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***LABORATORY OF PHARMACOLOGY AND TOXICOLOGY***

# Assessment of the effect of the aqueous extract of *Terminalia superba* on the reproductive function of male rats

Dissertation presented in partial fulfilment of the requirements for the obtention of a Master's degree in Biochemistry

Option: **Biotechnology and Development**



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**Academic year : 2024-2025**

**DEDICATION**

To my parents:

WELEPE Harris Dael and KAMENDJIE Cladis Yvonne.

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I express my deepest gratitude **to God Almighty**, for the breath of life, the strength, and the grace He has abundantly provided throughout this journey. Without His divine guidance, this work would not have been possible.

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## LIST OF ABBREVIATIONS

**AE:** Aqueous extract

**DW:** Distilled water

**EF:** Ejaculation frequency

**EL:** Ejaculation latency

**IF:** Intromission frequency

**IL:** Intromission latency

**IM:** Intramuscular

**MDA:** Malondialdehyde

**MF:** Mount frequency

**ML:** Mount latency

**NPM:** Non progressive mobility

**PM:** Progressive mobility

**ROS:** Reactive oxygen species

**SOD:** Super oxide dismutase

***T.superba*:** *Terminalia superba*

**TE:** Testosterone enanthate

**TS:** *Terminalia superba*

**ABSTRACT**

Male reproductive health is essential for fertility and also plays a key role in a fulfilling sex life. Increasing attention has been given to natural compounds these recent years for their potential to enhance reproductive function. The aim of this study was to evaluate the effect of the aqueous extract of *Terminalia superba* on the reproductive function of normal male rats. For this study, twenty-five (25) adult male rats (12 weeks old, 150–180 g) were randomly divided into five groups (n=5). Group I received distilled water (10 ml/kg) orally, Groups II–IV were administered *Terminalia superba* aqueous extract at doses of 43, 86, and 172 mg/kg orally, and Group V received testosterone enanthate via intramuscular injection once a week. Sexual behaviour was assessed before and after the treatment. On the 29th day, blood and androgen-dependent organs (testes, epididymis, seminal vesicles, and prostate) were collected for biochemical, histological, and sperm analyses. The extract significantly enhanced ($p < 0.05$) sexual performance, with the highest dose (172 mg/kg) producing the greatest increase in mounts, intromissions, and ejaculations. Biochemical analysis showed that treatment with 172 mg/kg of the extract increased cholesterol and fructose levels by 58.1% and 40.8%, respectively, and testosterone levels by over 5-fold compared to the control group. Histology of the testes showed an increase in the concentration of sperm at all extract doses. Furthermore, sperm analysis indicated enhanced motility, especially at higher doses (86 and 172 mg/kg). However, markers of oxidative stress (SOD, catalase, MDA) remained largely unchanged. Overall, these findings suggest that *Terminalia superba* aqueous extract improves male reproductive function, with stronger effects at higher doses.

**Key words:** Afrodisiac activity, androgenic activity, *Terminalia superba*, aqueous extract, male rats.

**RÉSUMÉ**

La santé reproductive masculine est essentielle pour la fertilité et joue également un rôle clé dans une vie sexuelle épanouie. Ces dernières années, une attention croissante a été accordée aux composés naturels pour leur potentiel à améliorer la fonction reproductive. Le but de cette étude était d'évaluer l'effet de l'extrait aqueux de *Terminalia superba* sur la fonction reproductive des rats mâles normaux. Pour cette étude, vingt-cinq (25) rats mâles adultes (âgés de 12 semaines, 150–180 g) ont été répartis aléatoirement en cinq groupes (n=5). Le groupe I a reçu de l'eau distillée (10 ml/kg) par voie orale, les groupes II–IV ont reçu l'extrait aqueux de *Terminalia superba* aux doses de 43, 86 et 172 mg/kg par voie orale, et le groupe V a reçu de l'énanthate de testostérone par injection intramusculaire une fois par semaine. Le comportement sexuel a été évalué avant et après le traitement. Au 29$^{e}$ jour, le sang et les organes androgéno-dépendants (testicules, épididyme, vésicules séminales et prostate) ont été prélevés pour des analyses biochimiques, histologiques et spermatiques. L'extrait a significativement amélioré ($p < 0,05$) la performance sexuelle, avec la dose la plus élevée (172 mg/kg) produisant la plus forte augmentation des montées, intromissions et éjaculations. L'analyse biochimique a montré qu'un traitement avec 172 mg/kg de l'extrait augmentait les niveaux de cholestérol et de fructose de 58,1 % et 40,8 % respectivement, et les niveaux de testostérone de plus de 5 fois par rapport au groupe témoin. L'histologie des testicules a montré une augmentation de la concentration de spermatozoïdes à toutes les doses de l'extrait. De plus, l'analyse du sperme a indiqué une motilité accrue, en particulier aux doses plus élevées (86 et 172 mg/kg). Cependant, les marqueurs de stress oxydatif (SOD, catalase, MDA) sont restés largement inchangés. Dans l'ensemble, ces résultats suggèrent que l'extrait aqueux de *Terminalia superba* améliore la fonction reproductive masculine, avec des effets plus prononcés à des doses plus élevées.

**Mots-clés** : Activité aphrodisiaque, activité androgénique, *Terminalia superba*, extrait aqueux, rats mâles.

## INTRODUCTION

Reproduction is the process by which organisms give rise to offspring. In multicellular eukaryotes such as animals, sexual reproduction is predominant and it involves the fusion of a male and female gamete **(Yadav *et al.*, 2023)**. The male gamete plays a crucial role in this process for it is the gamete responsible for fertilisation. The male reproductive function is ensured by a series of organs that produce, transport, and deliver viable sperm for reproduction **(Gurung *et al.*, 2023)**. The male reproductive system is made up of the external reproductive organs which are the penis and the scrotum and the internal reproductive organs which are the testes, epididymis, seminal vesicles, bulbourethral glands and prostate gland. Each of them has a particular role and a proper functioning of these organs will lead to the production of gametes **(Mjaess *et al.*, 2022).** However, a default in the structure and/or functioning of these organs might lead to certain disorders such as hypogonadism, erectile dysfunction, premature ejaculation and infertility (**Salonia *et al.*, 2021)**.

Among these conditions, erectile dysfunction and male infertility are particularly significant due to their high prevalence and profound effects on male reproductive health and quality of life. They can be caused by some factors such as diet, medications, genetic conditions, environmental toxins, and lifestyle habits (**Tesarik, 2025**). These disorders are statistically significant in terms of global prevalence and impact. The worldwide prevalence of erectile dysfunction goes up to 76.5% (**Kessler *et al.*, 2019**) and males are responsible for 50% of infertility cases, highlighting their significant role in reproductive health (**Leslie *et al.*, 2023**). These disorders can have many consequences such as depression, anxiety, lower self-esteem and general psychological distress, and (**Biggs *et al.*, 2023**).

In an attempt to address male reproductive disorders, various methods such as medications (e.g., Androtardyl, Sildenafil), surgery, and assisted reproductive techniques are commonly used. However, these approaches may not always be effective and can sometimes come with significant side effects. For instance, medications like Androtardyl can cause high blood pressure, Sildenafil can cause prolonged erections, which may be problematic. Additionally, these treatments are often expensive (**Azonbakin *et al.*, 202**). Hence, the World Health Organisation (WHO) recommends the use of medicinal plants because they are available, affordable and healthy and suggests that researchers define their rational use as a potential source of new treatments (**Ghutke *et al.*, 2023**). These medicinal plants represent a valuable therapeutic resource due to the secondary metabolites they contain, such as phenols, alkaloids, and terpenes. (**Hilal *et al.*, 2024**) Among these numerous plants, *Terminalia superba*, a plant

from the family of the Combretaceae has numerous therapeutic properties. The methanolic extract of the leaves of *T. superba* was shown to possess antibacterial and anti-inflammatory properties (**Ani *et al.*, 2023**). Also, the alcoholic extract of the barks of *T. superba* was shown to possess strong antioxidant properties (**Beboy *et* al., 2024**). In addition, the aqueous extract of the leaves has been found to possess anti-plasmodial properties (**Mbouna *et al.*, 2018**). Equally, the ethanolic extract of the barks of *T. superba* was shown to have anti-ulcer properties (**Savitha *et al.*, 2023**). Moreover, the aqueous extract of the barks of *T. superba* possess anti-pyretic properties (**N'Dia *et al.*, 2021**). The aim of this present study will be to determine if the aqueous extract of the barks of *Terminalia superba* has an effect on the male reproductive function.

**Research hypothesis**

The administration of the aqueous extract of the barks *T. superba* improves the reproductive function of normal male rats.

**General objective**

Evaluate the positive effect of the aqueous extract of the barks *T. superba* on the reproductive function of male rats.

**Specific objectives**

- Determine the effect of the aqueous extract of the barks of *T. superba* on the sexual behaviour of male rats by measuring mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency and ejaculation frequency;
- Determine the effect of the aqueous extract of the barks *T. superba* on sperm quality and some male reproductive markers such as cholesterol, testosterone and fructose;
- Determine the effect of the aqueous extract of the barks *T. superba* on some markers of oxidative stress namely catalase, superoxide dismutase and malondialdehyde.

## I-1. Overview of male reproductive anatomy and physiology

The male reproductive system (Figure 1) is a collection of organs which work together for sperm production, storage, ejaculation, and the production of important androgens like testosterone. It includes internal structures such as the testes, epididymis, vas deferens, and prostate, as well as external components like the scrotum and penis. Together, these organs sustain male fertility and sexual health. (**Gurung *et al.*, 2023**).

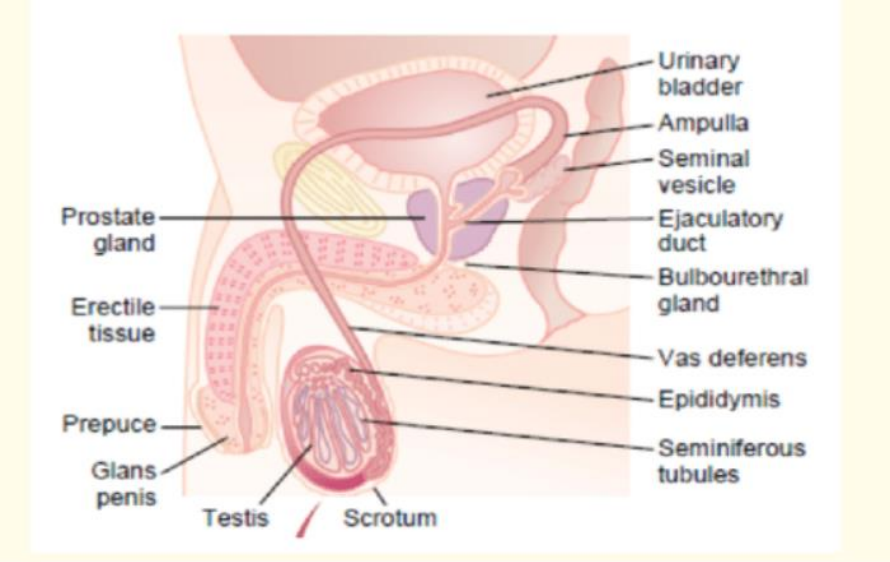


**Figure 1**: Male Reproductive System (**Udom *et al.*, 2021**)

### I-1.1. The Testes

The testes are paired, oval-shaped structures located outside the body within the scrotum, separated by the scrotal septum (Figure 2). Typically, they are about 4 to 5 cm in length and 2 to 3 cm in width. The testis is made up of three distinct layers: the tunica vaginalis, tunica albuginea, and the tunica vasculosa. They receive their blood supply from the testicular arteries (**Tiwana *et al.*, 2023)**. The testicle is sectioned into compartments by septa, which are extensions of the tunica albuginea. Each septum divides the seminiferous tubules and the interstitial tissue, the latter of which contains Leydig cells, blood vessels, lymphatics, mast cells, nerves, and macrophages. Each testis is divided into 200 to 300 lobules, with each lobule containing tightly coiled seminiferous tubules. The primary functions of the testes are the production of sperm and the secretion of androgens, particularly testosterone (**Goldstein *et al.*, 2023**).

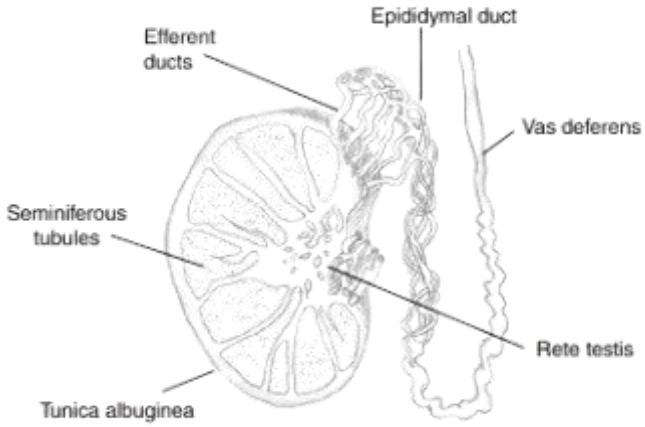


**Figure 2**: Transverse section of the testis (**Goldstein *et al.*, 2023**)

### I-1.1.1 Seminiferous Tubules

The seminiferous tubules are the site where sperm cells are produced. These are coiled tubules which are looped and connect to the rete testis, which is a network of small tubes. The rete testis then joins together to form the efferent ducts, which carry sperm and testicular fluid to the head of the epididymis. The seminiferous tubules are mainly made up of Sertoli cells and germ cells (which develop into sperm) (**Goldstein *et al.*, 2023**).

