cantharidin has been used historically to enhance sexual function, with its use documented for over 2,000 years in traditional Chinese and African medicine. In these practices, dried beetles were ground into a fine powder and dissolved in a solvent for oral administration (Ekane, 2021). Upon ingestion, cantharidin causes intense irritation of the urinary and genital tracts, which can lead to prolonged and painful erections (priapism) in both men and women. The underlying

mechanism involves inhibition of phosphodiesterase and protein phosphatase enzymes, leading to stimulation of β -receptors and vascular inflammation within the genitourinary system. However, these same effects can result in serious renal complications, including acute tubular necrosis and glomerular damage, making renal dysfunction a common outcome of cantharidin toxicity.

2.8.5 Muira puama (potency wood)

Ptychopetalum olacoides Benth (family Olacaceae), commonly known as *muira puama*, *mara puma*, or “potency wood,” is native to the Amazon region of Brazil and has a long history of traditional use among Amazonian peoples (Maxwell, 2022). It is widely marketed as a natural sexual enhancer, based on the traditional belief that root extracts can increase libido, improve erectile strength, and enhance overall sexual performance. Additionally, it has been used as a general body stimulant, nerve tonic, and remedy for chronic degenerative conditions.

Phytochemical investigations of *muira puama* have identified the presence of alkaloids, tannins, saponins, flavonoids, and several other bioactive compounds. Studies have shown that *muira puama* increases the intracellular levels of cyclic adenosine monophosphate (cAMP), which may explain its smooth muscle relaxant effects on the corpus cavernosum of rabbits and its potential prosexual effects in men. The observed smooth muscle relaxation was characterized by a rapid onset but short duration of action. Mauss (2018) reported that *muira puama* exhibits excitatory effects on the central nervous system, likely mediated through interactions with dopaminergic and/or noradrenergic neurotransmission, supporting its proposed psychostimulant and aphrodisiac properties.

Safety and toxicity data on *muira puama* remain limited. Most available information is derived from studies on herbal combinations containing *muira puama* along with other active ingredients.

One such formulation, Herbal vX, was evaluated in a clinical study involving 2,702 healthy women (Waynberg and Brewer, 2000, cited in Lammers *et al.*, 2021). Only eight participants reported mild adverse effects, including sweating, headache, irritability, vaginal thrush, and stomach cramps, with no repeated occurrence of the same reaction. Interestingly, several participants also reported beneficial side effects such as increased energy levels.

Another commercially available product, Catuama®, contains a combination of *Paullinia cupana* (guarana), *Trichilia catigua* (catuaba), *Ptychopetalum olacoides* (muira puama), and *Zingiber officinale* (ginger). Catuama® capsules are sold both in Brazil and internationally via online markets (Mark *et al.*, 2021).

2.8.6 Maca root

Maca (*Lepidium meyenii*), a member of the Brassicaceae family, is a plant native to the high altitudes of the Peruvian Central Andes. Its root has been consumed as both food and medicine for over 2000 years by local populations, who traditionally attribute to it properties such as enhancement of sexual function, increased libido, and improved fertility (Marshall and Rossman, 2019). Among herbal remedies promoted online, *maca* is frequently cited as one of the most popular natural products for improving sexual desire, particularly in women.

Phytochemical analyses of *maca* have identified several secondary metabolites, including glucosinolates, imidazole alkaloids, fatty acids, and macamides. However, limited scientific evidence exists on the acute side effects or toxicity of *maca* products, as most clinical trials involve small sample sizes and methodological limitations. Shin *et al.* conducted a systematic review on the efficacy of *maca* for enhancing sexual function and identified four randomized controlled trials. None of these studies, however, systematically evaluated the adverse effects of *maca* supplementation.

Kolawole (2019) reported that black *maca*, and to a lesser extent red *maca*, significantly reduced hemoglobin levels in high-altitude dwellers with elevated hemoglobin, while no effect was observed in lowlanders. Additionally, black *maca* lowered blood glucose levels, but no severe adverse effects were noted with *maca* extracts. Idoko *et al.* (2018) detected the presence of 1R,3S-1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (MTCA) in *maca* extracts, a compound proposed to inhibit monoamine oxidase (MAO) activity and act as a comutagen or precursor to mutagenic substances.

2.9 Viagra

Viagra is the trade name for the drug sildenafil citrate, which is used to treat erectile dysfunction in adult males (Ekeh, 2019). According to the Viagra Website, the drug works by increasing blood flow to the penis, which increases hardness and sexual satisfaction (Okodudu, 2022). It can be taken an hour before sexual activity and only works when the user is aroused (Oloruntoba-Oju, 2017).



Figure 2.3: Viagra Tablets (Ing, 2020)

2.9.1 Mechanism of Action

The molecular structure of sildenafil mimics that of cyclic guanosine monophosphate (cGMP). This similarity protects cGMP from degradation because sildenafil can bind to the catalytic site to act as a competitive inhibitor of cGMP-specific PDE-5, the enzyme that normally catalyzes the breakdown of vasodilatory cGMP (Giorgi, 2018). When PDE5 is active, cGMP is degraded, causing the vascular smooth muscle contraction, thereby limiting blood flow (Brown, 2023). When PDE5 becomes inhibited, the accumulation of cGMP leads to increased cGMP-dependent protein kinase activity, which phosphorylates multiple targets in the smooth muscle cell (Sekaran and Bougie, 2023). The result of smooth muscle cell target protein phosphorylation is a decrease in intracellular calcium, increased efflux of potassium, and deactivation of myosin light chain kinase, ultimately causing smooth muscle relaxation (Aidoo, 2019).

Penile erection occurs through the relaxation of vascular smooth muscle within the corpus cavernosum, allowing the cavernosal sinusoids to fill with blood and compress the subtunical veins against the tunica albuginea. Similarly, in patients with vasoactive pulmonary arterial hypertension, vasodilation of the pulmonary arteries decreases vascular resistance, resulting in a reduction of mean pulmonary arterial pressure (Sekaran and Bougie, 2023).

