

**ASSESSMENT OF SPERM QUALITY AND QUANTITY IN MALE WISTAR RATS
SUPPLEMENTED WITH AN HERBAL DRUG (LONG JACK XXXL) USED AS SPERM
ENHANCER**

BY

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

MARCH, 2024

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**BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL
LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY
SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.**

MARCH, 2024.

CERTIFICATION

This is to certify that this work carried out by OHIKHEMOTA MICHELE PRAISE with matriculation number BMS1802478, under the supervision of Prof. M.A. EMOPKAE, is being submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree.

Prof. M. A. EMOKPAE
Supervisor

DATE

Dr. Mrs. ZAINAB OMORUYI
Head of Department

DATE

DEDICATION

I dedicate this project work to God Almighty for his unending love and guidance throughout my stay in the University of Benin.

ACKNOWLEDGEMENT

I give thanks to the Almighty God for the guidance, love, comfort, and grace granted me towards the presentation of this work.

My sincere gratitude goes to my highly esteemed supervisor PROF. M.A EMOPKAE, for cutting through his tight schedule to supervise and inspect my work. Thank you for your fatherly concern, counsel and encouragement throughout this project

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ABSTRACT

The World Health Organization reported that over 186 million couples in developed countries suffered from infertility a condition that is more worrisome in the so-called infertility belt of sub-Saharan Africa and about 40-50 % of the problem is attributed to men. Long Jack XXXL Sperm Enhancer an herbal supplement containing long jack (*Eurycoma longifolia*) commonly referred to as Tongkat Ali, siberian ginseng, maca extract, fenugreek extract and horny goat weed. It has been revered for its aphrodisiac and fertility-enhancing properties. The aim of this study was to evaluate the effect of Long Jack sperm enhancer supplementation on the sperm quality and quantity in male Wistar rats. The rats were grouped into five and administered varying concentration of Long Jack XXXL excluding the control group for 28 days. Group 1 was given commercial rat feed and water, group 2 received 10 mg/kg Long Jack dissolved in 2 mL distilled water, group 3 received 20 mg/kg Long Jack dissolved in 2 mL distilled water, group 4 received 40 mg/kg Long Jack dissolved in 2 mL distilled water and group 5 received 80 mg/kg Long Jack dissolved in 2 mL distilled water orally by gavage. On day 29 the rats were sacrificed and the caudal epididymis was excised for semen analysis. The Sperm count increased from the control group 1(8.57±2.0), to 9.6±1.9 group 2, to 9.2±2.5 (group 3), to 9.9±2.2 (group 4), to 10.1±1.2 (group 5). The differences in the means were however not statistically significant ($p>0.5$) when compared with control. There was no difference in the mean progressive motility, non-progressive motility, immotile form, normal morphology and abnormal morphology when compared with control. Long Jack sperm enhancer did not significantly increase sperm quantity and quantity of male Wistar rat.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Infertility is becoming a serious global health issue that is particularly concerning in the so-called infertility belt in sub-Saharan Africa. 15% of couples who are of reproductive age have infertility; male infertility makes for up to half of these cases (Agarwal *et al.*, 2021) and there is an estimated 0.3% annual increase in the age-standardized prevalence of male infertility. (Sun *et al.*, 2019). About 40-50 % of the problem is attributed to men (Uadia and Emokpae, 2015), and the majority of male factor infertility was linked to either a complete lack of spermatozoa or a low sperm count (oligozoospermia). (Ilegogie and Emokpae, 2021). The occurrence of low sperm count has been linked to environmental factors such as lifestyle, seasonal changes, food, increasingly sedentary work habits, electromagnetic radiation, wearing tight clothing, and extended driving hours, according to researchers. (Emokpae and Brown, 2021). The prevalence of male infertility has led to the introduction of assisted reproductive technologies (ART), which are more direct and expedient treatment options in conventional medicine. (Tournaye *et al.*, 2017). These technologies include intrafallopian transfer (ITF) followed by intracytoplasmic sperm injection (ICSI), surgery, and hormone therapy. While ART is the primary method of treating infertility effectively, its accessibility, pricing, and availability vary by country, and problems after treatment are still a worry. (Tournaye *et al.*, 2017). As a result, more people are turning to plant-based or herbal medications to address a variety of illnesses and conditions. For many years, Long Jack sperm enhancer has been utilized and is frequently promoted and sold online as a miraculous herbal remedy to help male infertility. It might be well-liked in Nigeria due to the rising percentage of people who have either poor sperm counts or no spermatozoa at

all in their ejaculate. Long Jack is said to be a preparation from *Eurycoma longifolia* Jack, which is known by different names such as ‘Tongkat Ali’ in Malaysia, ‘Pasak Bumi’ in Indonesia and ‘Cay ba binh’ in Vietnam (Chan *et al.*, 2009). This small tree is a member of the Simaroubaceae family. Above all, *Eurycoma longifolia* Jack has long been used to treat male infertility and sexual dysfunction. Moreover, *E. longifolia* has other medicinal benefits that include improving muscular mass and athletic performance, decreasing obesity, increasing appetite, and treating a variety of illnesses like cancer, osteoporosis, diabetes, anxiety, weariness, and peptic ulcers. (Rehman *et al.*, 2016). Some classes of phytochemical constituents, including quassinoids, alkaloids, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans, have been identified in the plant's root. These constituents have been shown to increase male sexual behavior, improve the quality of male rats' sperm, and have some cytotoxic effects. (Adimoelja, 2000).

According to some authors, the plant's aqueous extract contains quercetin, polysaccharides, glycoprotein, mucopolysaccharides, and tannins. These compounds have been shown to have anti-malarial, anti-ulcer, anti-tumor, anti-parasitic, and anti-pyretic properties. (Chan *et al.*, 2009).

But the primary application for this herb is as a sexual enhancer, since research indicates that it can raise serum testosterone levels, which improves strength and power during intercourse and increases male virility. (Tambi *et al.*, 2012).

Maca (*Lepidium meyenii* Walp.), a component of long jack xxxl a specie of the family Brassicaceae, has been cultivated both as a crop and medicinal plant for 1300–2000 years in Peru. Native Andean inhabitants employed the plant for its energizing qualities, improving bovine and human fertility, and other therapeutic benefits like healing anemia and respiratory ailments..