### I-1.1.2. Sertoli Cells

Sertoli cells are special support cells found in the testes. They play an important role in how sperm cells are made. They release many different proteins and signals that help control each step of sperm development, from the early dividing cells to fully mature sperm (**Xiao *et al.*, 2025**).

### I-1.1.3. Exocrine function of the testes (Spermatogenesis)

Spermatogenesis is a complex process of cell differentiation that ultimately results in the formation of fully developed, haploid, motile spermatozoa. Spermatogenesis begins with spermatogonia which are immature germ cells which are found at the basement membrane of seminiferous tubules (**Ekementeabasi, 2024**). Spermatogonia will undergo a first mitotic division to form primary spermatocytes. Primary spermatocytes undergo a first meiotic division to form secondary spermatocytes. The latter will undergo a second meiotic division to produce spermatids. These spermatids eventually undergo a transformation called spermiogenesis, where they develop tails, condense their nuclei, and form acrosomes, essential for fertilization (Figure 3). Throughout this process, support and nourishment is provided by the Sertoli cells (**Obukohwo *et al.*, 2021**).

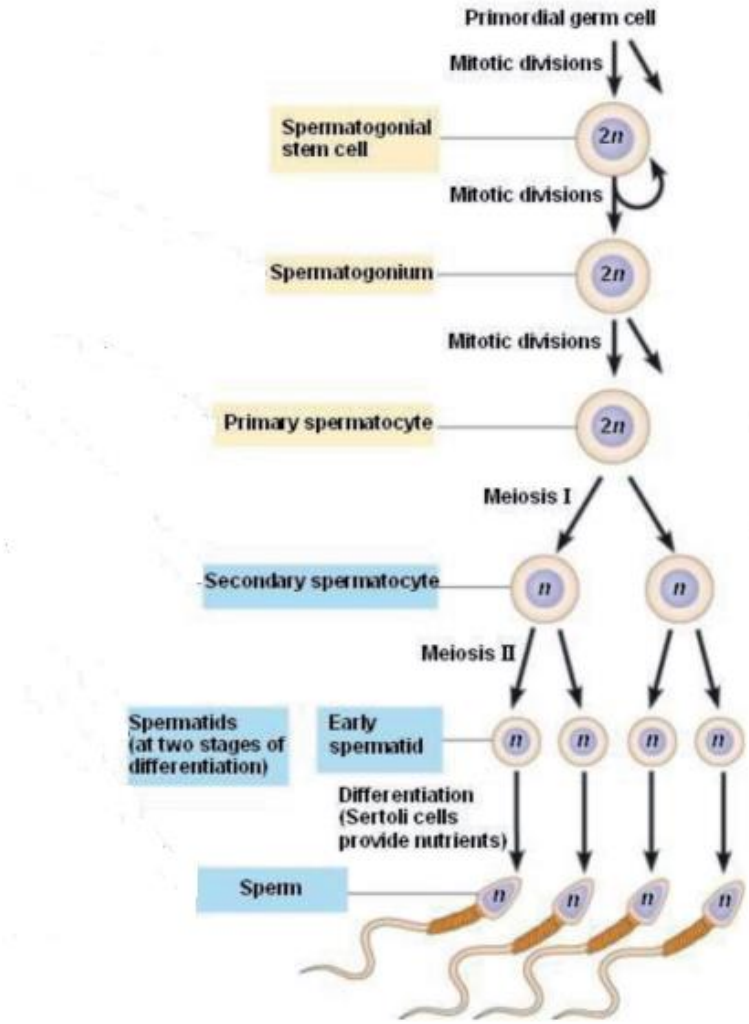


**Figure 3**: Spermatogenesis (**Goldstein *et al.*, 2023**)

### I-1.1.4. Endocrine function of the testes

This involves the production of male reproductive hormones known as androgens. Androgens are crucial for the development and maintenance of male reproductive organs, such as the testes, prostate, epididymis, seminal vesicles, and penis. Additionally, they contribute to the development of other male features, including increased muscle mass, hair growth and equally sexual activity. The primary androgen responsible for these effects is testosterone (**Emojevwe *et al.*, 2021**).

#### I-1.1.4.1. Testosterone

Testosterone or 17β-hydroxyandrost-4-en-3-one (Figure 4**)** is a steroid hormone produced by the Leydig cells of the testes. Testosterone production in the testes is controlled by the hypothalamic-pituitary axis. The hypothalamus releases gonadotropin-releasing hormone

(GnRH) into the bloodstream, which then signals the pituitary gland to release luteinizing hormone which reaches the testes and stimulates Leydig cells to produce testosterone (**Nassar *et al.*, 2023**).

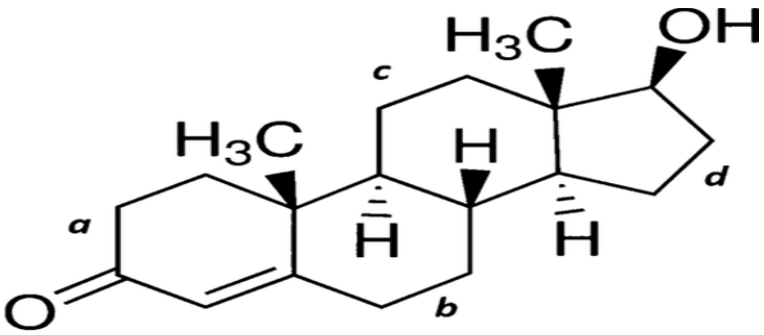


**Figure 4*:*** Structure of testosterone (**Leon *et al.*, 2021**)

**I-1.1.4.2. Biosynthesis of testosterone**

Testosterone is synthesized from cholesterol, a fat molecule that serves as a building block for various hormones in the body. Cholesterol can be obtained from the breakdown of low-density lipoproteins. The Cytochrome P450 enzyme, found in the inner mitochondrial membrane of Leydig cells, helps break down cholesterol by adding hydroxyl groups to the C22 and C20 positions, turning it into pregnenolone. This pregnenolone is then moved to the smooth endoplasmic reticulum (ER), where it is converted into testosterone through two different pathways; The Dehydroepiandrosterone (DHEA) pathway and Progesterone pathway (Figure5) (**Emojevwe *et al.*, 2021**).

**Dehydroepiandrosterone (DHEA) Pathway**: In this pathway, cholesterol is first converted to pregnenolone through the action of cAMP. Pregnenolone is then converted into 17α-hydroxypregnenolone by 17α-hydroxylase. In Leydig cells, this compound is further processed into dehydroepiandrosterone (DHEA) via the activity of 17, 20-lyase. Two subsequent processes occur. First, DHEA is transformed into androstenediol by 17-hydroxysteroid dehydrogenase (17-HSD), and then into testosterone through the combined activities of 3β-hydroxysteroid dehydrogenase (3β -HSD) and 5-4 isomerase. In a second pathway, DHEA is converted into androstenedione by the action of 3-HSD and 5-4 isomerase, and then converted to testosterone through 17-HSD activity (**Lawrence *et al.*, 2022**). ~**Progesterone Pathway**: In this pathway, most pregnenolone is converted to progesterone by the actions of 3β-HSD and 5, 4 isomerase. The enzyme 17-hydroxylase then converts progesterone into 17-hydroxyprogesterone, which is further processed into androstenedione by 17, 20-lyase. Finally, androstenedione is converted into testosterone through the activity of 17-hydroxysteroid dehydrogenase (17β-HSD) (**Emojevwe *et al.*, 2021**).

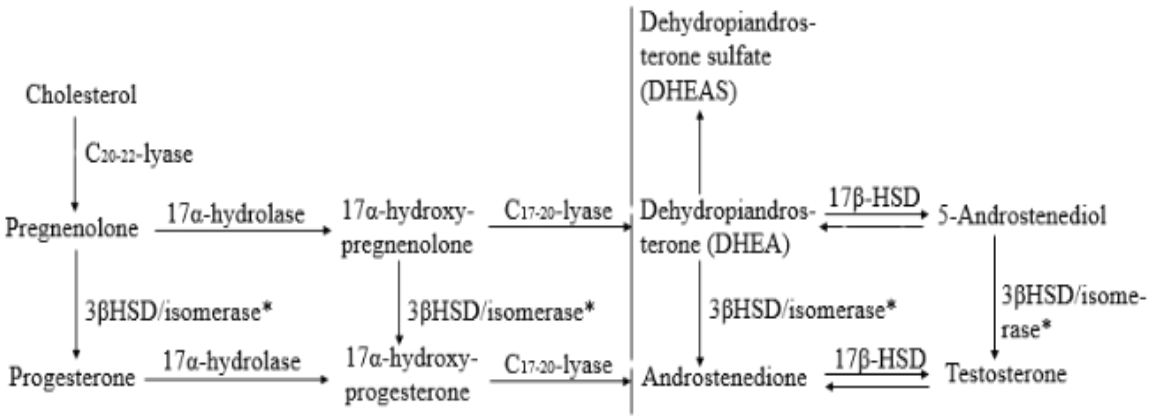


**Figure 5:** Biosynthesis of Testosterone (**Obukohwo *et al.*, 2021**)

### I-1.1.5. The sperm cell

The male gamete is called sperm cell. It consists of a head, a midpiece, and a tail. It plays a key role in continuing life by delivering the male partner's genetic material and proteins to the female egg during fertilization (**Teves *et al.*, 2022**). Figure 6 shows the structure of the sperm cell.

The sperm head has an elongated, oval shape and consists primarily of a nucleus, which houses the condensed chromatin and the acrosome. The acrosome is a membrane-enclosed structure that contains hydrolytic enzymes required for the sperm to penetrate the egg prior to fertilization. The sperm neck contains the sperm centrioles, which are vital for the early stages of embryo development. (**Alves *et al.*, 2020**).

The middle piece contains numerous mitochondria which produces ATP hence supplying the tail with energy necessary for movement of the sperm cell in the female reproductive tract to reach the oviduct for fertilisation (**Obukohwo *et al.*, 2021)**.

The tail has a flagellum which helps the sperm cells to displace itself in the female reproductive tract. The sperm tail is covered by the axoneme along its entire length, along with extra structures called peri axonemal structures, except for the short end piece, which lacks these additional parts. The end piece only has the axoneme surrounded by the plasma membrane. Under a microscope, the axoneme appears as a structure made of microtubules, consisting of nine outer pairs of microtubules and one central pair (9 + 2), connected by radial spokes and dynein arms (**Kumar *et al.*, 2020**).

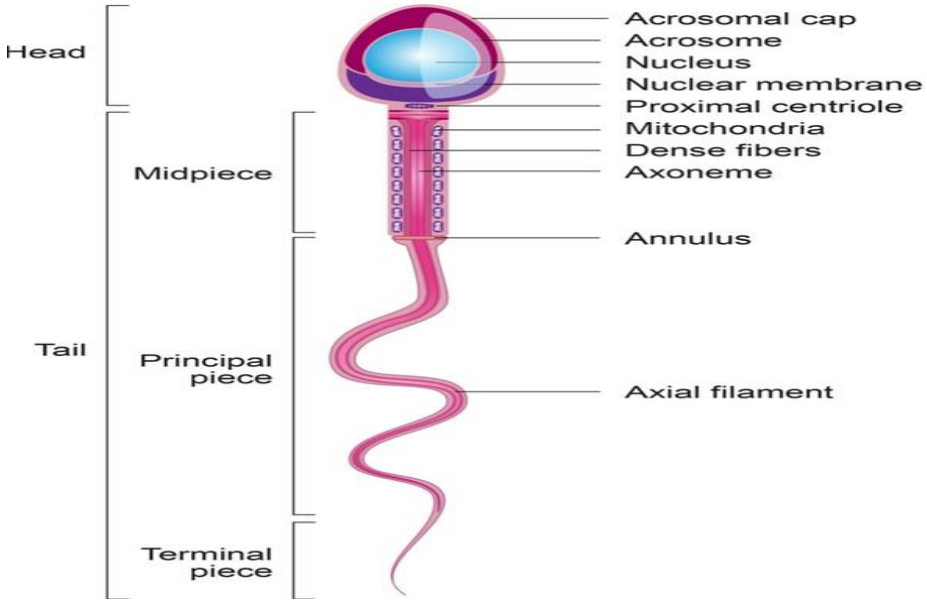


**Figure 6:** Sperm cell (**Alves *et al.*, 2020**)

### I-1.1.6. Capacitation and Acrosome reaction

Capacitation refers to a morphological change that sperm undergo, involving hyperactivation and an acrosome reaction, which is necessary for the sperm to be able to fuse with the egg. Sperm motility hyperactivation is marked by an increase in tail movement amplitude and an asymmetrical pattern of movement, which allows the sperm to penetrate the egg's zona pellucida. This process is also associated with the acrosome reaction, where enzymes like acrosin are released from the sperm head to break down the cumulus cells and help the sperm pass through the zona pellucida. (**Obukohwo *et al.*, 2021**).

### I-1.2. Epididymis

The epididymis is a single, elongated and highly coiled tube with three parts; head (caput), body (corpus) and tail (cauda) (**Gocht *et al.*, 2024**). The primary function of the epididymis is to transport and store spermatozoa produced in the testes. Within this duct, sperm undergo critical maturation processes, allowing them to develop the necessary motility and functionality for successful fertilization. Throughout this process, sperm become more concentrated as they move from the caput (proximal end) to the corpus and finally to the cauda (distal end) of the epididymis. The epididymis serves as a reservoir, holding the mature sperm until they are needed during ejaculation (**Wang *et al.*, 2024**).

### I-1.3. Prostate

The prostate is a fibromuscular elastic gland that is shaped like a donut. It plays a role by secreting a milky fluid which contains several important substances, including phosphate acids, zinc, and calcium. Each of these components is vital for the normal functioning of

spermatozoa, supporting their motility and overall health. The secretions from the prostate contribute approximately 30 percent of the total volume of seminal fluid (**Obukohwo *et al.*, 2021**).