2.9.2 Administration

2.9.2.1 Erectile Dysfunction

Sildenafil is administered orally in tablet form at strengths of 25 mg, 50 mg, and 100 mg for the treatment of erectile dysfunction (ED). The typical starting dose is 50 mg, taken as needed approximately one hour before sexual activity (Cate *et al.*, 2019). Depending on individual efficacy and tolerability, the dose may be adjusted up to a maximum of 100 mg or reduced to 25 mg (Lawrance and Byers, 2022; Glass and Staeheli, 2022). Patients are advised not to exceed

one dose per day. Sildenafil may be taken 30 minutes to 4 hours before intercourse, with an onset of action typically within 30 minutes and effects lasting up to 18 hours (Cate *et al.*, 2019).

For individuals with hepatic impairment (such as cirrhosis), severe renal impairment (creatinine clearance <30 mL/min), or those aged 65 years and older, a starting dose of 25 mg is recommended, as these groups tend to exhibit higher plasma concentrations of sildenafil. Similarly, patients taking concomitant CYP3A4 inhibitors should begin with the lower dosage (Ekeh, 2020).

2.9.3 Adverse Effects

Sildenafil exhibits systemic vasodilatory effects and should therefore be prescribed cautiously in patients who may be sensitive to blood pressure fluctuations, such as those with left ventricular outflow obstruction or impaired autonomic regulation of blood pressure. Caution is also warranted when sildenafil is used concurrently with alpha-adrenergic blockers or other antihypertensive agents.

Occasionally, sildenafil use may result in **priapism**, a prolonged erection lasting more than four hours, which can lead to irreversible penile tissue damage if not promptly treated. Extra care is required in patients with anatomical penile deformities or conditions predisposing them to priapism, such as sickle cell anemia or multiple myeloma. The use of phosphodiesterase type 5 (PDE5) inhibitors, including sildenafil, has also been linked to an increased risk of non-arteritic anterior ischemic optic neuropathy (NAION), particularly in men over 50 years of age. Thus, individuals with a history of NAION or a “crowded” optic disc should use sildenafil with caution. Sildenafil is metabolized primarily via the CYP3A4 enzyme pathway and, to a lesser extent, by CYP2C9. Strong inhibitors of these enzymes can elevate plasma sildenafil concentrations, potentially leading to toxicity. For example, coadministration with ritonavir increases sildenafil

exposure by approximately 11-fold due to inhibition of CYP3A4 and CYP2C9. Therefore, patients taking ritonavir should not exceed 25 mg of sildenafil within a 48-hour period. When used with the endothelin receptor antagonist bosentan for pulmonary arterial hypertension (PAH), sildenafil has been shown to increase bosentan levels while reducing its own plasma concentration. Similarly, concurrent use of CYP3A4 inducers may lower sildenafil concentrations, making such combinations unsuitable for chronic PAH therapy.

When initiating sildenafil in patients already on alpha-blockers, therapy should begin with the lowest possible dose due to the risk of systemic hypotension. Caution is also necessary when combining sildenafil with mixed alpha/beta-blockers unless coadministration safety has been established. Doses exceeding 100 mg do not enhance efficacy and are associated with a higher incidence of adverse effects.

Commonly reported side effects (approximately 2% of users) include headache, flushing, dyspepsia, nasal congestion, back pain, myalgia, nausea, dizziness, and rash. Visual disturbances such as color vision changes, altered light perception, and blurred vision have also been noted. Rarely, sildenafil has been associated with ototoxicity, including reversible hearing loss. Epistaxis occurred in about 13% of patients treated for PAH secondary to connective tissue disorders, particularly among those concurrently taking oral vitamin K antagonists, though it was not observed in idiopathic PAH.

The safety of sildenafil has not been established in patients with active peptic ulcers or bleeding disorders. In rare instances, users have reported sudden hearing loss or decreased hearing, sometimes accompanied by tinnitus or dizziness. While causality remains uncertain, patients should be advised to seek immediate medical attention if they experience any sudden changes in hearing while taking sildenafil.

2.9.4 Contraindications

Nitrates promote the formation of cyclic guanosine monophosphate (cGMP), while sildenafil inhibits its degradation. When used together, this synergistic interaction can cause severe and potentially life-threatening hypotension; therefore, the coadministration of sildenafil and nitrates is strictly contraindicated. Nitrates may be safely administered only after at least five sildenafil elimination half-lives equivalent to 24 hours or more have passed since the last sildenafil dose.

Sildenafil use is also contraindicated in cases of severe hypotension from other causes, such as volume depletion. Additional contraindications include hypersensitivity to any component of the formulation, pulmonary veno-occlusive disease, left ventricular outflow obstruction, pulmonary arterial hypertension (PAH) associated with sickle cell anemia, and multiple system atrophy.

Patients with ischemic optic neuropathy or inherited degenerative retinal disorders should not use sildenafil. In the event of sudden vision loss, discontinuation of sildenafil is strongly recommended.

2.9.5 Monitoring

Routine monitoring is generally not required for patients taking sildenafil. However, periodic assessment of blood pressure and pulse may be advised when the medication is first prescribed, following a dosage increase, or when CYP3A4 inhibitors are introduced. During clinical evaluations, it is recommended to assess patients for any visual disturbances. For those receiving sildenafil as treatment for pulmonary arterial hypertension, monitoring for signs and symptoms of pulmonary edema is also advised.

2.9.6 Toxicity

Studies in healthy individuals have shown that single doses of sildenafil up to 800 mg produce adverse effects similar to those observed at lower doses, although with greater frequency and severity. There is currently no specific antidote for sildenafil overdose; therefore, treatment is primarily supportive.

At toxic doses, dose-dependent visual disturbances occur more frequently, while changes in blood pressure show only a weak correlation with toxicity. Because sildenafil is highly bound to plasma proteins and is not significantly excreted by the kidneys, dialysis is ineffective in accelerating its elimination.

When assessing the duration of toxicity, it is important to consider the activity of N-desmethyl-sildenafil, a primary metabolite that retains phosphodiesterase type 5 (PDE5) specificity at a lower potency and can reach plasma concentrations of approximately 40% of those of the parent compound.

2.9.7 Advantages of Viagra

Viagra offers several reported benefits. It can be taken anywhere from 30 minutes to 4 hours before sexual activity, allowing flexibility in its use. The medication enhances penile firmness, facilitating easier penetration and a more satisfying sexual experience (Ahikire, 2021). Its effects diminish after sexual activity, ensuring that the penis does not remain erect for an extended period following intercourse (Glass and Staeheli, 2022).