(Beharry *et al.*, 2018). The biological active components include Alkaloids like lepedilins and macapirrolins; thiazoles; glucosinolates; polysaccharides; polyphenols; sterols; free fatty acids; flavonoids; tricin derivatives; complex polysaccharides; imidazole alkaloids; pyrrole alkaloids; β and polyphenols(Gonzales, 2012). Iron, calcium, copper, zinc, and potassium are among the important micro- and macroelements that it is a powerful store of. For centuries, the indigenous people have held great respect for Maca, utilizing it in their traditional medicine and culinary traditions to enhance a range of health benefits, such as libido, fertility, energy, mental clarity, emotional stability, and physical resilience. (Brown *et al.*, 2013).

Fenugreek (*Trigonella foenum-graecum Linn*) a component of long jack xxxl is a member of the Fabaceae family and it is renowned as an herbal remedy commonly employed as an aphrodisiac, enriched with a repertoire of active phytochemicals including trigonelline, steroidal saponins, salicylate, nicotinic acid, and flavonoids, which are purportedly responsible for its manifold therapeutic effects (Nagulapalli *et al.*, 2017). Numerous phytochemicals and chemical constituents, including carbohydrates, proteins, lipids, pyridine alkaloids, flavonoids, free amino acids, saponins, glycosides, cholesterol, sitosterol, and vitamins B, A, C, and nicotinic acid, are found in fenugreek seeds. Additionally, volatile oils, such as n-alkanes and sesquiterpenes, which are rich in linoleic acid, linolenic acid, oleic acid, neryl acetate, camphor, β -Pinene, β -caryophyllene, and 2, 5-dimethylpyrazine, are present in fenugreek seeds. (Nagulapalli *et al.*, 2017). Given that fenugreek has been shown to increase the frequency of erections in the morning and sexual activity, its effects on reproductive function are contradictory. (Rao *et al.*, 2016).

Eleutherococcus senticosus also known as Siberian ginseng is a perennial herb belonging to the Araliaceae family, it is mainly distributed in Russia, China, Korea, and Japan, especially in

Heilongjiang, Jilin, and Liaoning provinces of the northeast of China (Jia *et al.*, 2021). Lignons, flavonoids, flavones, phenolic acids, saponins, phenylpropanoids, essential oils, acanthoside, anthocyanins, polyphenols, chiisanoside, senticoside, triterpenic, sesamine, polysaccharides, phenylalanine, protocatechuic acid, chlorogenic acid, caffeic acid, isofraxidin, vitamins, and minerals like manganese, iron, zinc, and copper are among the multitude of bioactive substances found in Siberian ginseng. (Guo *et al.*, 2022) Siberian ginseng is one of the most well-known herbal supplements in the world. Widely used in conventional and alternative medicine, it is highly valued for its alleged advantages on immunological, neurological, endocrine, and cardiovascular systems. Current pharmacological research indicates that it tends to boost immunity, treat disorders of the cardio-cerebrovascular system, and prevent diseases brought on by stress. (Xie *et al.*, 2015).

Epimedium, belonging to the *Berberidaceae* family and often called "horny goat weed," has a long history in traditional Chinese medicine. There, its ability to "nourish the kidney and fortify the Yang" has earned it reverence. Botanical supplements made from *Epimedium*, which is well-known for its aphrodisiac properties, are often used to treat sexual dysfunction. Of the more than 260 known chemical compounds found in *Epimedium* species, prenyl flavonoids are the main components. Numerous scientific studies have examined the pharmacological range of *Epimedium*, including its ability to improve sperm count, boost sexual function, and protect the heart. (Edwards *et al.*, 2015).

Sperm quality, which encompasses several attributes like motility, morphology, and sperm count, is a major determinant of male fertility. Numerous studies have demonstrated the importance of these components for the successful fertilization of eggs and the subsequent growth of embryos. The primary objective of this study is to understand the potential impact of Long Jack on these

factors. Several studies examining the effects of herbal supplements on male fertility have brought attention to the need for evidence-based treatment regimens. (Ismail *et al.*, 2012) further investigation was done to shed more insight on *Eurycoma longifolia*'s capacity to enhance male reproductive function. The study suggests that Long Jack may affect testosterone levels, which are necessary for the growth and production of sperm. The lack of well-designed trials and the need for additional research to draw firm conclusions are both highlighted by the report. Long Jack has amazing potential, but its use needs to be considered carefully and put through a thorough scientific review. Animal research offers a crucial link between anecdotal evidence and human trials. A well-liked model in reproductive research, wistar rats offers a great way to assess Long Jack's effects on sperm quantity and quality in a controlled environment.

1.2 Statement of problem

E. longifolia may have sufficient evidence that elicit benefits on sexual performance and sperm enhancement in appropriate dosage and longer supplementation. However, this herb has shown to have other effects such as antianxiety properties. It was suggested that further studies could also focus on the herb's effects and determine if these effects are associated with an improvement in sperm quality and quantity (Khanijo and Jiraungkoorskul, 2016). This controlled animal trial is especially important to verify the claims of the marketers of “wonder” herb with regards to improving sperm quality indices. Although research on the effects of Long Jack on male reproductive function in humans has been reported (Ismail *et al.*, 2012) more research is needed to fully understand the impact of this supplement on sperm quality and quantity in animal models, particularly Wistar rats.

1.3 Justification of study

Infertility is a global health concern that requires evidence-based interventions to be addressed. This supports the study evaluating the amount and quality of sperm in Wistar rats fed with Long Jack (*Eurycoma longifolia*), a sperm enhancer. Long Jack's study has some serious flaws, even though it has been used for a very long time as a conventional treatment for male reproductive health issues. These include a scarcity of well-designed animal experiments, insufficient assessments of sperm quality metrics, and inadequate safety protocols. By carefully analyzing how Long Jack influences the quality of sperm in Wistar rats, our study aims to fill in these crucial gaps and contribute significantly to our understanding of the plant's potential sperm enhancer qualities. The results may offer empirically supported recommendations for addressing problems associated with male infertility and male reproductive health in general.

1.4 Aim

The aim of this study is to determine the effect of Long Jack sperm enhancer on sperm quality and quantity in Wistar rats.