### I-1.4. Seminal vesicles

The seminal vesicles are situated in the pelvis, positioned above the rectum, below the bladder's fundus, and behind the prostate. The typical seminal vesicle is a single, coiled, blind-ended tube with several irregular pouches branching off from it. It typically has a volume of about 14 mL, with a length ranging from 3 to 5 cm and a diameter of around 1 cm, though it measures approximately 10 cm when uncoiled. The seminal vesicles provide approximately 50-80% of the fluid that ultimately forms semen but does not store the latter. The fluid produced by the seminal vesicles provides spermatozoa nutrition and promotes mobility because it contains fructose which serves as a high energy source for sperm cells (**Gaspar *et al.*, 2024**).

### I-1.5. Penis

The penis is composed of three cylindrical columns of erectile tissue and the penile urethra, all encased in skin (Figure 7). Two of these columns, known as the corpora cavernosa, are positioned on the dorsal side, while the ventral corpus spongiosum encases the urethra (**Panchatsharam, 2023**). At its end the corpus spongiosum expands, forming the glans. The 2 main functions of the penis are intercourse and urination. For sexual intercourse to occur, it is typically necessary for the penis to be in an erect state. This involves the penis becoming firm and enlarged due to increased blood flow, which facilitates penetration and effective engagement in the sexual activity which can eventually lead to ejaculation (**Sam *et al.*, 2023**).

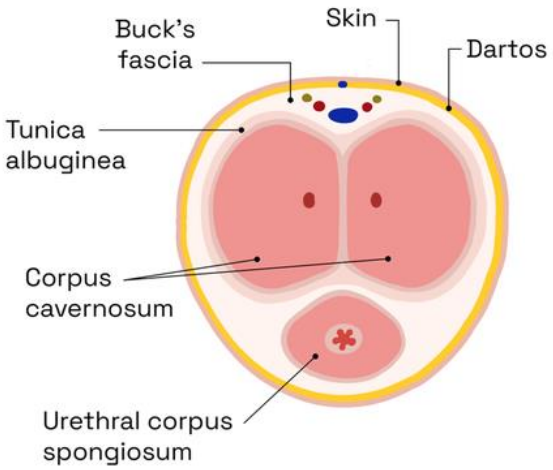


**Figure 7:** Cross section of penis (**Novacescu *et al.*, 2023**)

### I-1.5.1 Erection

Penile erection is the physiological process where the penis fills with blood and becomes engorged. It involves a series of neurologic and humoral events initiated by stimuli such as visual, auditory, and olfactory signals and local stimulation of the penis typically leading to sexual arousal (**Akorede *et al*., 2024**).

When the penis becomes erect, the body undergoes a series of changes; Initially, signals from the sympathetic nerves that typically inhibit arousal are reduced, while signals from the parasympathetic nerves that promote it are increased. This leads to the release of pro-erectogenic neurotransmitters from the cavernous nerves, which help relax the smooth muscles in the penis. As these muscles relax, the levels of calcium inside the cells decrease. This relaxation causes a significant increase in blood flow, expanding the small blood vessels in the penis by about 20 to 40 times. As these vessels enlarge, they compress the veins that normally drain blood from the penis, helping to maintain the erection by preventing blood from flowing out **(Sam *et al*., 2023).**

Muscle contractions around the penis help increase pressure in all parts of the penis, including the glans (tip), making it more rigid. During sexual activity, these muscles contract strongly, pushing more blood into the penis and increasing rigidity. This is called the rigid erection phase, where pressure can become very high. Later, as the veins expand and start to drain blood, the pressure decreases slowly. Finally, pressure drops quickly as normal blood flow returns and venous drainage is fully restored **(Sam *et al*., 2023)**.

### I-1.5.2. Ejaculation

Ejaculation occurs when semen is released and externally expressed from the male reproductive system. Normal ejaculation is a process which involves a series of steps. It typically happens in three phases: emission, expulsion, and orgasm (**Crowdis *et al*., 2023**).

- During emission, seminal fluid from the seminal vesicles, prostate, and bulbourethral glands moves into the posterior urethra. At the same time, the internal sphincter tightens to close off the bladder, stopping semen from going into the bladder instead of out through the penis. This phase relies on the contraction of smooth muscles. (**Mason *et al*., 2022**).
- Expulsion is the part of ejaculation where semen moves from the posterior urethra to the opening at the tip of the penis. This process is mainly a reflex controlled by the spine and happens when you reach the "point of no return". The pressure needed for this comes from contractions of the pelvic floor muscles and the rhythmic squeezing of other muscles around the base of the penis. These contractions, which happen about every 0.8 seconds, increase the

pressure in the urethra, pushing the semen out through the opening at the tip of the penis (**Mason *et al*., 2022**).

- Orgasm is a quite short extremely pleasurable event. It is primarily a brain or cerebral process associated with various physical events, including contractions of accessory sexual organs **(Crowdis *et al*., 2023).**

## I-2. Reactive Oxygen Species and its impact on male reproductive health

### I-2.1. Reactive Oxygen Species (ROS)

Reactive Oxygen Species are highly reactive molecules due to the presence of an unpaired electron on the outermost shell. The most common reactive oxygen species are; hydroxyl radicals (•OH), superoxide anion ($\bullet O_2^-$) and hydrogen peroxide ($H_2O_2$). Reactive oxygen species are generated as part of natural cellular processes and play a role in the normal functioning of cells. Reactive oxygen species can have endogenous sources and exogenous sources (**Castleton *et al*., 2022**).

The primary source of ROS originates from two metabolic processes that generate energy: glycolysis and oxidative phosphorylation. Mitochondria are essential for ATP production in cells, and during this process, they also produce ROS. The electron transport chain and oxidative phosphorylation facilitate ATP synthesis by transferring electrons across the inner mitochondrial membrane complexes. This movement of electrons leads to the pumping of protons into the intermembrane space, which can cause a localized accumulation of ROS, particularly from complexes I and III (**Annegowda *et al*., 2022**).

However reactive oxygen species can also originate from exogenous sources such as; environmental pollution, lifestyle factors such as alcohol and smoking, obesity, varicocele, bacterial/viral infections, sexual transmitted disorders (**Mannucci *et al*., 2022**).

The production of ROS begins with the formation of a superoxide anion radical ($O_2^-$), which then undergoes dismutation reactions to form hydrogen peroxide ($H_2O_2$). Hydrogen peroxide is a stable molecule that can easily cross the plasma membrane, reaching both intracellular and extracellular compartments. Although hydrogen peroxide itself is a non-radical species, it can produce highly reactive hydroxyl radicals in the presence of transition metals like copper and iron. Through the Haber–Weiss reactions, an excess of $H_2O_2$ can lead to the creation of highly reactive radicals such as hydroxyl (OH) and alkoxyl ($OH^-$) radicals (**De Luca *et al*., 2021**).

### I-2.2. Role of reactive oxygen species in male reproduction

Sperm production takes place in the testes through the hormone-controlled process of spermatogenesis. A key phase for achieving fertilization ability, motility, and full maturation occurs in the epididymis. During this time, sperm are naturally exposed to ROS which are involved in essential processes such as sperm capacitation and the acrosome reaction. These processes are crucial for effective fertilization and demand high energy levels, which are provided by metabolic pathways like glycolysis and oxidative phosphorylation (**De Luca *et al*., 2021**).

ROS equally stimulate the production of cyclic adenosine monophosphate (cAMP) in sperm cells, which leads to the inhibition of tyrosine phosphatases and promotes tyrosine phosphorylation. This molecular pathway triggers the activation of various transcription factors, initiating intracellular signalling cascades that regulate processes such as sperm motility, chemotaxis, and chromatin condensation in maturing spermatozoa (**Mannucci *et al*., 2022**).

Additionally, ROS can enhance the sperm's ability to bind to the zona pellucida as it oxidizes and extrudes cholesterols within the membrane, increasing its fluidity, thereby facilitating the fusion between sperm and oocyte (**Mannucci *et al*., 2022**).

### I-2.3. Pathological role of ROS

Physiological levels of ROS are essential for regulating sperm functions; however, an excess of ROS can lead to potential damage to sperm cells. These effects include an increase in lipid peroxidation and DNA damage, which can compromise the structural integrity and functionality of the sperm (**Chakraborty & Roychoudhury., 2022**). Additionally, elevated ROS levels can result in a reduction in sperm motility, abnormalities in sperm morphology, and a decline in sperm viability. As a result, these factors are closely linked to a decrease in sperm fertility, potentially impairing the ability to fertilize an egg and reduce overall reproductive success. (**De Luca *et al*., 2021**).

### I-2.4. Role of the antioxidant system

The antioxidant system is a set of enzymatic and non-enzymatic components that work together to maintain normal cellular function and provide protection against excess ROS. This system includes important enzymes such as superoxide dismutase (SOD) and catalase which play key roles in neutralizing excess ROS and ensuring optimal cellular defence. (**De Luca *et al*., 2021**). SOD is a metalloenzyme that catalyses the conversion of superoxide anions and plays a crucial role in protecting polyunsaturated fatty acids (PUFAs), which are components

of the plasma membrane, and in the breakdown of DNA. SOD transforms superoxide anions ($O_2^-$) into less harmful molecules, oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$). By transforming superoxide radicals into less aggressive entities, SOD inhibits their reaction with nitric oxide preventing the formation of peroxynitrite, a highly reactive nitrogen species detrimental to both the sperm membrane and its DNA (**Sengupta *et al*., 2024**). Catalase is an enzyme that plays a vital role in breaking down hydrogen peroxide into water and molecular oxygen. This process is crucial for preventing the accumulation of hydrogen peroxide, which, if left unchecked, can cause cellular damage. By efficiently converting hydrogen peroxide into harmless substances, catalase helps safeguard cells from oxidative stress and maintains overall cellular integrity (**De Luca *et al*., 2021**).

### I-3. Sexual health and male reproductive disorders

The World Health Organization (WHO) describes sexual health as a state of physical, emotional, mental, and social well-being concerning sexuality, emphasizing that it is more than just the absence of disease, dysfunction, or illness (**De Jonge *et al*., 2024**). Various factors including the use of medications, genetics, environmental exposures, and lifestyle habits, can significantly affect male reproductive health. These elements may disrupt the normal functioning of the reproductive system, leading to potential disorders which can affect sexual health such as hypogonadism, erectile dysfunction, premature ejaculation and infertility (**Salonia *et al*., 2021**). These disorders can lead to consequences such as anxiety, stress, depression, low self-esteem and relationship breakup (**Biggs *et al*., 2023**).

#### I-3.1. Hypogonadism

Male hypogonadism is a condition characterized by reduced activity of the testes, leading to a decrease in the production and/or function of androgens such as testosterone and/or impaired sperm production. This condition arises from either impaired testicular function or insufficient stimulation of the testes by the hypothalamic-pituitary-gonadal (HPG) axis which releases gonadotropin-releasing hormone, which stimulates the secretion of luteinizing hormone, ultimately leading to the synthesis of testosterone (**Ugo-Neff *et al*., 2022)**. Male hypogonadism can be categorized based on the cause into: Primary hypogonadism, when it results from testicular dysfunction which affects testosterone production. and Secondary hypogonadism, when it is caused by dysfunction of the pituitary or hypothalamus and consequently, insufficient stimulation of the gonads to produce androgens (**Sizar *et al*., 2024)**.

### I-3.2. Erectile Dysfunction

Erectile dysfunction (ED) is described as the inability to achieve or sustain an erection that is adequate for fulfilling sexual activity. Lack of attraction, depression, lifestyle habits, stress can be a cause. However, organic diseases such as cardiovascular diseases, diabetes, neurological disorders, kidney diseases play a key role in increasing the risk of erectile dysfunction. (**Mazzilli *et al*., 2022**).

### I-3.3. Premature Ejaculation

According to International Society for Sexual Medicine (ISSM), Premature ejaculation (PE), whether lifelong or acquired, is a male sexual dysfunction characterized by the following: (1) ejaculation that consistently occurs either before or within approximately two minutes of vaginal penetration (lifelong PE) or a significant and troubling decrease in latency time (refers to the period of time between the initiation of sexual arousal or stimulation and the onset of ejaculation), often to around three minutes (acquired PE) (**Crowdis *et al*., 2023**),; (2) the inability to control or delay ejaculation during most or all vaginal penetrations (**Salonia *et al*., 2021**).

### I-3.4. Infertility

Infertility is a medical condition of the reproductive system, characterized by the inability to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse between a couple. According to the World Health Organization (WHO), around 9% of couples face fertility issues worldwide, with male factors accounting for nearly 50% of the cases (**Gül *et al*., 2024**). Some causes of infertility are quality and quantity of semen (sperm abnormalities), genetics, lifestyle factors (smoking and alcohol), environmental factors (chemical dust, organic solvents, pesticides), medications (**Shah *et al*., 2021**).

## I-4. Methods of treatment of disorders of the male reproductive function

### I-4.1. Conventional treatment methods

Modern treatment methods for male reproductive disorders involve a combination of advanced strategies, including hormone therapies, medications, and surgical interventions. Hormone treatments, such as gonadotropins, are commonly used to stimulate testosterone production and restore hormonal balance, particularly in cases of hypogonadotropic hypogonadism (**Chan *et al*., 2023**). Medications like anabolic steroids such as testosterone enanthate and testosterone propionate are prescribed to regulate hormone levels and improve sperm production. Surgical options, such as varicocelectomy, address physical issues like varicocele or blockages in the reproductive tract. For cases of severe infertility, assisted

reproductive technologies (ART), such as invitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI), can provide effective solutions. Together, these treatments offer a comprehensive approach to enhancing male reproductive health and fertility (**Kaltsas *et al*., 2024**).