2.9.8 Disadvantages of Viagra

According to the manufacturer's official website, "Viagra can cause serious side effects." Although these effects are relatively uncommon, they may include the following: priapism, an erection lasting more than four hours that requires immediate medical attention to prevent

permanent penile damage (Glaser and Strauss, 2017); sudden loss of vision in one or both eyes, which may indicate non-arteritic anterior ischemic optic neuropathy (NAION); and sudden hearing loss or a decrease in hearing ability (Glass and Staeheli, 2022).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Laboratory Equipment

The laboratory tools and materials used in this study included: lancets, alcohol swabs, gauze pads, heparinized microhematocrit tubes, tube sealants such as “Seal-Ease” or “Crit-Seal,” a microhematocrit centrifuge and reader, tourniquet, 2 mL syringes, anticoagulant-containing tubes (EDTA and citrate), dissection tools, capillary tubes, a hot plate, cotton wool, knives, measuring cylinders, a digital scale, scissors, lithium heparin bottles, cover slips, stainless steel instruments, tissue blenders, gauze, 5 mL syringes, forceps, microscopes, dissection boards, universal containers, tissue cassettes, rotary microtomes, lead pencils, automatic tissue processors, and frosted-end glass slides.

3.2 Drugs and Chemicals

Viagra (sildenafil citrate) was obtained in tablet form (100 mg per tablet) from Airen Pharmaceutical Co., Benin City (Catalog No: T712515). Each tablet was dissolved in 20 mL of distilled water for oral administration. The dose administered was 20 mg/kg body weight per day, which is considered an equivalent effective dose for humans based on Paget and Barnes (2018).

3.3 Experimental Design and Preparation

A total of 18 adult male albino rats, each weighing between 200 g and 220 g, were sourced from the Anatomy Department's vivarium at the University of Benin, Edo State. The rats were housed in steel mesh cages, six per cage, and maintained on standard commercial feed and tap water

during a one-week acclimatization period. The animals were randomly allocated into three groups of six rats each (Groups A, B, and C).

- **Group A (Control):** Received only standard feed and water.
- **Group B:** Received an oral dose of Viagra at 5 mg/kg body weight daily for four weeks.
- **Group C:** Administered 10 mg/kg body weight of Viagra daily for four weeks, followed by a four-week withdrawal period without treatment.

Viagra doses were administered orally using a curved gavage tube over six weeks. At the end of the treatment, the rats were anesthetized using 4% isoflurane in 100% oxygen. Blood was collected via cardiac puncture into EDTA tubes for hematological assessment, and organs were excised and preserved in 10% neutral buffered formalin for histological processing.

3.4 Body Weight Monitoring

The body weights of all experimental rats were measured before starting treatment (week 0) and again on the final day of drug administration. The percentage change in body weight was calculated accordingly.

3.5 Hematocrit (Packed Cell Volume) Determination

Following chloroform-induced anesthesia, the rats were sacrificed. A midline incision was made to access the abdominal cavity, and blood samples were obtained directly from the heart using 5 mL disposable syringes. Blood was transferred into EDTA tubes to analyze hematological parameters such as hemoglobin (Hb) and packed cell volume (PCV).

For PCV measurement, blood was drawn into a capillary tube to about two-thirds to three-quarters of its length after proper mixing. Excess blood was wiped off the outside of the tube, which was then sealed at one end with a sealant. Care was taken to avoid air bubbles, as incomplete sealing can lead to leakage and inaccurate readings during centrifugation. Tubes were spun in a microhematocrit centrifuge at high speed for 3–5 minutes. The ratio of red cell column length to total blood column (cells + plasma) was measured using a hematocrit reader, then multiplied by 100% to calculate hematocrit values. Multiple readings were averaged for accuracy.

3.6 Tissue Processing

Excised tissues were placed in tissue cassettes and processed using a 22-hour cycle on an automated tissue processor. During this process, tissues underwent dehydration, clearing, and infiltration. They were then embedded in paraffin, sectioned, floated, dewaxed, and stained.

3.7 Hematoxylin and Eosin (H&E) Staining

The left testis of each rat was fixed in 10% formalin and gradually dehydrated using graded alcohols. Following treatment with xylene, tissues were embedded in paraffin blocks. Thin sections (5 μm thick) were cut with a microtome and stained using hematoxylin and eosin for histological examination.

3.8 Testicular Histopathology

Histological processing of testicular tissue was conducted using standard protocols described by Drury *et al.* (2017). The testes were excised, sliced into smaller sections, and fixed in 10%

buffered formalin for 24 hours. After rinsing to remove formalin, tissues were stored in 70% alcohol. They were then dehydrated through alcohol gradients, embedded in paraffin, and sectioned into 5 µm slices for mounting on glass slides. These were stained with H&E and mounted for microscopic evaluation. Each testis was assessed across multiple cross-sections (20–50 tubules) for histological signs such as interstitial edema, degeneration of seminiferous tubules, and vascular congestion.

3.9 Slide Preparation and Microscopy

Cardiac tissues were fixed in 10% formalin, embedded in paraffin, and sectioned into 5 µm slices. These sections were stained with hematoxylin and eosin and then examined under a light microscope by the study supervisor for histopathological analysis.

3.10 Photomicrography

Representative sections of stained tissue samples were photographed and compiled as image plates for visual documentation.

CHAPTER FOUR

4.0 RESULTS

Sildenafil administration for 6 weeks reveals changes in weight, haematological parameters with no histopathological damages. The results are explained separately in different sections as

follows. The collated values were expressed in mean \pm SD. ($P < 0.05$) was considered significant. The average weight of animal after the 6 weeks administration of Sildenafil shows significant reduction ($P < 0.05$) when compared with the control as presented in table 4.1. The hematocrit and white blood cell count value were significantly reduced ($P < 0.05$) in the test group when compared to the control. There were no significant changes in lymphocytes of the experimental group when compared with control. There was significant increase in monocytes count ($P < 0.05$) in the test group in comparison with the control group

The histological sections of the heart and testes did not present any degenerative changes as presented in the plates below.