1.4.1 Specific objectives

1. To determine the effect of long jack on sperm motility and morphology
2. To determine the effect of long jack on sperm count and compare with controls

1.5 Research questions

1. Does Long Jack administration influence sperm motility and morphology in Wistar rats?
2. Does Long Jack administration alter sperm cell count?

1.6 Hypothesis

1.6.1 Null Hypothesis (H_0)

1. There is no significant difference in sperm motility and morphology of Wistar rats treated with Long Jack and those in the control group.
2. There is no alteration in sperm cell count in Wistar rats treated with Long Jack sperm enhancer

1.6.2 Alternate Hypothesis (H_A)

1. There are significant differences in sperm motility and morphology of Wistar rats treated with Long Jack and those in the control group.
2. There are alterations in sperm cell count in Wistar rats treated with Long Jack sperm enhancer

CHAPTER TWO

LITERATURE REVIEW

2.1 Long Jack Sperm Enhancer

Long jack sperm enhancer is a mixture of herbs such as Tongkat Ali, Maca, Fenugreek, Siberian ginseng and horny goat weed.

2.2 Tongkat Ali (*Eurycoma Longifolia*)

Long Jack, scientifically known as *Eurycoma longifolia*, is a medicinal plant native to the rainforests of Southeast Asia, particularly found in countries like Malaysia, Indonesia, and Thailand (Rehman *et al.*, 2016). Commonly referred to as Tongkat Ali, Long Jack has a long history of traditional use in folk medicine, particularly in Southeast Asian cultures, where it has been revered for its purported aphrodisiac and fertility-enhancing properties. The roots of the Long Jack plant are the primary source of its medicinal compounds and have been utilized for centuries to address various health concerns, including male reproductive health issues such as erectile dysfunction, low libido, and infertility. Traditionally, Long Jack preparations were consumed as decoctions or infusions, but modern formulations are available in various forms such as extracts, capsules, and powders, making it more accessible to consumers worldwide. Long Jack's possible effects on male fertility and reproductive function have drawn interest recently from both the scientific community and the general population. Research attempts to examine the pharmacological characteristics and mechanisms of action of Long Jack extracts and its bioactive components have surged as a result of this growing interest.

It is thought that the abundance of bioactive substances found in long jack, including as flavonoids, alkaloids, saponins, and quassinoids, contributes to its therapeutic qualities. Among them, eurycomanone is one of the most researched substances and is believed to have a range of biological effects, such as pro-androgenic, antioxidant, and anti-inflammatory properties.

Additionally, Long Jack has a reputation for regulating hormone levels, especially testosterone, which is essential for healthy male reproduction. According to studies, Long Jack may increase the synthesis of testosterone by inducing the pituitary gland to release more luteinizing hormone (LH), which would then promote spermatogenesis and improve the quality of sperm (Mohamed *et al.*, 2015).

2.2.1 Traditional Uses

In traditional folk medicine, *Eurycoma longifolia*, also referred to as Tongkat Ali, has a variety of medicinal applications. Its uses include the treatment of conditions like dropsy, lumbago, indigestion, fever, jaundice, cachexia, and as a postpartum tonic (Rehman *et al.*, 2016). Known for its aphrodisiac qualities and effectiveness in treating malarial intermittent fever, *E. longifolia* is a common ingredient in traditional medicine. While its fruits are used to treat diarrhea, the leaves of *E. longifolia* are used in decoctions to wash away skin disorders like itches. In addition, the bark is mostly used as a vermifuge; taproots are used to treat hypertension; and root bark is used to cure fever and diarrhea. Extracts derived primarily from the roots of *E. longifolia* are used in traditional medicine to treat a wide range of ailments, such as syphilis, leukemia, osteoporosis, aging, diabetes, anxiety, aches, constipation, exercise recovery, fever, and glandular swelling. It is also recognized as a health supplement, appetite stimulant, antibacterial, and aphrodisiac. It has long been customary to drink a water infusion made from the roots of *E. longifolia*. Modern times have brought about the emergence of more practical formulas, usually

in the form of tea and coffee additions. There are already more than 200 items available in the health-food market, ranging from powdered raw root to capsules mixed with various herbs. The National Pharmaceutical Control Bureau of Malaysia registered over 200 *E. longifolia* products in 2016, indicating a significant demand for the plant's products due to its many traditional and scientifically proven benefits. As a result, *E. longifolia* is now formally acknowledged in Malaysia and offered for sale as a traditional herbal medicine. About 21,000 kg of *E. longifolia* are harvested each year by collectors yet the demand exceeds this figure, reaching an estimated >54,000 kg annually. (Rehman *et al.*, 2016)

2.2.2 Phytochemical constituent

It is well known that long jack contains bioactive substances such eurycomanone, peptides, alkaloids, and quassinoids. These substances fall into several types, including eurycolactone, laurycolactone, eurycomalactone, canthin-6-one alkaloids, β -carboline alkaloids, squalene derivatives, tirucallane-type triterpenes, biphenyl neolignan, and bioactive steroids. (Rehman *et al.*, 2016)

2.3 Maca (*Lepidium Meyenii* Walpers) Extract

For 1300–2000 years, Peruvians have grown maca (*Lepidium meyenii* Walp.), a member of the Brassicaceae family, as a crop and medicinal herb. Native Andean inhabitants employed the plant for its energizing qualities, improving bovine and human fertility, and other medical benefits like healing anemia and respiratory ailments. (Beharry *et al.*, 2018). Iron, calcium, copper, zinc, and potassium are just a few of the vital micro- and macroelements that it effectively stores. For centuries, the indigenous people have held great respect for Maca, utilizing it in their traditional medicine and culinary traditions to enhance a range of health

benefits, such as libido, fertility, energy, mental clarity, emotional stability, and physical toughness. (Brown *et al.*, 2013)

2.3.1 Phytochemical Constituents of Maca Plant

The vital nutrients proteins, carbohydrates, lipids, minerals (iron, calcium, copper), amino acids, fatty acids, sterols, alkaloids, and glucosinolates are all included in the chemical composition of maca. Among the biologically active substances are Flavonoids, tricin derivatives, β and polyphenols, thiazoles, glucosinolates, polysaccharides, polyphenols, sterols, complex polysaccharides, imidazole alkaloids, pyrrole alkaloids, and polysaccharides (Gonzales, 2012).