#### 1.4.1.1. Limits of conventional methods

These treatments provided by modern medicine are often expensive, difficult to access, and their effectiveness is sometimes questionable. Furthermore, these treatments can have numerous side effects, making them less desirable. Given these limitations, phytotherapy presents a promising and potentially safer alternative for addressing male reproductive health concerns (**Azonbakin *et al*., 2021**).

### I-4.2. Phytotherapy

For centuries, medicinal plants have been used as remedies for various health conditions. Their therapeutic benefits have been acknowledged and utilized by cultures worldwide. In recent years, there has been a renewed interest in medicinal plants as natural alternatives to synthetic medications, owing to their potential effectiveness and fewer side effects. For example, Turmeric (*Curcuma longa*) is known for its analgesic and anti-inflammatory properties, garlic (*Allium sativum*) possesses antimicrobial and antiviral effects, and green tea (*Camellia sinensis*) is rich in antioxidants, offering anti-aging benefits (**Ghutke *et al*., 2023**).

## I-5.Generalities on *Terminalia superba*

### I-5.1. Description

*Terminalia superba* Engl. & Diels is a species belonging to the family of Combretaceae. It is a deciduous tree, medium to large in size, reaching up to 50 meters tall and is generally straight, with a diameter of up to 150 cm. Picture 1 shows a picture of the bark of *Terminalia superba*. The bark usually has a grey-brown colour, the leaves are arranged alternately and gather at the ends of the branches and can be 12 to 18 cm long, and 5 to 8 cm wide (**Kougnimon *et al*., 2015**).

**Picture 1**: Photograph of *Terminalia superba* **(**Photograph taken by **Jignoua, 2020**)

### I-5.2. Geographical distribution of the plant

*Terminalia superba* is found in several countries and is known by a variety of local names according to the country, as shown in Table 1 below (**Kougnimon *et al.*, 2015**);

**Table 1:** Geographical distribution of *Terminalia superba* and local names

| **Country** | **Local name** |
|---|---|
| Cameroon | Akom |
| Benin | Afan |
| Ghana | Ofram |
| Gabon | Fraké |
| Ivory Coast | Fraké |
| Nigeria | Afara |
| Democratic Republic of Congo | Limba |
| Equatorial Guinea | Akom |
| France | Limba |
| Germany | Limba |
| USA | Korina |

### I-5.3. Phytochemical composition of the plant

*Terminalia superba* is characterized by a variety of phytochemicals namely alkaloids, phenols, flavonoids, coumarins, tannins and glycosides with the plant exhibiting high concentrations of phenols, flavonoids, coumarins, and glycosides while tannins are present in moderate amounts (**Keumedjio *et al*., 2023**).

### I-5.4. Pharmacological properties of the plant

Various studies have highlighted the pharmacological properties of *Terminalia superba*, revealing its wide range of therapeutic effects. As a matter of fact, the methanolic extract of the leaves has demonstrated strong antibacterial, and anti-inflammatory activities (**Ani *et al*., 2023**). In addition, the aqueous extract of the leaves has been found to possess anti-plasmodial properties (**Mbouna *et al*., 2018**). Also, the alcoholic extract of the barks of *T. superba* was shown to possess strong antioxidant properties (**Beboy *et* al., 2024**). Equally, the ethanolic extract of the barks of T. superba was shown to have anti-ulcer properties (Savitha et al., 2023). Moreover, the aqueous extract of the bark exhibits anti-pyretic effects (**N'Dia *et al*., 2021**). All of these emphasizing on the plant's diverse therapeutic potential

## II-1. Material

### II-1.1. Plant material

The barks of *Terminalia superba* were collected in 2020 at Eloundem I and were cut, dried, ground that same year and conserved at room temperature. The plant was identified at the National Herbarium of Cameroon under the number 655546.

### II-1.2. Animal material

The animals used were male and female wistar rats. Male rats were twenty-five (25) in number and weighed between 150-180g. Female rats used were ten (10) in number and they weighed between 110-120g. The rats were provided by the Laboratory of Biology and Animal organisms. The experience started with the approval of the Joint Institutional Review Board of Human and Animal Bioethics N$^{o:}$ **BTC-JIRB2024-110** (Appendix 1).

### III-I.3.Laboratory material and reagents

The reagents used were obtained provided by the MONOCENT Laboratory and the Q-LINEBIOTECH Laboratory. The equipment used, as well as the reagents and solution preparation protocols, are provided in the appendix

## II-2. Methods

### II-2.1. Preparation of plant extract

The plant extract was prepared using 750g of *Terminalia superba* powder, which was decocted with 7.5L of water, resulting in a 1:10 ratio. Seventy-five grams of the powder were added to a 1L round-bottom flask containing 750 mL of distilled water, and heated with a flask heater for 30 minutes. The mixture was subsequently filtered using cotton and then centrifuged at 1620 g at 4°C for 15 minutes. Following centrifugation, the supernatant was filtered again using filter paper. The resulting filtrate was dried in an oven at 60°C until the solvent evaporated completely. The extraction yield was calculated using the formula below:

$$\text{Yield (\%)} = \frac{\text{Mass of extract obtained (g)}}{\text{Mass of powder obtained (g)}} \times 100$$

### II-2.2. Dose determination

The dose determination was based on the traditional practitioner's recommendation of administering 3 glasses per day to a man weighing an average of 70kg. To achieve this, the extract was divided in 3 glasses and placed in an oven. After complete evaporation, the mass of the extract in one glass was found to be 2g, making a total of 6g for all 3 glasses. This enabled the calculation of the dose administered by the traditional practitioner, which is equivalent to 0.086g/kg or 86mg/kg. Using this initial dose, the second and third doses were

subsequently calculated to be 43mg/kg and 172mg/kg which are half of the initial dose and the double of the initial dose respectively.

### II-2.3. Experimental design

The experimental design lasted for 28 days and it involved twenty-five (25) male wistar rats divided into 5 groups as follows: (**Orieke *et* al., 2019**)

- Group I (negative control) received distilled water (10 mL/kg) orally;
- Group II received 43 mg/kg of aqueous extract of *T. superba* orally;
- Group III received 86 mg/kg of aqueous extract of *T. superba* orally;
- Group IV received 172 mg/kg of aqueous extract of *T. superba* orally;
- Group V (positive control) received 5mg/kg testosterone enanthate once a week by intramuscular injection.

Throughout the 28-day study period, the animals' body weights were recorded daily. On the 28$^{th}$ day, the animals underwent a muscular strength test followed by a sexual behaviour assessment. On the following day (the 29$^{th}$ day), the animals were terminated after being anaesthetized with an ether solution. Blood was then collected into dry tubes. The collected blood was subsequently centrifuged at 1620 g for 15 minutes at 4 °C to obtain serum. The resulting serum was carefully transferred into labelled Eppendorf tubes and stored at -20 °C for further analysis.

Organs such as the penis, testes, epididymis, prostate, and seminal vesicles were carefully dissected and weighed to assess any changes induced by the experimental conditions. Some of these organs—including the testes, seminal vesicles, prostate, and epididymis—were used to prepare tissue homogenates for biochemical analysis. The distal portion of the epididymis was specifically reserved for semen analysis, while the remaining organs were preserved in Bouin's solution for the preparation of histological sections and subsequent microscopic examination.

### II-2.4.Determination of the effect of *Terminalia superba* on the sexual behaviour of male rats

#### II-2.4.1. Sexual behaviour test

This test was conducted according to the method described by **Ali *et al*. (2012)**. Male rats were individually placed in a cage for a 10-minute acclimatization period. Following this, a receptive female rat was introduced into the cage for a duration of 30 minutes. After this time, the following copulation parameters were recorded;

- Mount latency (ML): Time interval in seconds from introduction of the female in the cage till first mount.
- Mount frequency (MF): The number of observed mounts.
- Intromission latency (IL): Time interval in seconds between the introduction of the female in the cage and the first intromission
- Intromission frequency (IF): The number of observed intromissions from the time of introduction of the female.
- Ejaculatory latency (EL): Time interval in seconds between the introduction of the female in the cage and the first ejaculation.
- Ejaculation frequency (EF): It is the number of times there was expulsion of semen after vaginal penetration.

### II-2.5. Determination of the effect of *Terminalia superba* on sperm quality and male reproductive markers

#### II-2.5.1. Sperm analysis

##### II-2.5.1.1. Sperm count

Immediately after the rats were killed, the tail of the right epididymis was removed and minced with the help of a pair of scissors in a beaker containing 10 ml of 0.9 % NaCl previously incubated in a water bath at 34 °C following the method of **Ngoula *et al*. (2007)**. Twenty microliters of the solution were placed on a slide which was covered with a cover-slip and left for 2 minutes so that the sperms should settle. The slide was observed using a magnification of ×400. The mobile and immobile sperm were then counted randomly in 4 squares of the haemocytometer**)**. The number of sperm cells per ml was given by the formula below;

$$\textbf{Total sperm count} = \frac{N \times df}{4 \times V} \times 1000$$

Where; Df (dilution factor) = 20; N=number of sperm cells counted in 4 squares

V=volume of 1square ($1cm^3$)

##### II-2.5.1.2. Sperm motility

Using a haemocytometer, sperm cells were observed and classified based on their motion into three categories **(Ngoula *et al*., 2007)**:

- Progressively motile (P): Sperm that move actively forward in a straight line or large circles.
- Non-progressively motile (NP): Sperm that show movement but do not progress forward effectively; they may move in place or in tight circles.

➢ Immotile (I): Sperm that show no movement.

- Percentage of progressively motile sperm

$$= \frac{Number\ of\ progressive\ motile\ sperm}{Total\ number\ of\ counted\ sperm} \times 100$$

- Percentage of non-progressively motile sperm

$$= \frac{Number\ of\ non-progressive\ motile\ sperm}{Total\ number\ of\ counted\ sperm} \times 100$$

- Percentage of immotile sperm

$$= \frac{Number\ of\ immotile\ sperm}{Total\ number\ of\ counted\ sperm} \times 100$$

**II-2.5.1.3. Sperm viability**

Ten microliters of the solution containing the minced epididymis were pipetted and deposited on a slide. Then, 10 µl of 0.5% eosin were added, and the two solutions were thoroughly homogenized. The slide was subsequently covered with a cover-slip and observed under a microscope at ×400 magnification **(Ngoula *et al.*, 2007)**. Viable spermatozoa appeared clear, whereas non-viable spermatozoa appeared dark. This distinction occurred because intact (living) cell membranes do not permit the diffusion of stains, while compromised (dead) membranes allow stain penetration. The number of viable sperm cells was then counted.

**II-2.5.2. Determination of *Terminalia superba* on some male reproductive markers**

**II-2.5.2.1. Assessment of cholesterol levels**

➢ **Principle**

Cholesterol is present in the body in the form of cholesterol esters and hydrogen peroxide. It reacts with phenol and 4- Aminophenazone to form quinonimine. The intensity of the pink coloration is directly proportional to the cholesterol level present in the sample.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + H_2O \xrightarrow{\text{Cholesterol oxydase}} \text{4-Cholestenone} + 4H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{Peroxydase}} \text{Quinonimine} + 4H_2O$$

- **Procedure**

An assay kit from Q-lineBiotech was used to perform this assay. In each test tube, 10 µl of the serum was added, followed by the addition of 1 ml of reagent. The mixture was then shaken and incubated at 25°C for 10 minutes. The absorbance was measured using a spectrophotometer at 500 nm against a blank. The concentration of cholesterol in the different tubes was proportional to the optical density (Table 2).

**Table 2**: Assessment of cholesterol levels

| Tubes | Test | Standard | Blank |
|---|---|---|---|
| **Sample (µL)** | 10 | - | - |
| **Standard (µL)** | - | 10 | - |
| **Reagent (mL)** | 1 | 1 | 1 |
| The mixtures are then agitated and incubated at room temperature for 10minutes. The absorbance is then measured using a spectrophotometer at 505nm against the white | | | |

$$\text{Concentration of the sample}\left(\frac{mg}{dl}\right) = \frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank} \text{ x Concentration of sample}$$

Where; (A)Sample= Absorbance of sample;

(A)Standard= Absorbance of standard;

(A)Blank= Absorbance of blank;

Concentration of standard=200mg/dL;

[Cholesterol] (mmol/L) = [Cholesterol](mg/dL) ×0,0258

**II-2.5.2.2. Assessment of testosterone levels**

- **Principle**

This technique is based on a competition reaction for binding to a monoclonal antibody fixed in the wells of a microplate between the labelled antigen (testosterone conjugated to peroxidase) from the kit and the antigen to be measured (testosterone in the sample). The addition of the substrate, Tetramethylbenzidine (TMB), allows for an enzymatic reaction that results in the formation of a coloured complex absorbing at 450 nm. The intensity of the coloured complex is inversely proportional to the amount of testosterone in the sample.

- **Procedure**

The dosage was performed using the MONOCENT Testosterone ELISA kit (Catalog No. EL1-1263) according to the manufacturer's protocol (Table 3). All reagents were brought to room temperature before beginning the procedure. Fifty microliters of standard or supernatant were added to the wells of the microplate, followed by 100 µL of 1X conjugated testosterone and 50 µL of biotinylated testosterone in each well. The microplate was gently shaken using a vortex, covered, and incubated for 60 minutes. The contents were then discarded. After three washes with washing buffer, the wells were dried using absorbent paper, and 100 µL of TMB were added to each well. The microplate was agitated and covered again, then incubated at room temperature for 30 minutes. After the second incubation, the reaction was stopped by adding 50 µL of stop solution. The microplate was gently shaken again, and the optical density of each well was measured at 450 nm using an ELISA plate reader. Testosterone concentrations were determined by extrapolating the optical densities from the standard curve.