Table 4.1: Showing the Effect of Sildenafil on the body weight across group (Experimental and Control)

Weight (control group)	Weight (Exp. group)	t-value	p-value
210.0 \pm 5.30	202.7 \pm 3.11*	5.987	0.0001

Table 4.2: The effect of Sildenafil on hematological parameters across group (Experimental and Control)

	Control	Experimental group	t-value	p-value
HCT	45.6 ± 1.50	41.0 ± 1.66*	20.917	0.0001
WBC	7.4 ± 1.92	2.3 ± 0.55*	9.039	0.004
LYM	52.2 ± 2.49	46.7 ± 12.98	0.925	0.369
MON	7.4 ± 1.14	17.2 ± 2.38*	-8.684	0.0001
GRAN	40.4 ± 1.82	32.8 ± 3.27*	4.876	0.0001

Key

HCT = Hematocrit

WBC= White blood count

LYM = Lymphocytes

MON = Monocytes

GRAN= Granulocytes

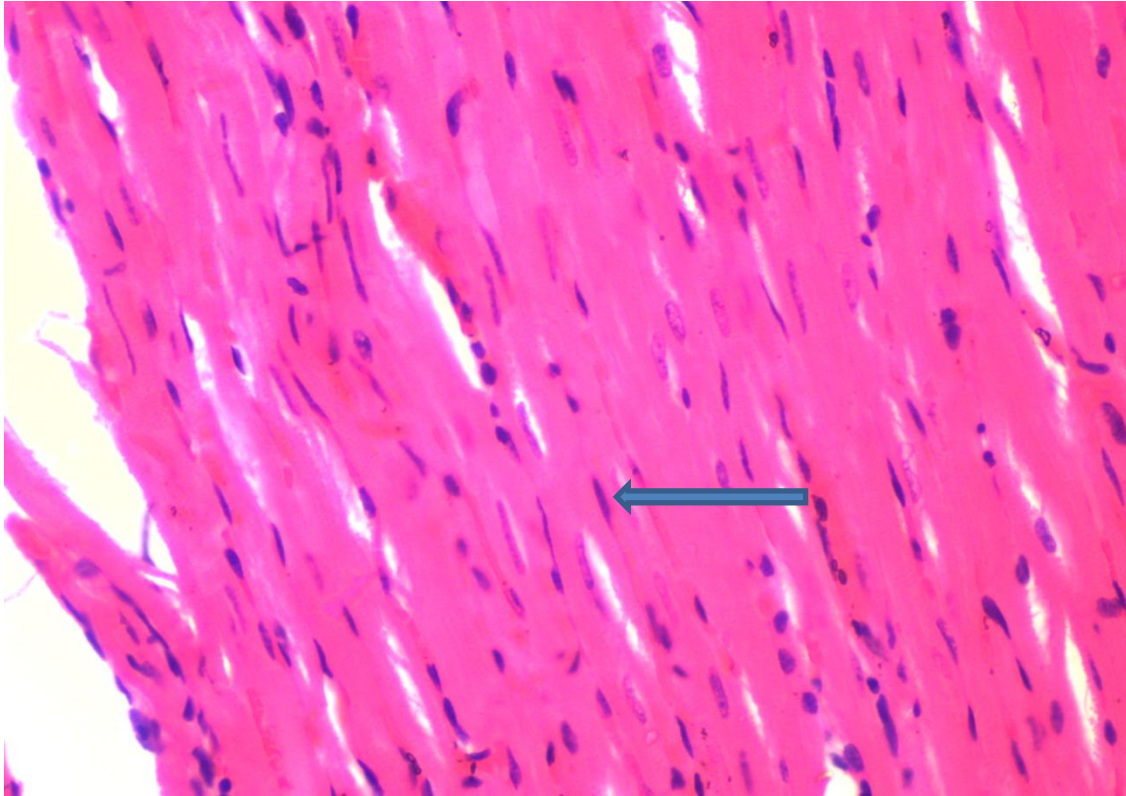


Plate 1: Control Section of cardiac muscle shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm. NORMAL HEART MUSCLE Haematoxylin and Eosin X 400

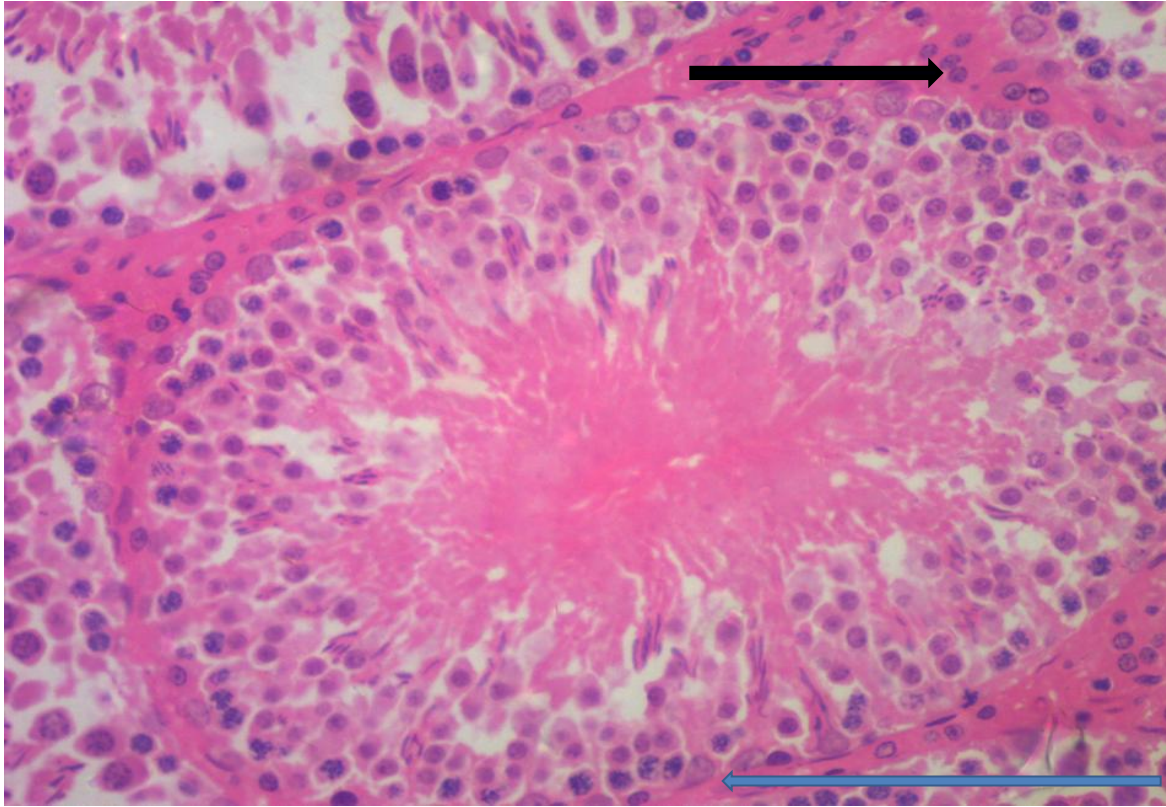


Plate 2: Control section of the testis shows normal seminiferous tubules (blue arrow) containing sertoli cells and spermatozoa. The interstitium contains Leydig cells (black arrow). Features of NORMAL TESTIS. H AND E X400