2.4 Fenugreek (*Trigonella Foenum-Graecum* Linn) Extract

Trigonella foenum-graecum Linn, also known as fenugreek, is a well-known herbal remedy that is frequently used as an aphrodisiac. It is a member of the Fabaceae family and is rich in active phytochemicals, such as salicylate, nicotinic acid, trigonelline, and flavonoids, which are thought to be responsible for a variety of its therapeutic effects. (Nagulapalli *et al.*, 2017). Beyond its aphrodisiac qualities, fenugreek seeds are widely used in the Middle East and South Asia as a spice in food preparation as well as a traditional remedy for gastrointestinal ailments, diabetes, hypercholesterolemia, and inflammation. (Belguith-Hadriche *et al.*, 2010). Given that fenugreek has been shown to increase the frequency of erections in the morning and sexual activity, its effects on reproductive function are contradictory. (Rao *et al.*, 2016), contrasted with data indicating no appreciable changes in male fertility or reproductive efficiency (Hind *et al.*, 2017) As a result, it is apparent that although fenugreek is widely used in traditional medicine, its aphrodisiac properties are not well supported in the literature.

2.4.1 Medicinal and Pharmacological Properties of Fenugreek

Scientists have documented fenugreek's many benefits. Anti-diabetic, hypoglycaemic, anti-hyperlipidemic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic, lactation stimulant, galactagogue, hypocholesterolemic, immunomodulatory, anti-carcinogenic, anti-helminthic, anti-nociceptive, anti-oxidant, anti-ulcer, gastroprotective, and hepatoprotective effects are just a few of its many therapeutic qualities. When soaked overnight, fenugreek seeds improve the texture of hair and skin while lowering the risk of myocardial damage and abnormal blood clotting linked to heart attacks and strokes. (Al-Asadi, 2014). Moreover, fenugreek is a great provider of vital nutrients like calcium, iron, sulfur, chlorine, and vitamins A and C. It has historically been used as a tonic to cure weakness and leg edema. It is also frequently used to promote lactation and ease menstruation cramps. (Varshney *et al.*, 2012). By controlling sex hormones, sperm viability, sperm count, and treating sexual dysfunction in both men and women, fenugreek has a positive impact on reproductive health. Its bioactive substances have anti-sterility and anti-androgenic qualities. Examples of these include steroidal saponins like yamogenin and diosgenin. According to research, saponins modulate E2 activity in rat models of ovarian hyperstimulation syndrome by binding to estradiol receptors. (Ben *et al.*, 2019). Diosgenin stimulates the pituitary gland's growth hormones, which in turn controls sexual behavior. (Mansour *et al.*, 2021). Furthermore, diosgenin functions as a precursor for a number of commercially available steroids, including progesterone, cortisone, and pregnenolone. Research on rats exposed to fenugreek powder, oil extract, and aqueous extract has revealed changes in sperm viability, count, and sex hormone levels. (Mansour *et al.*, 2021).

2.4.2 Phytochemical Constituent of Fenugreek

Numerous phytochemicals and chemical constituents, including carbohydrates, proteins, lipids, pyridine alkaloids, flavonoids, free amino acids, saponins, glycosides, cholesterol, sitosterol, and vitamins B, A, C, and nicotinic acid, are found in fenugreek seeds. Additionally, volatile oils, such as n-alkanes and sesquiterpenes, which are rich in linoleic acid, linolenic acid, oleic acid, neryl acetate, camphor, β -Pinene, β -caryophyllene, and 2, 5-dimethylpyrazin, are present in fenugreek seeds. (Nagulapalli *et al.*, 2017)

2.5 Siberian Ginseng (*Eleutherococcus Senticosus*) Extract

A perennial herb of the Araliaceae family, *Eleutherococcus senticosus* is mostly found in China, Korea, Japan, and Russia; it is particularly prevalent in the northeastern Chinese provinces of Heilongjiang, Jilin, and Liaoning. (Jia *et al.*, 2021) It holds a prominent position among herbal supplements worldwide. Employed extensively in traditional and folk medicine, it is esteemed for its purported benefits on cardiovascular, immune, nervous, and endocrine functions. Modern pharmacological studies have shown that it tends to stimulate immunity, prevent diseases caused by stress, and treat diseases of the cardio-cerebrovascular system (Xie *et al.*, 2015).

2.5.1 Phytochemical Constituents of Siberian Ginseng

Siberian ginseng contain a variety of bioactive compounds such as lignans, flavonoids, flavones, phenolic acids, saponins, phenylpropanoids, essential oils, acanthoside, anthocyanins, polyphenols, chiisanoside, senticoside, triterpenic, sesamine, polysaccharides, phenylalanine, protocatechuic acid, chlorogenic acid, caffeic acid, isofraxidin, vitamins, and minerals like manganese, iron, zinc, and copper. (Guo *et al.*, 2022)

2.5.2 Antioxidant Potential

Due to the synergistic interactions between its chemical ingredients, *E. sessiliflorus* exhibits outstanding antioxidant capacities (Munteanu *et al.*, 2021). Significant hydroxyl radical scavenging ability is demonstrated by the flavonoids found in *E. sessiliflorus* preparations (Min *et al.*, 2007).

2.6 Horny Goat Weed (*Epimedium Spp.*)

Phlegm-producing goat weed, or epimedium, is a member of the Berberidaceae family and has a long history in traditional Chinese medicine. It is valued for its ability to "nourish the kidney and fortify the Yang". Botanical supplements made from Epimedium, which is well-known for its aphrodisiac properties, are often used to treat sexual dysfunction. Of the more than 260 known chemical compounds found in Epimedium species, prenyl flavonoids are the main components. Numerous scientific studies have examined the pharmacological range of Epimedium, including its ability to improve sperm count, boost sexual function, and protect the heart. (Edwards *et al.*, 2015)

2.6.1 Traditional Uses

It enhances sexual desire and performance and has been used traditionally as an aphrodisiac. It is said to work by increasing testosterone levels, which are the main sex hormone for men, which in turn promotes better sexual function. Significantly, a small-scale, double-blind clinical study using an epimedium herbal complex supplement on 25 healthy men and 13 sildenafil (Viagra) users showed that taking the herbal complex supplement on a daily basis for at least 45 days produced higher levels of sexual satisfaction than sildenafil. (Ma *et al.*, 2011) It has been shown that icariin, a phytoestrogenic substance, activates aromatase to promote the manufacture of

estrogen. Additionally, it has lipid-lowering benefits, as shown by decreased platelet adhesiveness and aggregation in atherosclerotic rabbits, as well as lower serum levels of total cholesterol and low-density lipoprotein cholesterol. (Zhang *et al.*, 2013).