**Table 3**: Assessment of testosterone levels

| | Concentration of standards | | | | | | Serum sample |
|---|---|---|---|---|---|---|---|
| | 0 | 0.2 | 0.5 | 2 | 6 | 18 | |
| Standard (uL) | 50 | 50 | 50 | 50 | 50 | 50 | - |
| Serum (uL) | - | - | - | - | - | - | 50 |
| Testosterone-Enzyme Conjugate (µL) | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Anti-Testosterone Biotin (µL) | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| After the reagents above were added, the microplate was gently shaken for 30 seconds, then covered and incubated at 25°C for 60 minutes.<br>After incubation, the supernatant was removed, and all wells were rinsed 3 times with wash buffer and dried thoroughly. | | | | | | | |
| TMB Substrate (µL) | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| After adding the substrate, the microplate was shaken, then covered and incubated at 25°C for 30 minutes. | | | | | | | |
| Stop Solution (µL | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| | | | | | | | |

The concentration of testosterone in the sample is obtained from the equation of the line (Appendix 4)

### II-2.5.2.3. Assessment of fructose levels

- **Principle**

In an acidic medium, indole reacts with fructose in solution to form a brown-coloured complex that absorbs at 470 nm. The intensity of the colour is proportional to the concentration of fructose in the medium **(OMS, 1993)**.

- **Procedure**

The sample preparation was carried out in two main steps. The first step involved diluting the samples (seminal vesicles homogenates). Each sample (400 µL) was diluted with 1.96 mL of distilled water, and the resulting solution was mixed for homogenization. The second step involved centrifuging the samples to deproteinize them. To 1 mL of the diluted sample, 0.3 mL of 1.8% zinc sulphate and 0.2 mL of 0.1 M NaOH were added sequentially. After mixing, the tubes were allowed to rest and then centrifuged for 20 minutes at 2000 g. The supernatant was then used for the assay. The different reactants used are stated in Table 4 below:

**Table 4**: Assessment of fructose levels

| Tubes | Blank | Standard 1 | Standard 2 | Sample |
|---|---|---|---|---|
| Fructose 0,14 mM (mL) | / | 0.5 | / | / |
| Fructose 0,28 mM (mL) | / | / | 0.5 | / |
| Supernatant (mL) | / | / | / | 0.5 |
| Distilled water (mL) | 0.5 | / | / | / |
| Indole (mL) | 0.5 | 0.5 | 0.5 | 0.5 |
| Concentrated HCL (mL) | 0.5 | 0.5 | 0.5 | 0.5 |
| All the tubes were sealed with glass beads and incubated in a water bath at 50°C for 20 minutes. After rapid cooling in an ice water bath, the optical density was measured using a spectrophotometer (Urit-810) at 470 nm against the white. | | | | |

The fructose concentration in the samples was calculated using the following formula:

$$[\text{Fructose}] = As \times F \times f$$

With: $F = 12\left(\frac{0,14}{S1} + \frac{0,28}{S2}\right)$

f = Dilution factor (50) ; S1 = Absorbance C1 (0,14 mM); S2 = Absorbance C2 (0,28 mM)

As = Absorbance of sample

### II-2.5.2.4. Assessment of quantity of proteins

- **Principle**

In a basic medium, sodium and potassium tartrate forms a soluble complex with copper ions. The addition of a protein displaces the copper bound to tartrate, resulting in the formation of a violet copper-protein complex which absorbs at 540 nm. The intensity of the colour is proportional to the amount of protein present in the solution **(Gornall *et al*., 1949)**.

Protein violet complex

- **Procedure**

The various reagents were added as indicated in the table below. After dissolving all the products, the tubes were shaken and incubated at 25°C for 30 minutes, then the optical density was read using a spectrophotometer at 540 nm against the white (Table 5).

**Table 5**: Assessment of quantity of proteins

| | Blank | Standards | | | | Test | |
|---|---|---|---|---|---|---|---|
| Tubes | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| BSA(ml)3mg/mL | 0 | 0.1 | 0.2 | 0.4 | 0.6 | 1 | 0 |
| NaCl 0.9% (µL) | 3 | 2.9 | 2.8 | 2.6 | 2.4 | 2 | 2.95 |
| Sample (µL) | 0 | 0 | 0 | 0 | 0 | 0 | 0.05 |
| Buiret's reagent (µL) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Quantity of protein (mg) | 0 | 0.3 | 0.6 | 1.2 | 1.8 | 3 | |
| Homogenize and allow at room temperature away from light for 30mins. The optical density is then read at 540nm | | | | | | | |

The quantity of proteins in each tube was determined using the calibration curve.

**II-2.5.2.5. Muscular Strength Test**

The muscular strength test was done the day before the experimentation starts and on the last day that is on the 28$^{th}$ day of the experimentation. During this test, the rats were suspended from a horizontal bar securely fixed in place by an appropriate apparatus. Each animal's duration (in seconds) spent on the bar was meticulously recorded. Every rat went through three trials of the test, and the best time achieved in these trials was selected **(Plaznik *et al.*, 1993)**.

**Picture 2**: Muscular strength test (Photograph taken by **Charfradine, 2023**)

**II-2.5.2.6. Histological cuts**

Histological sections of testis, epididymis, prostate, seminal vesicles and penis were done in order to observe structural changes due to the treatment given to animals. Organs were removed after sacrifice and fixed in Bouin's liquid. Organ sections were prepared according to the following protocol (**Cannet, 2004**).

- Step 1: Trimming

Each fixed organ was sliced thinly and evenly using a scalpel. The slices obtained were stored in plastic cassettes.

- Step 2: Dehydration

Dehydration was carried out in 3 stages: dehydration proper, brightening and impregnation.

- Step 3: Inclusion

The tissues were placed in moulds filled with molten paraffin, which were placed on cooling plates for solidification.

- Step 4: Cuts

The 5µm thick sections were cut using a microtome. The sections obtained were unfolded in a water bath at 40 °C, then the best sections were collected on slides and placed in a heat chamber to dry for 24 hours.

- Step 5: Coloration

The haematoxylin-eosin staining technique was used.

- Step 6: Mounting, microscopy and photography

Once out of the xylene, few drops of resin were applied to the sections which were then covered with a thin slide for microscopic observation. The stained sections were observed using an Olympus photonic microscope connected to a computer. Microphotographs were taken using Minisee version 1.0 image-taking software.

### II-2.6. Determination of the effect of *Terminalia superba* on some markers of oxidative stress

#### II-2.6.1. Assessment of catalase activity (EC 1.11.1.6)

- **Principle**

Hydrogen peroxide is decomposed in the presence of catalase and binds with potassium dichromate to form an unstable blue-green precipitate of perchloric acid. This precipitate is then decomposed by heat, producing a green complex that absorbs at 570 nm **(Sinha, 1972)**.

- **Procedure**

In the test tube, 50 ml of each sample and distilled water were introduced respectively. Subsequently, 750 ml of phosphate buffer (0.1 mM, pH 7.5) and 200 ml of hydrogen peroxide (50 mM) were added. The mixture was homogenized and the reaction was stopped after 60 seconds by adding 2 ml of a dichromate/acetic acid mixture (0.5%). The solution was then heated to 100°C for 10 minutes. After cooling, the absorbance was measured at 570 nm against the blank using a spectrophotometer. For each tube, the amount of peroxide remaining in the solution after the addition of perchloric acid was evaluated using the calibration curve obtained according to the protocol indicated in Table 6 below:

**Table 6**: Assessment of catalase activity

| Tubes | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| H2O2 (50mM) (µL) | 0 | 20 | 40 | 80 | 160 |
| Solution of 0,5% potassium dichromate/ acetic acid (mL) | 2 | 2 | 2 | 2 | 2 |
| After the formation of the blue precipitate, the solutions will be heated until boiling for 10mins and cooled down at room temperature | | | | | |
| Distilled water (µL) | 1000 | 980 | 960 | 920 | 840 |
| The absorbance of the tubes will be read at 570nm | | | | | |
| Concentration of $H_2O_2$ (mM) | 0 | 2 | 4 | 8 | 16 |

The calibration curve as a function of H2O2 concentrations has been plotted, and the specific activity of the catalase has been determined using the following formula:

$$\textbf{Act CAT} = \frac{\triangle DO \times f}{a \times t \times m}$$

Act CAT = Catalase activity (mM of $H_2O_2$/min/g of organ tissue);

ΔOD= OD of the sample – OD of the blank;

f=dilution factor (10); a= Slope of the calibration curve;

t= Duration of the reaction (1 minute);

m= Mass of the organ (g).

**II-2.6.2. Assessment of superoxide dismutase activity (EC 1.15.1.1)**

- **Principle**

The presence of superoxide dismutase (SOD) in the sample inhibits the oxidation of adrenaline to adrenochrome. The increase in absorbance, which is proportional to SOD activity, is measured between 20 and 80 seconds at 480 nm (**Misra and Fridovich, 1977**).

- **Procedure**

The different reagents were mixed as indicated in Table 7 below:

**Table 7**: Assessment of superoxide dismutase activity

| Tubes | Blank | Sample |
|---|---|---|
| Distilled water (µL) | 134 | - |
| Homogenate (µL) | - | 134 |
| Carbonate buffer (0.05M) pH 10.2 (µL) | 1666 | 1666 |
| Adrenaline 0.03mM (µL) | 200 | 200 |
| After homogenization, the absorbance of the test tubes at 480 nm was measured at 20 and 80 seconds against the blank. | | |

The activity of the SOD was determined as follows:

Change in absorbance: ΔA (min) = A20S – A80S

**% inhibition = 100 – (ΔAtest x 100/ΔAblank) = n units of SOD**

The specific activity of the SOD (units of SOD/g of tissue) is calculated as follows:

**Specific activity of SOD = (number of SOD units/mL/g of tissue × f)**

where: $A_{20S}$ = Absorbance measured at 20 seconds;

$A_{80S}$ = Absorbance measured at 80 seconds;

ΔAtest = Change in absorbance of the sample;

ΔAblank = Change in absorbance of the blank;

f = dilution factor (10);

50% inhibition corresponds to one unit of SOD.

### II-2.6.3. Assessment of malondialdehyde levels

- **Principle**

Malondialdehyde (MDA) formed during lipid peroxidation reacts with thiobarbituric acid in an acidic and hot environment to produce a pink complex that has a maximum absorption at 530 nm (**Hoyland et al., 1991**).

- **Procedure**

In the test and white tubes, 500 µL of the organ homogenate and 500 µL of 1.15% KCl were added, respectively. Subsequently, 250 µL of 20% trichloroacetic acid (TCA) and 500 µL of 0.67% thiobarbituricacid (TBA) were added to each tube. The tubes were then sealed with glass beads and incubated for 19 minutes at 90°C in a water bath. After incubation, the tubes were placed in an ice bath and centrifuged at 3,000 rpm for 15 minutes at 25°C. The

supernatant was pipetted (Table 8), and the absorbance was measured at 530 nm against the blank.

**Table 8**: Assessment of malondialdehyde levels

| Tubes | White | Sample |
|---|---|---|
| KCL 1.15% (µL) | 500 | - |
| Homogenate (µL) | - | 500 |
| TCA 20% (µL) | 250 | 250 |
| TBA 0.69% (µL) | 500 | 500 |
| The tubes were sealed with glass beads, heated in a water bath at 90°C for 10 minutes, then cooled under tap water and centrifuged at 3000 rpm for 15 minutes at 25°C. The supernatant was pipetted, and the absorbance was measured at 530 nm against the blank using a spectrophotometer (Urit-810). | | |

The malondialdehyde content was calculated using the molar extinction coefficient and expressed in µmol/g using the formula below;

$$[\mathbf{MDA}] = \frac{\triangle \boldsymbol{DO} \times \boldsymbol{Vts} \times \mathbf{103}}{\varepsilon \times \boldsymbol{l} \times \boldsymbol{m}}$$

[MDA] = Concentration of MDA (mol/g of organ);

ΔDO = Optical density (OD) of the sample - OD of the blank;

l = Optical path length (1 cm);

Ɛ = Molar extinction coefficient (15,600 $mol^{-1}.cm^{-1}$); m = Mass of the organ (g).

**II-2.7. Statistical analysis**

The results were processed as mean ± standard deviation using Microsoft Excel version 2016, while graphical representations and statistical analysis were performed using GraphPad Prism version 8.0.1. The results were compared using the Kruskal-Wallis analysis test, followed by Dunn's multiple comparison post hoc test, with differences considered significant for $p < 0.05$.

## III-1-RESULTS

### III-1.1. Yield of extraction

The extraction yield of the plant extract was found to be 13.19%, reflecting the proportion of extract obtained from the initial plant material.

### III-1.2. Effect of *Terminalia superba* on the sexual behaviour

Sexual behaviour tests conducted before and after the treatment revealed an increase in the frequencies of mount, intromission, and ejaculation across all groups following treatment. Notably, a significant increase ($p < 0.05$) in mount and intromission frequencies was observed in the group that was administered the aqueous extract at a dose of 172 mg/kg as compared to the normal group. In contrast, there was a reduction in mount, intromission, and ejaculation latencies at the end of the treatment. This decrease was also statistically significant ($p < 0.005$) in the group treated with the 172 mg/kg dose of the extract as compared to the normal group. (Table 9).