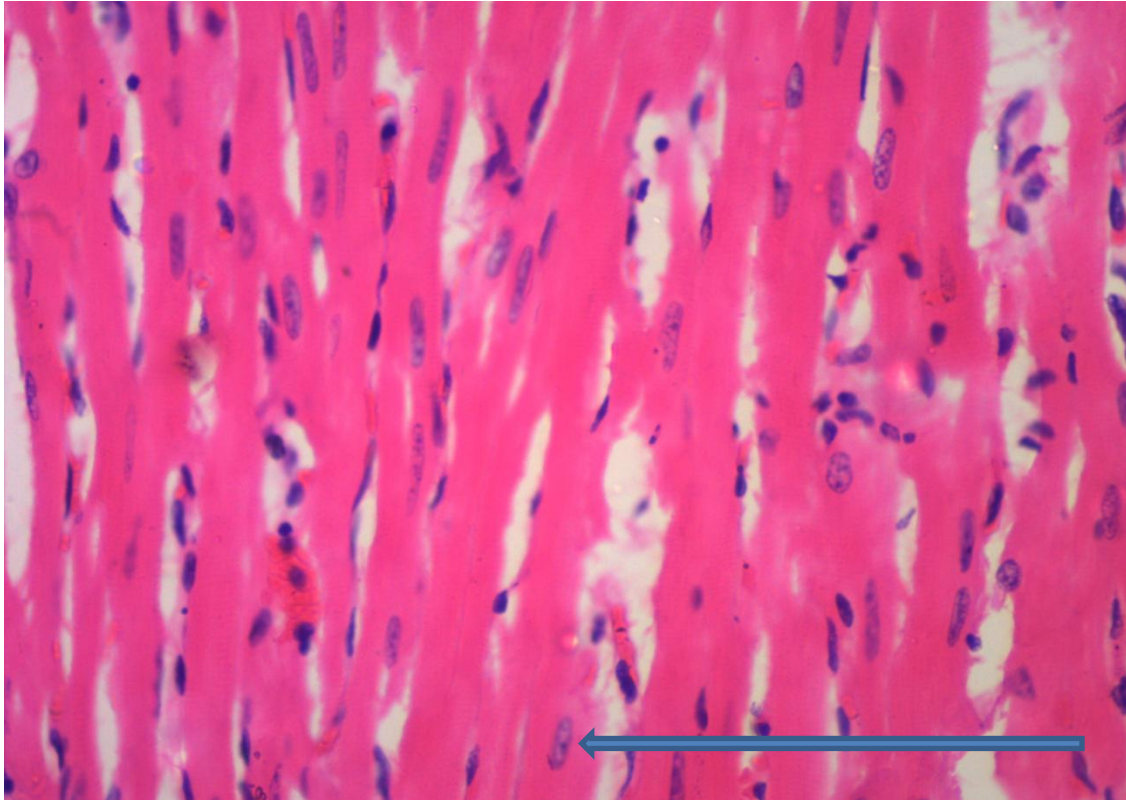


Plate 3: Section of rat cardiac tissue from the experimental Group B, Treated with 5mg shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm. NORMAL HEART MUSCLE

Haematoxylin and Eosin X 400

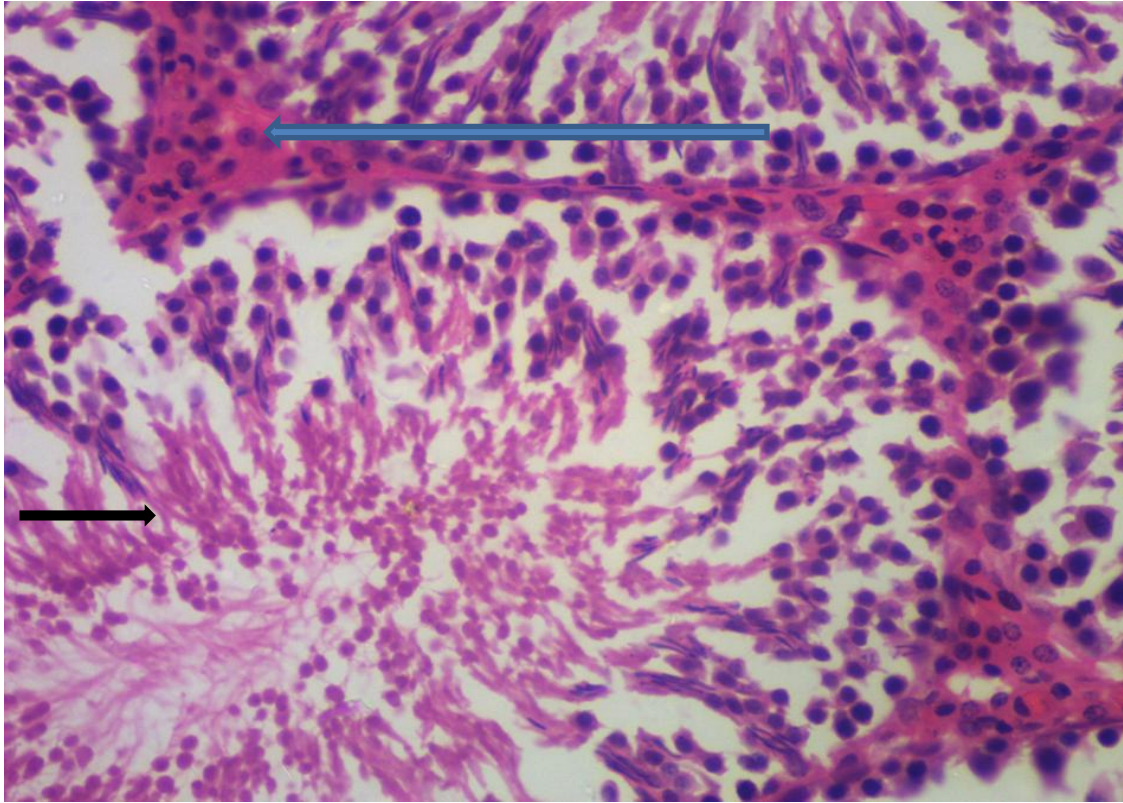


Plate 4: Section of the testis administered with 5mg/kg body weight from the experimental group B. see normal seminiferous tubules (black arrow) containing Sertoli cells and spermatozoa. The interstitium contains Leydig cells (blue arrow). Features of NORMAL TESTIS. H AND E X400