2.6.2 Phytochemical Constituents of Horny Goat Weed

Numerous substances have been found in Horny Goat Weed, such as alkaloids, phytosterols, terpenoids, chlorogenic acid, lignans and their glycosides, ionones and their derivatives, phenethyl alcohol glycosides, xanthenes, aldehydes, acids, and Epimedium polysaccharides. (Zhuang *et al.*, 2023).

2.7 The Testis

An organ that is paired is called a testicle or testis. According to Wein et al. (2012), the testes measure 4 to 5 cm in length, 3 cm in width, and 2.5 cm in depth. A robust capsule made up of the visceral tunica vaginalis, the tunica albuginea, and the tunica vasculosa encloses them. Attached to the posterolateral portion of the testis is the epididymis. The mediastinum testis, which is where ducts and vessels cross the testicular capsule, is formed by the tunica albuginea projecting inward beneath it. From the mediastinum, septa extend outward and adhere to the inner surface of the tunica albuginea, forming 200–300 cone-shaped lobules that have one or more convoluted seminiferous tubules within each. The cells that produce testosterone are called interstitial (Leydig) cells, and they are found in the loose tissue that surrounds the tubules. The seminiferous tubules become straight (tubuli recti) toward the lobule apices and enter the mediastinum testis to form an anastomosing network of tubules. This network, referred to as the rete testis, divides into 12 to 20 efferent ductules before entering the caput, which makes up the majority of the epididymis. The efferent ductules expand, get more coiled, and produce conical

lobules in this location. Each lobule's duct empties into a single epididymal duct, which forms the body and tail of the epididymis by winding through its fibrous sheath for around 6 m. The duct becomes the vas deferens when it gets closer to the tail, thickening and straightening. (Wein *et al.*, 2012).

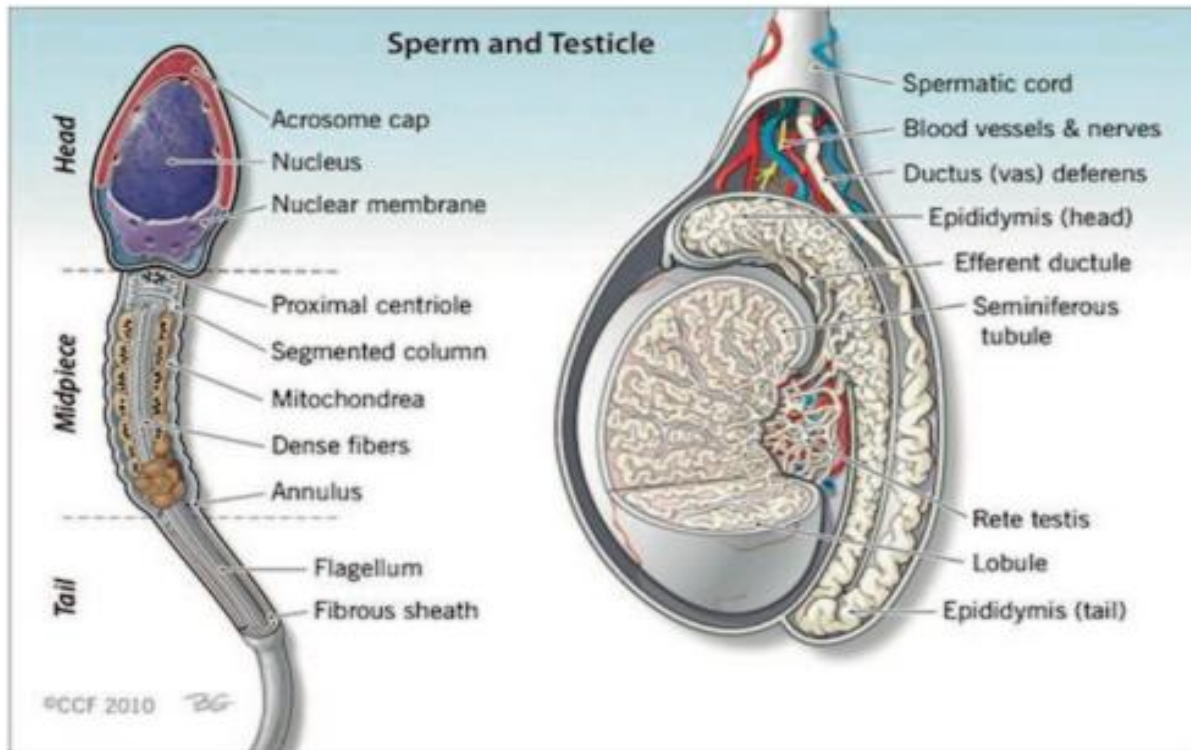


Fig. 2.1 The human testis, epididymis, and spermatozoa. A fully developed human spermatozoon with its head, midpiece, and tail portions visible is seen on the left. The human testis, seminiferous tubules, and epididymis are seen on the right, with the corpus (head) and caudal (tail) parts visible. (Durairajanayagam *et al.*, 2015).

2.7.1 Function of the Testis

The testis performs two basic functions which are sperm production and testosterone secretion

2.7.2 Anatomy and Location

The fibrous covering of the tunica albuginea encloses the oval-shaped testis. The scrotum is external to the body, and its placement aids in maintaining the lower temperature required for sperm generation.