**Table 9**: Effect of different treatments on sexual behaviour before treatment (Day 0) and after treatment (Day 28)

| Parameter | Day | Treatment | | | | |
|---|---|---|---|---|---|---|
| | | DW | AE 1 | AE 2 | AE 3 | TE |
| ML | 0 | 469.30 ± 39.32 | 555.00 ± 39.69 | 780.00 ± 34.64 | 727.00 ± 23.09 | 598.30 ± 2.88 |
| | 28 | 58.00 ± 9.16 | 37.00 ± 1.00 | 26.33 ± 3.51 | 17.33 ± 3.05 [b] | 20.00 ± 7.21 |
| IL | 0 | 470.00 ± 39.69 | 559.00 ± 35.12 | 793.00 ± 11.55 | 727.00 ± 23.09 | 798.00 ± 2.89 |
| | 28 | 60.13 ± 9.16 | 38.00 ± 2.00 | 27.00 ± 2.64 | 17.33 ± 3.05 [b] | 20.00 ± 7.21 |
| EL | 0 | 1495.00 ± 42.00 | 1615.00 ± 37.00 | 1712.00 ± 15.00 | 1745.00 ± 25.00 | 1510.00 ± 5.00 |
| | 28 | 189.57 ± 12.00 | 114.00 ± 6.00 | 60.21 ± 6.00 | 52.00 ± 5.00 [b] | 60.00 ± 6.50 |
| MF | 0 | 27.33 ± 6.65 | 19.67 ± 5.03 | 20.33 ± 3.51 | 27.67 ± 3.58 | 29.33 ± 4.50 |
| | 28 | 33.00 ± 2.64 | 29.67 ± 7.23 | 31.67 ± 2.88 | 41.33 ± 5.85 [a] | 43.00 ± 6.24 |
| IF | 0 | 27.33 ± 6.65 | 20.00 ± 2.00 | 27.67 ± 3.59 | 27.67 ± 3.58 | 29.33 ± 4.50 |
| | 28 | 33.00 ± 2.65 | 28.00 ± 3.00 | 33.00 ± 3.50 | 41.00 ±5 .00 [a] | 43.00 ± 6.00 |
| EF | 0 | 0.66 ± 0.57 | 0.00 ± 00.00 | 0.66 ± 1.15 | 0.33 ± 0.57 | 1.00 ± 1.00 |
| | 28 | 1.66 ± 0.57 | 1.66 ± 0.57 | 1.66 ± 0.57 | 2.33 ± 0.57 | 2.00 ± 1.00 |

The results are expressed as mean ± standard deviation (n=5). a: $p<0.05$; b: $p<0.005$ compared to normal group; b: $p<0.005$ compared to normal group ;DW: normal group of rats; AE 1: group of rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: group of rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3:group of rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group; ML: Mount Latency; IL: Intromission Latency; EL: Ejaculation latency; MF: Mount frequency; IF: Intromission frequency; EF: Ejaculation frequency.

### III-1.3. Effect of *Terminalia superba* on the quality of sperm cells

The effects of the aqueous extract of *T.superba* on sperm quality are summarized in Table 10. It shows the viability of sperm cells and the motility of sperm cells. It can be seen from table 10 that there is no significant change in the percentage viability of sperm cells. It equally shows us a significant increase ($p<0.01$) in progressive motility of sperm cells in the group which the aqueous extract at a dose of 172 mg/kg was administered compared to the normal group.

**Table 10**: Effect of *Terminalia superba* on the quality of sperm cells

| Group | Viability (%) | PM (%) | NPM (%) | Immobility (%) |
|---|---|---|---|---|
| DW | 83.28 ± 4.25 | 31.49 ± 1.52 | 10.24 ± 2.68 | 58.27 ± 6.66 |
| AE 1 | 91.90 ± 7.33 | 31.64 ± 2.43 | 6.34 ± 2.08 | 62.02 ± 5.03 |
| AE 2 | 91.57 ± 5.10 | 39.85 ± 2.87 | 5.68 ± 2.62 | 54.47 ± 3.51 |
| AE 3 | 91.92 ± 6.16 | 50.47 ± 5.11 [a] | 9.30 ± 1.72 | 40.23 ± 3.06 |
| TE | 90.50± 3.28 | 46.46 ± 3.67 [a] | 13.23 ± 1.83 | 40.31 ± 4.51 |

The results are expressed as mean ± standard deviation (n=5). a: $p<0.01$ compared to normal group; DW: normal group of rats; AE 1: group of rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: group of rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: group of rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group; PM: progressive motility; NPM: non progressive motility

### III-1.4. Effect of *Terminalia superba* on some male reproductive markers

#### III-1.4.1. Effect of *Terminalia superba* on cholesterol levels

The administration of the aqueous extract of *Terminalia superba* at a dose of 172 mg/kg induced a significant increase ($p < 0.005$) in serum cholesterol levels compared to the normal group (Figure 8).

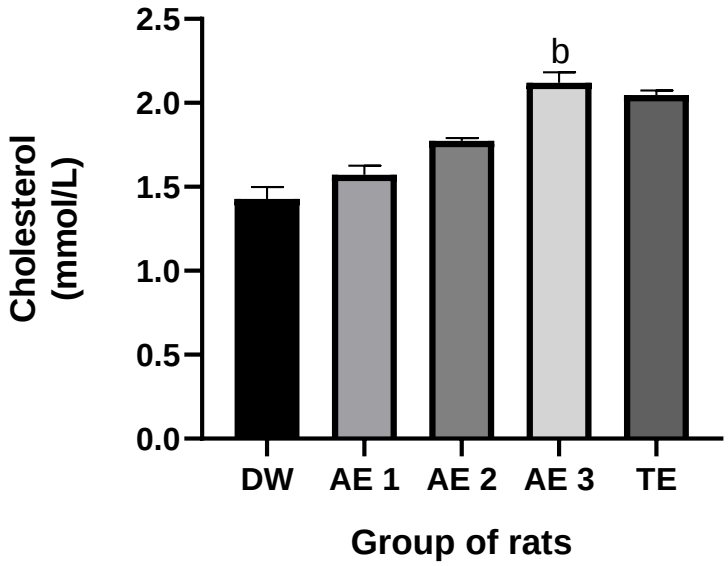


**Figure 8**: Effect of the aqueous extract of *Terminalia superba* on cholesterol levels

The results are expressed as mean ± standard deviation (n=5). b: $p<0.005$ compared to normal group; DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group

### III-1.4.2. Effect of *Terminalia superba* on testosterone levels

Figure 9 illustrates the effects of aqueous extract of *T. superba* on serum testosterone levels in experimental rats. The group to which the extract at the dose of 172 mg/kg was administered and the group to which testosterone enanthate was administered showed a significant increase ($p < 0.005$) in serum testosterone levels compared to the normal group of rats.

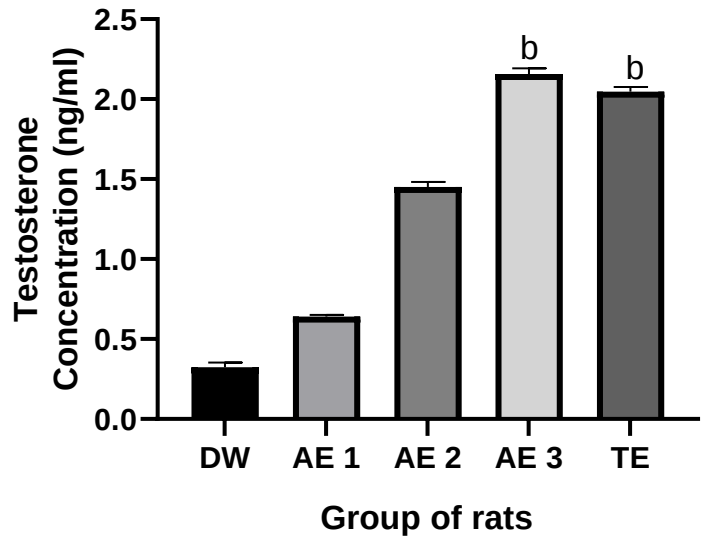


**Figure 9:** Effect of the aqueous extract of Terminalia superba on testosterone levels

The results are expressed as mean ± standard deviation (n=5). b: $p<0.005$ compared to normal group; DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group.

### III-1.4.3. Effect of *Terminalia superba* on fructose levels

Figure 10 shows the effects of the aqueous extract of *T. superba* on the vesicular fructose levels in the experimental rats. The administration of the aqueous extract of *T. superba* at a dose of 172mg/kg induced a significant increase ($p < 0.005$) in vesicular fructose levels compared to rats treated with distilled water.

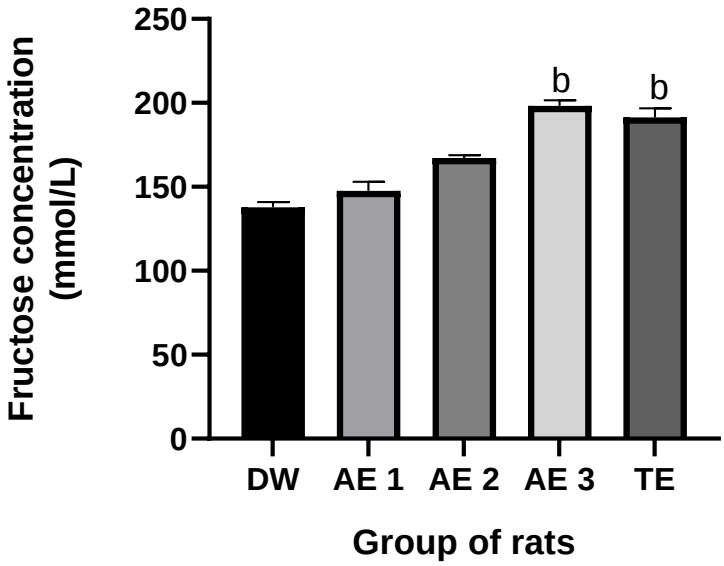


**Figure 10:** Effect of the aqueous extract of *Terminalia superba* on fructose levels

The results are expressed as mean ± standard (n=5). b: $p<0.005$ compared to normal group; DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group

### III-1.4.4. Effect of *Terminalia superba* on quantity of proteins

Figure 11 presents the effects of *T.superba* on proteins levels. The administration of the aqueous extract of *T.superba* did not cause any significant changes in the total testicular protein levels compared to the normal group.

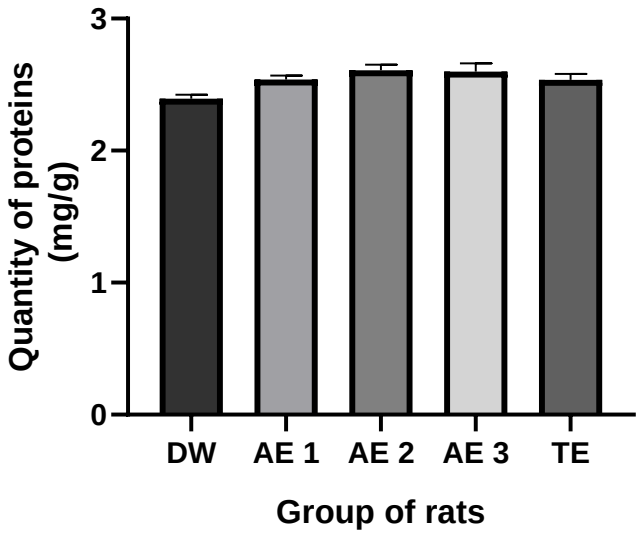


**Figure 11:** Effect of the aqueous extract of *Terminalia superba* on quantity of proteins

The results are expressed as mean ± standard deviation (n=5). DW normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group.

### III-1.4.5. Effect of *Terminalia superba* on muscle strength

Table 11 presents the effects of the aqueous extract of *Terminalia superba* on the muscle strength of the experimental rats. It shows that the administration of the aqueous extract of *Terminalia superba* led to a significant increase ($p < 0.005$) in muscle strength in animals treated with extract doses of 86 mg/kg, 172mg/kg and testosterone enanthate compared to those that received distilled water and the aqueous extract at a dose of 43mg/kg. This increase in muscle strength, characterized by an increased time hanging on the bar.

**Table 11**: Effect of *Terminalia superba* on muscle strength

| Groups | Before treatment | After treatment |
|---|---|---|
| Group 1 | 9.03 ± 0.35 | 25.66 ± 5.25 |
| Group 2 | 12.70 ± 3.13 | 28.11 ± 2.91 |
| Group 3 | 11.86 ± 1.24 | 42.55 ± 4.33 |
| Group 4 | 7.69 ± 2.23 | 66.89 ± 10.06 [b] |
| Group 5 | 9.62 ± 2.20 | 53.54 ± 11.61 [b] |

The results are expressed as mean ± standard deviation (n=5). b: $p<0.005$ compared to normal group; DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group

### III-1.4.6. Effect of *Terminalia superba* on the structure of androgen-dependent organs

Figure 12 below presents the effects of *T. superba* on the microarchitecture of the testis, epididymis, and prostate. In the cross-sections of the testis, it was noted in all animals that the seminiferous tubules contained germ cells at different stages of development, ranging from spermatogonia to spermatozoa. In the cross-sections of the epididymal tail, it was observed in the histological sections of all animals that spermatozoa were present in the lumen of the epididymal tube. In the prostate sections, the presence of secretions in the seminal lumen was noted in animals from all groups. However, it can be noted that the density of spermatozoa in both the epididymis and testis, as well as the density of eosinophilic secretions in the prostate, is higher in rats that received the aqueous extract at doses of 86 and 172 mg/kg.