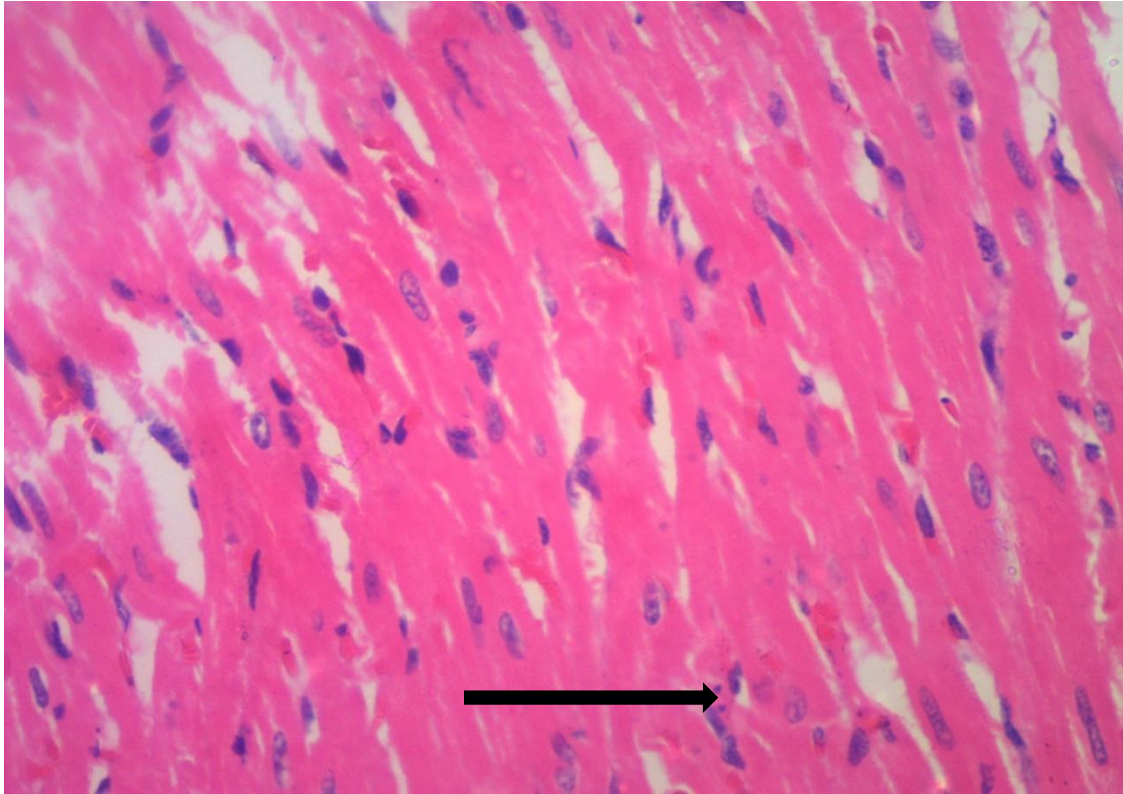


Plate 5: Section of rat cardiac tissue from the experimental Group C; Treated with 10mg/kg shows myocytes with peripherally placed nuclei surrounded by eosinophilic cytoplasm.

NORMAL HEART MUSCLE

shows slight degeneration of both the cardiac fibers (CF), and the nucleus (N). Haematoxylin and