2.7.3 Sperm Production

The process of producing sperm is known as spermatogenesis, and it is a continual, tightly controlled process of male germ cell differentiation and multiplication. This mechanism occurs in the testes' seminiferous tubules to enable sperm production all throughout an individual's life. When a mammal reaches adolescence, spermatogenesis begins with the development of undifferentiated spermatogonia and their meiotic entrance. (Feng *et al.*, 2014). Spermiogenesis, meiotic cell division I, meiotic cell division II, and mitotic division are among the intricately intertwined processes involved. The primary germ cell (PGC) is the source of the germ cell lines. PGCs begin to arise between endoderm cells in humans at the end of the third week of development. By the end of the fifth week, the PGCs move to the genital tract, where the Y chromosome causes the genital tract to proliferate and change into the primary male sexual organs. During the first trimester of mitosis, PGCs are often referred to as gonocytes. They cease in the G3 phase of the cell cycle and don't speak again until delivery (i.e., when they transform into spermatogonia). Spermatogonia don't speak until they reach puberty. After birth, spermatogonia proliferate mitotically, marking the start of spermatogenesis. (Emad *et al.*, 2020). Spermatogonia multiply and are maintained during mitosis. Spermatogonia (types A and B) and

initial spermatocytes (spermatocytes I) are involved in the mitotic phase. Developing germ cells that are joined by intracellular bridges during a sequence of mitotic divisions give rise to primary spermatocytes. An early spermatocyte in the basal compartment and all subsequent germ cells in the adluminal compartment are supported by the tight junction barrier. To create primary, secondary, and spermatid spermatocytes, type B spermatogonia go through mitosis. Type B spermatogonia differentiate into preleptotene at stage VII of the epithelial cycle, and then leptotene spermatocytes, which are the main diploid spermatocytes that cross the BTB and differentiate into zygotene spermatocytes at stages VIII–IX. During stage XIV, spermatocytes go through two rounds of meiosis in succession after entering the adluminal chamber. The primary spermatocytes differentiated into secondary spermatocytes during the first meiotic division, also known as the reduction division. It takes the spermatocyte around 26 days to develop. Type I spermatocytes have the longest lifespan because they go through a lengthy prophase during the first division. Because the prophase of the second meiotic division is so brief, secondary spermatocytes have short lifespan (Sharma *et al.*, 2011). Meiosis I and II are two processes that primary spermatocytes in the basal compartment go through to produce haploid spermatids, which are discharged from the seminiferous epithelium at the spermiation region. This process is known as the spermatocytogenesis phase. (Houda *et al.*, 2022)

Gonadotropins are necessary for undifferentiated spermatogonia to be committed to differentiating spermatogonia and proper spermatogenesis. The hypothalamus gonadotropin-releasing hormone (GnRH) regulates the pituitary gland's secretion of gonadotropins, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Gonadotropins' effects on the Leydig and Sertoli cells, which express the luteinizing hormone receptor (LHR) and follicle stimulating hormone receptor (FSHR), respectively, provide signals that control spermatogenesis.

The only cells found in the testis' seminiferous tubules when FSH and LH are absent are undifferentiated germ cells and Sertoli cells. During the prepubertal or juvenile phase of testis development, there is a prolonged hypogonadotropic condition in which undifferentiated germ cells and Sertoli cells make up the whole epithelium of seminiferous tubules. In the premature or juvenile testis, all germ cells are undifferentiated spermatogonia, which are largely gonadotropin-independent in their proliferation. (Khanehzad *et al.*, 2021).

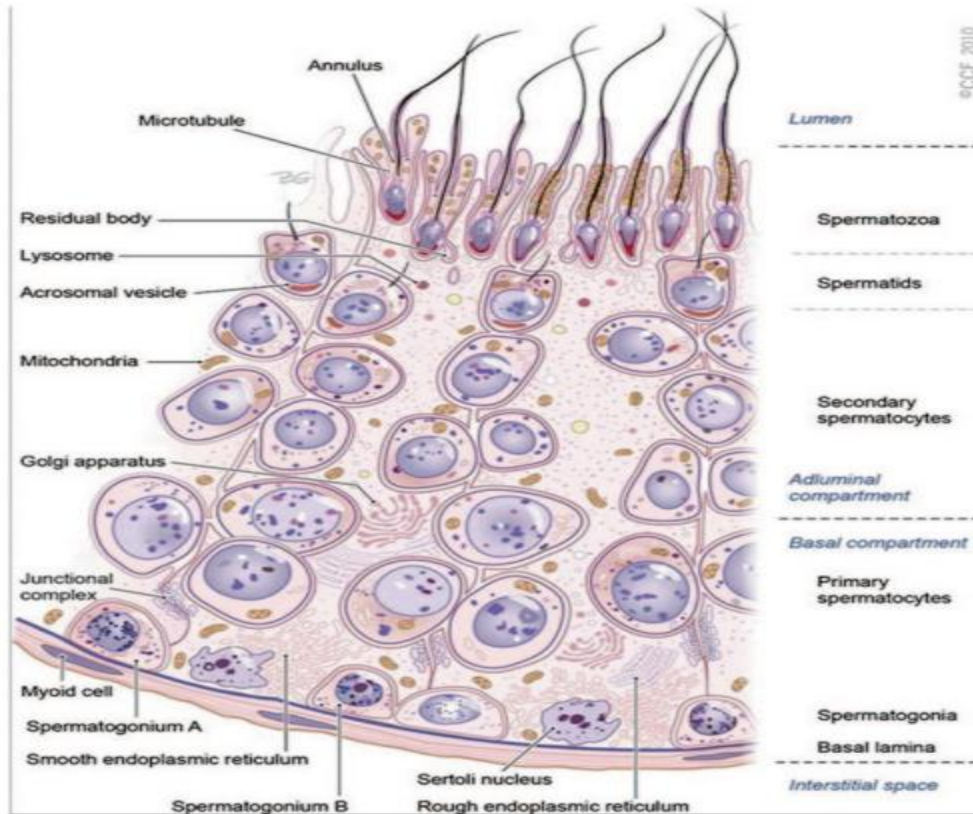


Fig. 2.2 Seminiferous tubules. A sectional view of the seminiferous tubule's germinal epithelium. The basal and adluminal compartments are the divisions made by the Sertoli cell between the germinal epithelium. Spermatozoa that are complete are discharged into the lumen. (Durairajanayagam *et al.*, 2015).