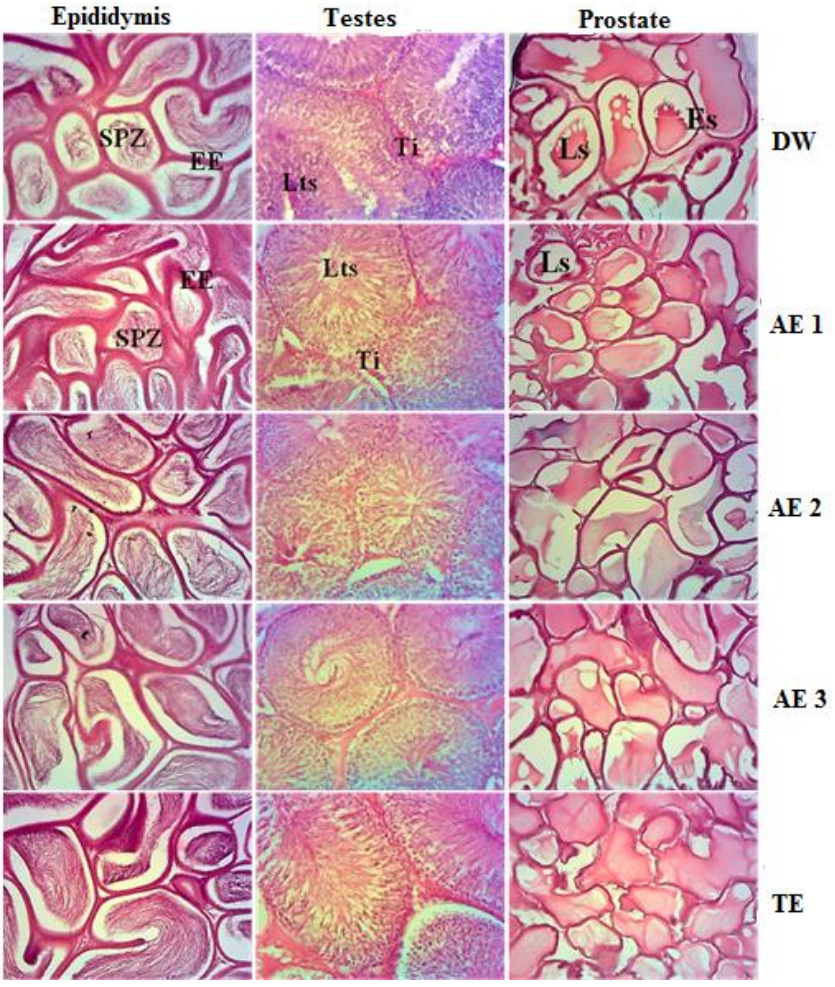


**Figure 12:** Microphotographs of the testes (×100), epididymis (×40), and prostate (×40); stained with hematoxylin-eosin

DW: normal group of rats ;AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group. Lts = seminiferous tubule lumen; Es = seminal epithelium; Spz = spermatozoa; Ti = interstitial tissue; Ls = seminal lumen.

### III-1.5. Effect of *Terminalia superba* on some parameters of oxidative stress

#### III-1.5.1. Effect of *Terminalia superba* on catalase activity

Figure 13 shows the effects of the aqueous extract of *Terminalia superba* on the testicular catalase enzymatic activity in the experimental rats. The administration of the aqueous extract of *Terminalia superba* did not cause any significant changes in the activity of catalase.

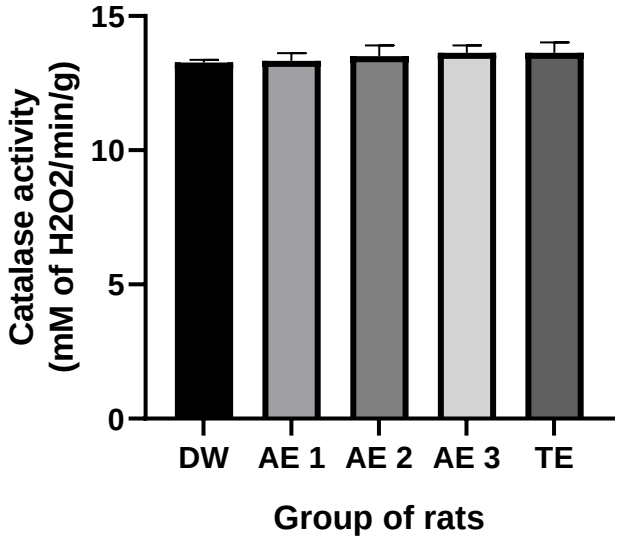


**Figure 13:** Effect of the aqueous extract of *T. superba* on catalase activity

The results are expressed as mean ± standard deviation (n=5). DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group.

#### III-1.5.2. Effect of *Terminalia superba* on SOD activity

Figure 14 shows the effects of the aqueous extract of *Terminalia superba* on the testicular SOD enzymatic activity in the experimental rats. The administration of the aqueous extract of *Terminalia superba* did not cause any significant changes in the activity of SOD.

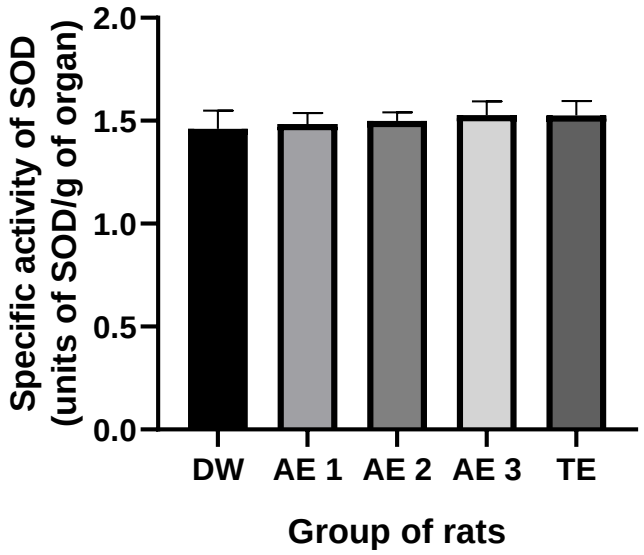


**Figure 14:** Effect of the aqueous extract of Terminalia superba on SOD activity

The results are expressed as mean ± standard deviation (n=5). DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group

### III-1.5.3. Effect of *Terminalia superba* on malondialdehyde levels

Figure 15 shows the effects of the aqueous extract of *T. superba* on the testicular malondialdehyde levels in the experimental rats. The administration of the aqueous extract of *T. superba* did not cause any significant changes in the level of malondialdehyde.

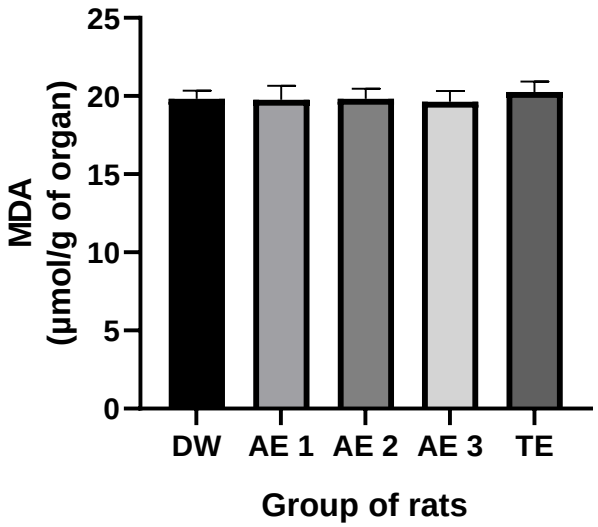


**Figure 15:** Effect of the aqueous extract of *Terminalia superba* on MDA levels

The results are expressed as mean ± standard deviation (n=5). DW: normal group of rats; AE 1: rats which received aqueous extract of T.S at a dose of 43 mg/kg; AE 2: rats which received aqueous extract of T.S at a dose of 86 mg/kg; AE 3: rats which received aqueous extract of T.S at a dose of 172 mg/kg; TE: positive control group.

## III.2. DISCUSSION

In recent years, increasing attention has been directed toward plant-based compounds with potential therapeutic effects on reproductive health. *Terminalia superba*, a species widely recognized in traditional medicine is known to have therapeutic properties. Despite its traditional use, scientific data supporting its effects on male reproductive function remain limited. The present study was therefore conducted to evaluate the effects of the aqueous extract of *Terminalia superba* on the reproductive function of normal male rats. To achieve this, animals received distilled water, the aqueous extract of *Terminalia superba* at three different doses (43 mg/kg, 86 mg/kg, and 172 mg/kg), and testosterone enanthate over a period of 28 days.

The aphrodisiac effect of the extract was evaluated based on the sexual motivation of rats. This sexual motivation is determined with a series of parameters such as mount latency (ML), mount frequency (MF), intromission latency (IL), intromission frequency (IF), ejaculation latency (EL) and ejaculation frequency (EF) (**Ali *et al*., 2012**). Mount frequency, intromission frequency and ejaculation frequency are a reflection of libido, potency, vigour and sexual performance in male rats (**Kpomah *et al*., 2012**). Treatment with *T.superba* at the dose of 172 mg/kg led to a significant increase in the complete mounts and intromissions. The other 2 doses (43 & 86 mg/kg) also demonstrated an increase in MF and IF but those two groups did not reach statistical difference. This highest dose was also able to increase significantly the number of ejaculations as compared to the normal group. All doses of *T.superba* caused a reduction in ML and IL but more significant at the dose 172 mg/kg. This indicates that the hesitation time for male rats to move towards the female rats was reduced. Ejaculation latency reduced in a dose dependent manner after administration of the extract. However, only the dose 172 mg/kg was able to influence EL significantly. All of this serves as clear evidence that sexual motivation and performance were enhanced in extract treated rats particularly at the dose 172 mg/kg. These results suggest that the extract could stimulate the NO production by activating the NO/cGMP signalling pathway which is involved in the penile erection. This effect may also be explained by the presence of some secondary metabolites identified (phenols, alkaloids, flavonoids, saponins, coumarins, glycosides) during the phytochemical screening conducted in an *in vitro* study on *Terminalia superba* (**Keumedjio *et al*., 2023**). Precisely alkaloids which enhance the activity of endothelial nitric oxide synthase at the level of the penis increasing the production of nitric oxide which is the molecule responsible for vasodilation. It will cause vasodilation of the penile arteries which will lead to increase blood

flow which fills and engorges the corpora cavernosa, leading to and maintaining an erection (**Owaba *et al.*, 2024**). Equally, flavonoids play a significant role by relaxing the corpora cavernosa smooth muscle in the copulatory organ of male rats and inducing vasodilation thereby leading to an erection (**Casisdy *et al.*, 2016**). Moreover, this effect may also be due to the extract's ability to stimulate dopaminergic activity at the level of the brain increasing sexual motivation and arousal.

For successful fertilisation to occur, a sperm cell must be viable, meaning it must be alive and able to perform progressive, forward movements towards the egg in order to fertilise it (**Tanga *et al.*, 2021**). These two factors (viability and motility) were evaluated. The results showed a non-significant change in the percentage of viable sperm in all the groups. This shows that the plant extract might not be a threat to sperm cells even at higher doses. However, there was a significant increase ($p<0.05$) in the percentage of progressively motile spermatozoa in the group which took the extract at the dose of 172 mg/kg. This effect may be due to saponins which enhance the activity of key enzymes in the Krebs cycle and oxidative phosphorylation, the primary processes that generate ATP in the mitochondria. By boosting the efficiency of these metabolic pathways, there will be an increase the overall energy supply available for the sperm's tail to beat, thus improving motility (**Shehadeh *et al.*, 2021**). This can also be associated with high fructose level significant in that group (group administered with the 172 mg/kg extract dose) since fructose is known to be the primary source of energy for sperm cells. Fructose is metabolised by the sperm mitochondria which powers flagellum movement. With sufficient fructose, sperm cells will have enough energy needed for progressive movement compared to weaker or non-progressive movement when there is insufficient fructose. The increase in fructose might be related to testosterone, which controls the function of accessory sex organs, like the seminal vesicles. Since seminal fructose production depends on androgens, higher testosterone levels will directly lead to a rise in fructose (**Ambiye *et al.*, 2013**). In order to assess the impact of the treatment on the structure of androgen-dependent organs (testes, epididymis, prostate), histological sections of these organs were prepared and revealed that rats that received the extract at doses of 86 mg/kg and 172 mg/kg of had a visibly higher amount of sperm in both the testes and epididymis, as well as more secretions in the prostate. All these results suggests that the extract enhances sperm production, sperm motility and reproductive organ function.

Testosterone is the principal androgen responsible for the development of secondary sexual characteristics, including increased muscle mass (**Emojevwe *et al.*, 2021**). In the present

study, testosterone levels were significantly elevated in the group treated with the extract at a dose of 172 mg/kg as well as in the group where testosterone enanthate was administered. This rise in testosterone levels was further supported by the observed increase in muscle strength in both groups. The raise in testosterone levels may be attributed to the concomitant increase in cholesterol levels, since cholesterol being the biochemical precursor of testosterone was also significantly higher in these groups. This observed enhancement in steroid hormone synthesis may be linked to flavonoids, which can upregulate key enzymes and proteins is in the steroidogenic pathway such as the StAR protein responsible for the cholesterol transport and the enzyme CYP11A1 which catalyses the conversion of cholesterol to pregnenolone, a key step to testosterone production (**Li *et al*., 2022**). These results are in line with those of **Keumedjio *et al.*** (**2023**) which showed that the aqueous extract of *T.superba* increased the production of testosterone by primary interstitial cells invitro.

Reactive oxygen species (ROS) have a deleterious effect on sperm cells when their levels are above normal, they can cause DNA fragmentation, lipid peroxidation and even spermatozoa apoptosis. But under normal physiological levels, these ROS are important in processes such as capacitation, acrosome reaction and fertilisation (**De Luca *et al*., 2021**). To always maintain this level of ROS in a normal range, antioxidants help regulate them. We measured the levels of key antioxidants involved in protecting against ROS. Specifically, we assessed catalase, an enzyme that helps detoxify excess hydrogen peroxide. Superoxide dismutase, which neutralizes reactive oxygen. Additionally, we measured the level of malondialdehyde, an indicative marker of lipid peroxidation. It was seen that there was no significant change in the level of catalase, super oxide dismutase and malondialdehyde in all the groups that were administered the extract compared to the group that was administered only with distilled water. This could be explained by the fact that the animals were not exposed to certain factors which could have impacted reproductive health negatively by altering the oxidant/antioxidant balance (**Mannucci *et al*., 2022**). The absence of significant variations in oxidative stress markers assessed could also indicate that the extract does not induce oxidative stress, a condition that could potentially harm sperm cells by causing cellular damage or destruction. This suggests that the extract may not contribute to oxidative damage that could negatively impact reproductive functions.