Eosin X 400

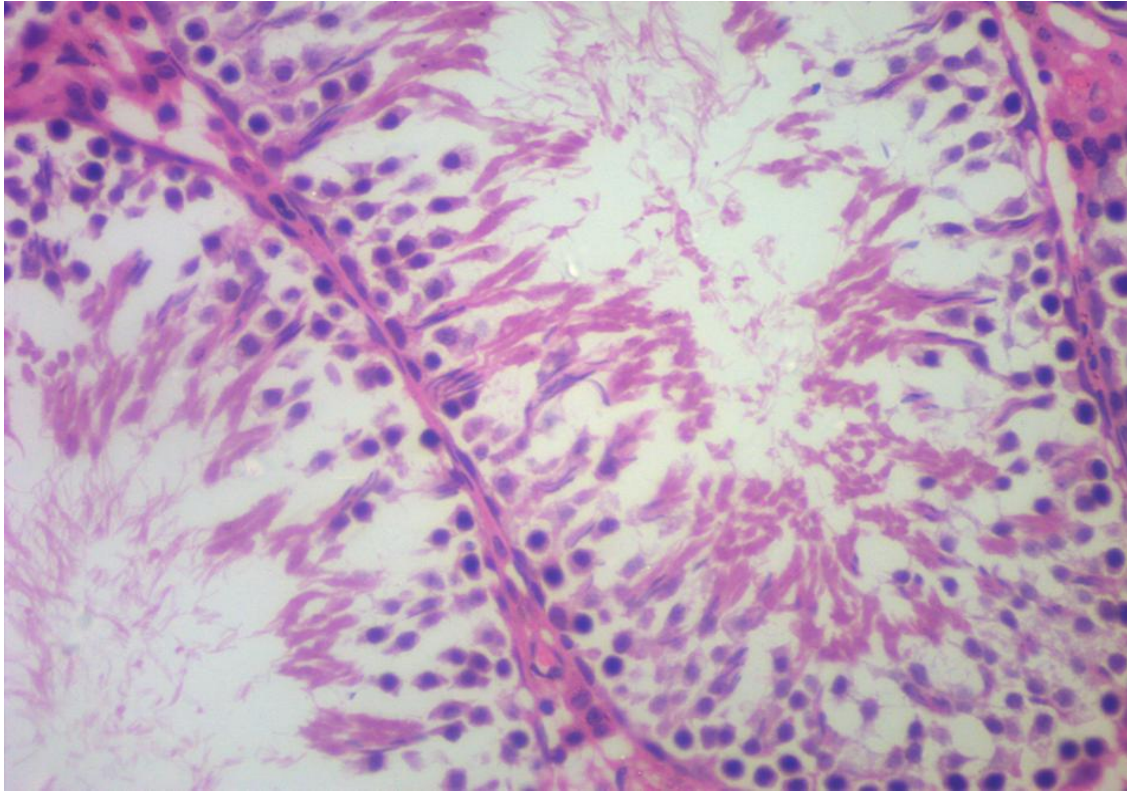


Plate 6: Section of the testis administered with 10mg/kg from the Experimental group C; see normal seminiferous tubules containing Sertoli cells and spermatozoa. The interstitium contains Leydig cells . Features of NORMAL TESTIS. H AND E X400

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 Discussion

The use of sildenafil (Viagra) has become increasingly common, particularly among young adult males experiencing premature ejaculation (Alkali *et al.*, 2019; Beres, 2017). The results of the present study showed that hematocrit testing revealed a general reduction in red blood cell (RBC) count following sildenafil administration. This may be attributed to the drug's potential inhibitory effect on erythropoiesis. The decline in RBC count and hemoglobin (Hb) levels likely led to reduced oxygen delivery to tissues, resulting in lower energy production. These findings are consistent with those of Bella and Shamloul (2020) and Cheng *et al.* (2021), who reported a decrease in RBC count and an increase in white blood cell (WBC) count following sildenafil treatment at doses between 50 mg/kg and 100 mg/kg. Weight analysis after four weeks showed a slight reduction in body weight in Viagra-treated rats (202.7 ± 3.11 g) compared to the control group (210.0 ± 5.30 g). Variations in dosage, duration of administration, diet, sex, or environmental conditions may account for this difference. Agunbiade and Ayotunde (2022) similarly observed a significant decrease in weight gain by day 14 in sildenafil-treated groups compared to controls. The notably lower percentage difference in groups A, E, and G (all treated with sildenafil) suggests that the drug may slow the rate of weight gain in both diabetic and non-diabetic rats. In a related study, Scorgie *et al.* (2019) reported that albino rats on a high-fat diet maintained stable body weight after being administered Viagra, possibly due to increased metabolism of stored fat. This was not a result of elevated physical activity but rather a transformation of white adipose tissue to brown fat known for its fat-burning properties.

McCarberg (2020) similarly observed significant weight gain in male Wistar rats administered aphrodisiacs. In contrast, organ weight loss was reported in all groups receiving 30 mg/kg of sildenafil. This contradicts Bowling (2018), who found no significant changes in body weight, testicular weight, or relative tissue weight index in any of the study groups, concluding that sildenafil citrate did not influence these parameters.

Sperm structure and function are critical for successful fertilization and reproductive success. Sperm quality and count play pivotal roles in male fertility, especially in assisted reproductive techniques like in vitro fertilization. Short-term use of sildenafil has been shown to enhance sperm function and trigger the acrosomal reaction (Vilakati, 2019). However, the present study observed that long-term administration of 10 mg/kg of Viagra resulted in mild histopathological changes in the testes and cardiac tissue. Three testis samples from the sildenafil group showed mild necrosis in cardiac fibers, degeneration of seminiferous tubules, interstitial congestion, Leydig cell hypertrophy, and spermatogonial degeneration (Plates 4, 5, and 6). In contrast, control rats showed normal testicular architecture (Plate 1). These findings align with those of Balasubramani *et al.* (2021), who reported no significant cardiac histological changes in sildenafil-treated Wistar rats. Their heart tissue samples showed normal cardiac myocytes in organized arrays. However, our results differ from those of Zhang (2021), who reported significant necrotic and inflammatory effects of Viagra on cardiac tissue in a Wistar rat model. Similarly, Bello and Isah (2018) divided 20 male albino Wistar rats into four groups (n=5). One group received distilled water, while the others received sildenafil at 50, 100, or 200 mg/kg for 28 days. Following the treatment period, significant reductions in sperm count and normal morphology, along with increased abnormalities, were observed in all Viagra-treated groups. Histopathological analysis revealed testicular alterations at all dose levels. This supports the

current study's conclusion that prolonged use of sildenafil can adversely affect sperm quality and fertility. Del Mar Sánchez-Fuentes *et al.* (2022) also investigated the effects of both short- and long-term sildenafil exposure in rats. In their study, 8 groups were treated with either single or continuous doses of sildenafil and ethanol for up to 45 days. Observable morphological changes in the testes were reported compared to the control group, indicating tissue structural disruption.

Our current findings also showed that administering 10 mg/kg of sildenafil significantly increased monocyte (MON) and granulocyte (GRAN) levels. Total WBC and lymphocyte counts were also elevated, indicating immune system activation. This is consistent with Brena and Sander (2021), who suggested that elevated WBC levels may reflect an active cell-mediated immune response beneficial for survival.

A similar trend was observed by Ezumah (2023), who found increased WBC counts in both diabetic and non-diabetic rats treated with sildenafil, particularly in Groups A and B. This supports our findings, confirming that sildenafil citrate can stimulate leukocyte production in treated rats.

5.1 Conclusion

Administration of sildenafil citrate at a dose of 10 mg/kg body weight resulted in:

An increase in monocytes (MON) and total white blood cell (WBC) count, indicating immune system activation. A decrease in granulocytes (GRAN) and lymphocytes at certain stages. A general reduction in red blood cell (RBC) count and hemoglobin levels, which may impair oxygen delivery and energy production. Histopathological changes in cardiac and testicular tissues, suggesting possible impacts on spermatogenesis and male reproductive function.

5.2 Recommendations

Gaining insight into the molecular downstream effects of long-term exposure to PDE5 inhibitors through basic and clinical research can aid in guiding the appropriate use of aphrodisiac substances. Such understanding may help optimize corrective strategies by supporting the short-term administration of aphrodisiac agents at low doses.