2.7.4 Intrinsic and Hormonal Regulation

Spermatogenesis travels through a complex regulatory process inside the testis. Leydig cells regulate this process by the release of growth factors, neuroendocrine compounds that function as neurotransmitters, and testosterone. Growth factors are also necessary for the health of blood vessels, the lamina propria of seminiferous tubules, Sertoli cells, and adjacent Leydig cells. (Barry *et al.*, 2018). Leydig cells are also essential for maintaining Sertoli cells and controlling blood flow in the intertubular microvasculature. Myofibroblast contractility is regulated by peritubular tissue cells, which in turn controls spermatozoa transit through peristaltic motions of seminiferous tubules. Conversely, Sertoli cells distribute diverse growth factors, involving distinct germ cells in the maturation and control of their respective counterparts. The complex interaction between the pituitary gland, the gonads, and the hypothalamus, referred to as the hypothalamus–pituitary–gonadal (HPG) axis, controls spermatogenesis by hormonal pathways. The hypothalamus acts as an integrating center, starting with signals from higher centers and coordinating the pulsatile release of gonadotropin releasing hormone (GnRH), with peaks happening around every 1.5 hours. Next, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), two gonadotropins, are produced when GnRH activates on the anterior pituitary. Desensitization of gonadotropin receptors caused by prolonged GnRH secretion reduces the release of LH and FSH. (Kelly *et al.*, 2011)

While inhibin controls the release of FSH, LH secretion follows the pulsatile pattern of GnRH. When LH reaches the testes, it activates Leydig cells, which then convert cholesterol into testosterone. A negative feedback mechanism is activated by rising testosterone levels at the pituitary (short loop) and hypothalamus (long loop), which ultimately suppresses the synthesis of GnRH and controls testosterone levels. Simultaneously, FSH stimulates the Sertoli cells in the

testes, causing them to secrete androgen-binding protein (ABP) and inhibin. By reducing FSH release through the pituitary's negative feedback loop, elevated inhibin levels regulate inhibin levels. (Kelly *et al.*, 2011).

2.7.5 Testosterone

The primary and secondary sexual features are mostly determined by testosterone, which is the main androgen in circulation in males and is produced in the testis' Leydig cells. Produced from cholesterol found in Leydig cells, it controls the growth, maturation, and movement of sperm cells as well as the unique morphological and behavioral characteristics that set males and females apart. (Nassar *et al.*, 2018)

2.8 Role of Sperm in Fertilization

The fertilization process is a meticulously orchestrated sequence of events that culminates in the fusion of spermatozoa with the female oocyte, resulting in the formation of a viable embryo. Sperm play a pivotal role in this process by delivering the paternal genetic material necessary for embryo development. Upon ejaculation, spermatozoa are released into the female reproductive tract, where they undergo capacitation, a series of biochemical and physiological changes that render them capable of fertilization (Suarez, 2008). The process of sperm capacitation includes the elimination of cholesterol from the sperm membrane, an increase in sperm motility, and the development of the ability to carry out the acrosome reaction, which is an essential step in fertilization. (Shadan *et al.*, 2007). Sperm motility and velocity are necessary for them to travel through the female reproductive canal and reach the fertilisation location, which is usually the fallopian tubes ampulla, where the oocyte is waiting to be fertilized. (Dcunha *et al.*, 2022). Spermatozoa must traverse the cervical mucus, penetrate the cervix, and ascend through the uterine cavity to reach the fallopian tubes, where they encounter the oocyte. The acrosome

reaction, triggered by binding to the zona pellucida surrounding the oocyte, involves the exocytosis of enzymes stored within the acrosome, facilitating the penetration of the zona pellucida and the binding of sperm to the oocyte membrane (Florman and Ducibella, 2006). Sperm with normal morphology are better equipped to undergo the acrosome reaction and successfully fuse with the oocyte membrane, leading to fertilization. Once fusion occurs, the sperm delivers its genetic payload, consisting of the haploid paternal genome encapsulated within the sperm nucleus, into the oocyte cytoplasm. This union of genetic material from both parents initiates the process of embryogenesis, ultimately giving rise to a new organism with a unique combination of genetic traits.

2.9 Semen analysis Parameters

The first evaluation of male fertility usually depends on a conventional semen analysis, which is considered the "gold standard." These characteristics of semen, particularly sperm count, motility, and morphology, are predictive of male fertility and may provide insight into possible issues related to the testicles that may contribute to infertility. (Esteves *et al.*, 2012).

2.9.1 Sperm Morphology

The sperm is divided into the head, midpiece and tail. The head is an elongated, oval shape that is also relatively flat, with some variation on an individual basis. The head consists of the acrosome, the post-acrosomal lamina, and the nucleus. The acrosome covers the anterior portion of the sperm head and contains hydrolytic enzymes which are released in order for the sperm to penetrate an oocyte. In addition, it is theorized that the proteases and hydrolases contained within the acrosome play a role in the penetration of the oocyte cumulus complex, in addition to the zona pellucida. The post-acrosomal lamina covers the caudal nucleus, which contains the highly condensed male genome. Species specific traits of the stallion sperm head include a

characteristic asymmetrical head, a paraxially inserted tail, and a small acrosome relevant to other species. (Foster and Gerton, 2016). The midpiece contain the mitochondria which supplies the energy the tail needs to move. The principal piece of the propelling tail consists of the continuation of the axoneme and tapering dense fibers. The distinguishing feature of the principal piece is a protein-rich fibrous sheath that provides structure and flexibility for tail movements. The end of the fibrous sheath indicates transition from the principal piece to the end piece, which solely consists of the axoneme. All of these components are covered superficially by the sperm plasma membrane. Although parameters of a morphologically normal sperm may vary significantly on an individual basis, abnormalities in the sperm anatomy may be an indication of subfertility or a problem with spermatogenesis. (Morgan *et al.*, 2021).

2.9.2 Sperm Motility

The human spermatozoon is a highly specialized motile cell with a little amount of cytoplasm and a highly condensed nucleus. Despite being transcriptionally and translationally inert, it possesses specific metabolic pathways that are essential for the process of fertilization to occur. The sperm matures in the epididymis following production in the seminiferous tubules, and it then passes via the female reproductive tract to enable the transfer of the paternal genome into the oocyte. The ampullary site of the fallopian tube is the location of fertilization for spermatozoa, which are deposited in the vagina during coitus. The female reproductive tract, which is controlled by female reproductive hormones, aids in the forward progressive motility that leads to the ampulla. As a result, sperm motility is thought to be the most reliable indicator of male reproductive potential among all the semen metrics. Human spermatozoa are thought to travel an average of around 19 cm and go through a number of physiological and biochemical changes before encountering an oocyte, according to research done on excised human uterus and

tubes. Spermatozoa can be classified as progressively motile, non-progressive, or immotile based on their pattern of movement and velocity. (Dunham et al., 2022).