## CONCLUSION

The objective was to evaluate in vivo the impact of the aqueous extract of *Terminalia superba* on the reproductive function of normal male rats. At the end of this study, the results obtained showed that:

- The aqueous extract of *Terminalia superba* enhances sexual behaviour in male rats significantly at a dose of 172 mg/kg.

- The aqueous extract of *Terminalia superba* at a dose of 172 mg/kg significantly increases fructose and testosterone levels and motility of sperm cells.

-The aqueous extract of *Terminalia superba* does not cause oxidative stress even at higher doses.

## PERSPECTIVES

-Conduct a high-performance liquid chromatography (HPLC) to identify precisely and quantify the bioactive compounds responsible for the beneficial effects observed with the aqueous extract.

-Determine the mechanism of action responsible for the effect of the aqueous extract on biochemical parameters such as Luteinising hormone and Follicle stimulating hormone.

-Evaluate the sub-chronic effect of the aqueous extract on some markers of oxidative stress providing insight to longer term impact.

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**Centre de Recherche et Formation Doctorale, Science de la Vie, Santé et Environnement (CRFD-SVSE)**
***Centre for Research and Graduate Studies in Life, Health & Environmental Sciences***
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**Joint Institutional Review Board for Animal & Human Bioethics (JIRB)**
**Email: jirbsecretariat@gmail.com**

15 April 2024

**WELEPE YONTA NISSE CYBELLE**
University of Yaoundé 1, Cameroon
Faculty of Science, Department of Biochemistry

**Ethical Clearance Reference N°: BTC-JIRB2024-110** (please use this reference number for all correspondences)

**Protocol Title: "ASSESSEMENT OF THE EFFECTS OF THE AQUEOUS EXTRACT OF *TERMINALIA SUPERBA* ON THE REPRODUCTIVE FUNCTION OF MALE RATS"**

**Investigator(s) Name(s)**: WELEPE YONTA NISSE CYBELLE, MSc Student, Dr. BEBOY EDJENGUELE Sara Nathalie, Supervisor.

The proposed research protocol and related documents have been reviewed and deliberated by the JIRB of the Centre for Research and Graduate Studies in Life, Health & Environment Sciences (CRFD-SVSE) at the Biotechnology Centre, University of Yaoundé 1, on the 23th February 2024.
The JIRB is satisfied with the current version of the research protocol and supporting documents and agrees that there is no objection on ethical grounds to the proposed study. The JIRB is therefore pleased to approve it on the consideration that the research team will strictly abide by the conditions of the approval below:

- Investigators must strictly follow the plan of the approved protocol. Any changes to the approved protocol will require prior JIRB approval.
- Must be promptly reported to the JIRB: (1) Any deviations from or changes to the protocol that are made to eliminate immediate hazards to the fauna or study participants; (2) All risks that may be rare or remote and especially those that may entail serious consequences or compromise potential benefits or that would affect the conduct of the research.
- Must prepare and submit a standard progress report of the research to the JIRB at completion or one year from the date of issuance of the approval letter and thereafter, on an annual basis.
- Must notify the JIRB when the research is completed. Failure to submit an annual progress report on the study may affect the conditions of approval.

The current ethical clearance is for one-year renewable, on the condition that a progress report is submitted to the JIRB. While the JIRB has given its approval for this study on a satisfactory ethical basis, it is necessary for the investigators to obtain research permit from the Ministry of Scientific Research and Innovation (MINRESI, Cameroon).
Sincerely,

**Professor Jude D. Bigoga, M.Sc, Ph.D.**
JIRB Chair
Faculty of Science, University of Yaoundé I, Cameroon

**Professor Frederic Nico Njayou, M.Sc., Ph.D.**
Coordinator for Animal Research Bioethics
Faculty of Science, University of Yaoundé I. Cameroon

## Appendix 1: Ethical Clearance Authorization Form

## Appendix 2 : Preparation of solutions

- **Sodium Chloride solution 0.9%**

  Prepared by dissolving 9 g of NaCl in 1 L of distilled water.

- **Bouin solution**

  For 500ml of Bouin solution, 22 g of picric acid was dissolved in 100 ml of acetic acid and 400 ml of formol 40 % was added.

- **Eosin solution 0.5 %**

  Prepared by dissolving 0,25 g of eosin in 50 ml of distilled water.

- **NaOH solution 0.1 M**

  Prepared by dissolving 0,2 g of NaOH in 50 ml of distilled water.

- **Sodium Phosphate buffer (0.1 M; pH 7.3)**

  3.6g of $NaH_2PO_4$ was dissolved in 300 ml of distilled water. 5.34 g of $Na_2HPO_4$ was equally dissolved in 300 ml of distilled water. The two solutions were mixed and the pH was adjusted using a pHmeter, with NaOH (0.1 M).

- **Hydrogen peroxide solution 50M**

  Prepared by diluting 0.95 ml of hydrogen peroxide 0.89 M in distilled water to a final volume of 50 ml.

- **Adrenaline solution 0.06 mg/ml**

  Prepared in the absence of light by diluting 3 ml of adrenaline in distilled water to a final volume of 100 ml.

- **Indole reagent**

  To prepare 100 mL of this reagent, 200 mg of benzoic acid were dissolved in 100 mL of distilled water by repeated agitation in a water bath at 60°C. After complete dissolution, 25 mg of indole were added to the solution, and the resulting mixture was filtered through Whatman paper No. 3.

- **Stock solution of fructose**

  This solution was prepared by dissolving 25.2 mg of fructose in 50 mL of distilled water. The standard fructose solutions for the assay were prepared by diluting the stock solution to concentrations of 0.14 mM and 0.28 mM.

- **Potassium dichromate solution 5% / Glacial acetic acid 100%**

  The 5 % potassium dichromate solution was prepared by dissolving 2.5 g of potassium dichromate in distilled water to a total volume of 50 ml. Then, 150 ml of glacial acetic acid was added to the solution.

- **Carbonate buffer (0.05 M; pH 10.2)**

  The buffer was prepared by dissolving 4.5 g of sodium carbonate ($Na_2CO_3$; 10 $H_2O$) and 4.2 g of sodium bicarbonate ($NaHCO_3$) in 500 mL of distilled water. The pH of the solution was adjusted to 10.2 with 1 M sodium hydroxide, and the volume was completed to 1000 mL with distilled water.

- **Potassium phosphate buffer (0.1 M; pH 6.8)**

  The potassium phosphate buffer was prepared by separately dissolving 3.40 g of $KH_2PO_4$ and 4.35 g of $K_2HPO_4$ in 250 ml of distilled water. The solution of pH of $KH_2PO_4$ was titrated with the solution of $K_2HPO_4$ until pH of 6.8 was obtained.

- **Sodium phosphate buffer (0.1 M; pH 7.3)**

  In 350 mL of distilled water, 1.175 g of $NaH_2PO_4$, 5.8 g of $Na_2HPO_4$, and 4.4 g of NaCl were dissolved. Gelatin (0.5 g) was dissolved in 25 ml of warm distilled water and added to the mixture. The pH was adjusted using a 0.1 M NaOH solution, and the final volume was brought to 500 mL with distilled water. The resulting buffer solution (Buffer S) was stored at 4°C.

- **Trichloroacetic acid (TCA) solution 20 %**

  The solution was prepared by dissolving 20 g of TCA in distilled water to a total volume of 100 ml.

- **Thiobarbituric acid (TBA) solution 0.67 %**

  The solution was prepared by dissolving 0.67 g of TBA in distilled water to a total volume of 100 ml.

- **Potassiun Chloride (1.15 %)**

  Prepared by dissolving 11.5 g of KCl in distilled water to a total volume of 1000 ml.

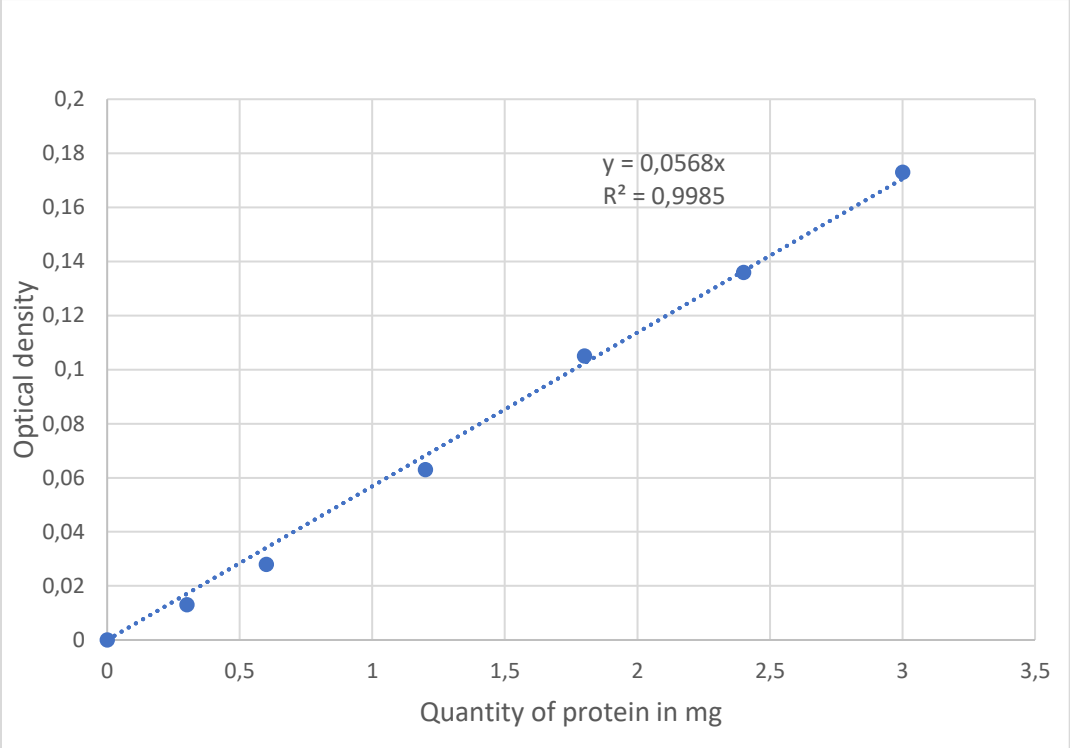
- **Bovine serum albumin (BSA) solution (3 mg/mL)**

  The BSA solution at was prepared by dissolving 150 mg of BSA in distilled water to a final volume of 50 mL.

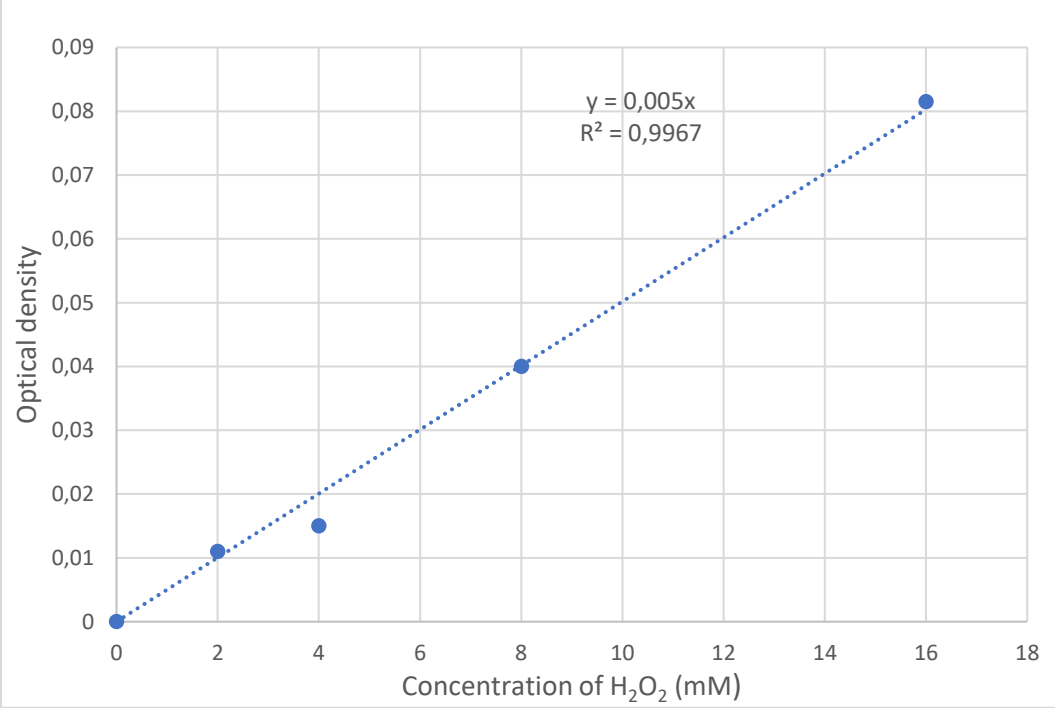
- **Biuret reagent**

- The Biuret reagent was prepared by dissolving 0.75 g of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) and 3 g of sodium potassium tartrate in distilled water to a total volume of 250 mL (Solution A). Then, 15 g of sodium hydroxide were dissolved in 250 mL of distilled water (Solution B). Solutions A and B were then mixed.

## Appendix 3: Protein calibration curve




## Appendix 4 : Catalase calibration curve



## Appendix 5 : Testosterone calibration curve