The use of scanning electron microscopy can potentially make a histopathological study to be more apparent. The use of scanning electron microscope for evaluation of the changes in the testis is recommended for more suitable observation of the surface and morphological shapes of the tissue structures. Further study using graded dose of sildenafil may reveal a better relationship between the effect of sildenafil and the measured parameters in this study.

5.3 Contribution to knowledge

1. **Internal Organs and Cells Effects:** This study has affirmed that the use of substances perceived to be aphrodisiacs such as Viagra (sildenafil) has proportionally increased the risk of internal organ complications such as necrosis of cardiac fibers, semeniferous tubules and the interstitial tissue, congested blood vessels, hypertrophy of the interstitial leydig cells and degeneration of the spermatogonial cells.
2. **Potential Weight Flunctuation and Complication Effects:** It has also affirmed the possibility of the drugs to contribute to weight gain and weight loss. This implies that users of Viagra are likely to develop one or more complications from the usage such as the weight issue.
3. **Histopathological Insight:** The histopathological findings provided by this study offers confirmation that consistent use of Viagra can potentially contribute to this growing

testicular problem and testicular issues. In southern part of Nigeria alone, there are 26 cases of testicular and para-testicular issues with an average incidence of 1.5 cases per year. The incidence of testicular cancer in some studies was 0.55 per 100,000 population (95% CI, 0.52–0.57) and accounted for 1.1% of all male cancers. Rhabdomyosarcomas were the most common variety (70% of the paratesticular tumors and 26.8% of all tumors of the testis). Seminomas comprised 50% of the germ cell tumors and 15.4% of all testicular tumors in this series.

Overall, the study shows that consistent use of Viagra and further study may potentially contribute to further histopathological findings and changes

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.
 - a. Feeding flat plate
 - b. Feeding water bottles
 - c. Feed (pellets)
 - d. ISOL disinfectant
 - e. Digital thermometer
 - f. Plastic cage
 - g. Weighing balance
 - h. Indian ink and plate

2. For Sacrificing
 - a. Hand gloves
 - b. Sterile Lancet
 - c. Cotton wool
 - d. Chloroform
 - e. Plastic container sterile with a cover
 - f. Dissenting set
 - g. Sterile containers
 - h. Formalin

3. Histology Laboratory
 - a. Scrape blade

- b. Spatula
- c. Block holder
- d. Automatic tissue processor
- e. Molten basket
- f. Tissue basket
- g. L-shaped mould
- h. Rotary type microtome
- i. Water bath
- j. Hot plate
- k. Metal pencil
- l. Slides and cover slip
- m. Stain (Haematoxylin and eosin)
- n. Binocular microscope
- o. Dibutylphthalate polysterene xylene (DPX),
- p. Xylene, alcohol and water

APPENDIX II

PROCEDURE FOR TISSUE PROCESSING

Histopathologically. the whole organ (that is the testis) were autopsied, stained using hematoxylin and eosin staining techniques to demonstrate general tissue structure and then viewed microscopically. The procedure involved includes:

TISSUE (testis) processing using manual method. Sequences for manual tissue processing were as follows:

Harvesting Tissue: The required tissues (testis) were harvested from the animals and immediately put in a fixative. All parts of the required tissue that showed obvious microscopic changes were essentially selected for sampling. Tissues were cut into thin slices of 3mm by size.

Selection of Tissue: The testis (oval-shaped) and were colored. They were pinkish to light brown in the scrotum. It is part of the male reproductive system. It is located outside the body, suspended in the scrotal sac, and is connected to the spermatic cord, lying between the epididymis and the start of the vas deferens.

Fixation: The fixation used was 10% Bouin fluid (prepared using a saturated picric acid solution by dissolving 13.6 g picric acid in 100 mL distilled water, mix 75 mL saturated picric acid solution with 25 mL 40% formaldehyde solution, add 5 mL glacial acetic acid), was carried out for 24 hours to ensure proper fixing of the testicular tissues.

Dehydration: Tissues was dehydrated by using increasing strength of alcohol from 70%, 90% and absolute alcohol. All at varying interval of time to ensure proper dehydration. The volume of alcohol used was 50 - 100 times of that of tissues.

70% alcohol	2hours
90% alcohol	2hours

95% alcohol	2hours
Absolute alcohol I	2hours
Absolute alcohol II	2hours
Absolute alcohol III	2hours

Clearing: Tissues was cleared by passing the tissue through two changes of xylene.

Xylene I	90 minutes
Xylene II	90 minutes

Impregnation with Wax: This was carried out at the melting point temperature of paraffin wax; volume of wax was about 25 - 30 times the volume of tissues. The duration of impregnation lasted for two hours each in two changes of wax to ensure proper impregnation.

Paraffin wax I	2hours
Paraffin wax II	2hours

Embedding: Impregnated tissues were placed in molds (tissue cassette) with their labels and then fresh melted wax was poured in it and allowed to settle and solidify. Afterwards they were immersed in cold water to cool it rapidly. After the blocks were completely cooled, they were cut into individual blocks and each trimmed.

Staining of Processed Tissues Principle: Hematoxylin is a basic dye and thus has affinity for the acidic part of the cellular component which is the nucleus. Therefore, the nucleus stains blue while eosin on the other hand is an acidic dye thus has affinity for the basic component of the cells which is the cytoplasm therefore it stains it pink which is the color of the dye. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

APPENDIX III

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

1. The section was dewaxed in two changes of xylene for 2minutes each.
2. The section were taken through descending grades of alcohol. From absolute alcohol for 2minutes to 90% alcohol for 1minutes, 70% alcohol for 1minutes
3. The slides were washed in running tap water for one minutes.
4. Tissue sections were stained in hematoxylin for 10minutes
5. The sections was rinsed in distilled water for 30 seconds.
6. The sections was then differentiated in 1% acid alcohol for 15seconds
7. After that, the sections were rinsed in distilled water for 5minutes.
8. The sections was counterstained with 1% eosin for 5minutes
9. The sections was washed in running tap water for 30seconds
10. Sections was dehydrated by passing through ascending grades of alcohol (70%, 90%, and 100%) for 1minutes each.
11. The section was cleared in two changes of xylene for 2minutes each
12. The section was mounted with DPX and viewed microscopically using the objectives lens.