2.9.3 Sperm Count

Sperm count, sometimes referred to as sperm concentration, is the quantity of sperm cells in a specific volume of semen. It is a crucial component of male fertility since it is necessary for fertilization. Sperm count is primarily influenced by two factors: the duration since the last ejaculation and the quantity of sertoli cells in the testes. (Sharpe, 2012). Between 15 million to 200 million sperm per milliliter of semen is generally accepted as the reference range for sperm count. Having less than 15 million sperm per milliliter of semen is known as oligospermia, a medical disorder marked by low sperm count. (WHO, 2010)

2.10 Sperm Quality and Fertility

Sperm quality refers to the overall health and functionality of spermatozoa, encompassing characteristics such as morphology and motility. Each of these parameters plays a critical role in the fertilization process. Sperm morphology, which evaluates the size, shape, and structural integrity of sperm cells, is indicative of their potential to penetrate the oocyte's protective layers and achieve fertilization. Abnormal sperm morphology, characterized by defects such as head or tail abnormalities, can significantly impair fertility by reducing the likelihood of successful fertilization (Auger *et al.*, 2016). Additionally, sperm motility, defined as the ability of spermatozoa to move progressively through the female reproductive tract, is essential for navigating towards the oocyte and penetrating its outer membrane. Studies have consistently demonstrated a strong correlation between sperm motility and fertility outcomes, with poor motility associated with decreased chances of natural conception (Cooper *et al.*, 2010).

2.11 Sperm Quantity and Fertility

Sperm quantity, often quantified by sperm count per ejaculate, is a fundamental aspect of male fertility. A sufficient sperm count is necessary to ensure an adequate number of spermatozoa reach the female reproductive tract and successfully fertilize the oocyte. Low sperm count, known as oligozoospermia, is a common cause of male infertility and can significantly reduce the chances of conception. Moreover, severe oligozoospermia or azoospermia (absence of sperm in ejaculate) may necessitate assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), to achieve pregnancy. Research has shown a concerning trend of declining sperm counts over the past few decades. . Environmental factors such as exposure to endocrine-disrupting chemicals (EDCs), pesticides, and heavy metals have been associated with adverse effects on sperm production and quality. These substances can disrupt the endocrine system, interfere with hormonal balance, and impair spermatogenesis, leading to reduced sperm counts and fertility (Jurewicz and Hanke, 2011). Lifestyle factors also play a significant role in sperm quantity and fertility. Habits such as smoking, excessive alcohol consumption and recreational drug use have been linked to decreased sperm counts and impaired sperm function (Povey *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This is an experimental study design to assess the sperm quality and quantity of wistar rat treated with Long Jack XXL sperm enhancer.

3.2 Study Subjects

In this study, animal (rats) model was used. A total of twenty five (25) healthy adult males of the Albino Wistar strain were purchased from the animal holdings of the Department of Anatomy, University of Benin, Benin city, Nigeria. The rats were housed at the animal housing wing of the department of Anatomy, University of Benin and had free access to commercial pellets of feed and water.

3.3 Ethical approval

Ethical clearance was obtained from the Animal Research Ethics Committee of the Faculty of Pharmacy at the University of Benin, Benin City

3.4 Drug Procurement

Long Jack XXL Men Power Booster supplements was procured from Saint Peters Pharmacy in Benin City, Edo State, and used for this study. The capsules were dissolved in 2 mL of distilled water and administrated to the experimental groups.

3.5 Animal Care

The rats were housed at the animal housing wing of the department of Anatomy, University of Benin and had free access to commercial pellets of feed and water. They were kept in clean and well ventilated plastic cages. The wood shavings in the cages were frequently changed to ensure proper hygienic conditions.

3.6 Inclusion Criteria

Apparently healthy adult male Wistar rats weighing between 150-200g were included in this study

3.7 Exclusion Criteria

Immature males Wistar rats, female rats and rats weighing less than 150g (<150g) were excluded from this study

3.8 Experimental Design

A total of twenty-five (25) adult male Wistar rats were divided into five groups, each comprising five animals. The animals were selected and an appropriate dose of the long jack supplement was administered orally via an orogastric tube for a supplementation period of 28 days which is as follows:

Group 1: This control group was fed only commercial feed and water throughout the period of the experiment.

Group 2: Animals were fed normal pellet of feeds and water ad libitum and administered 10 mg/kg of *long jack* supplement in 2 mL distilled water orally.

Group 3: Animals were fed normal pellet of feeds and water ad libitum and administered 20 mg/kg of *long jack* supplement in 2 mL distilled water orally.

Group 4: Animals were fed normal pellet of feeds and water ad libitum and administered 40 mg/kg of *long jack* supplement in 2 mL distilled water orally.

Group 5: Animals were fed normal pellet of feeds and water ad libitum and administered 80 mg/kg of *long jack* supplement in 2 mL distilled water orally.

3.9 Preparation of *long jack* supplement

In this study, the long jack supplement capsule will be dissolved in 2 mL of distilled water according to the various concentrations.

3.10 Collection of Semen

Animals in all five (5) groups were sacrificed on day 29 and their semen samples were collected for seminal fluid analysis.

3.11 Laboratory Analysis

The sperm parameters estimated in this study include sperm count, sperm morphology and sperm motility.

3.12 Sperm Count

Sperm cells were collected from the caudal epididymis of the sacrificed rats; the rats was sacrificed and the caudal epididymis located and ligated with a minimum of 36mm length, both extremities of the caudal epididymis was ligated, cut and placed in a sterile petri dish. To the petri dish, 6 µl of normal saline already adjusted to $37\pm 2^{\circ}\text{C}$ was added. caudal epididymis was teased to allow the sperm cell diffuse out of it

3.12.1 Reference range

15 – 200 million per milliliter (mL)

3.12.2 Procedure

The semen was mixed and a 1:20 dilution was prepared while the Neubauer counting chamber was loaded to allow the spermatozoa to settle in a humid chamber the samples were assessed within 10–15 minutes to prevent evaporation which has noticeable effect on sperm positioning. It was placed on the microscope and at least 200 spermatozoa per replicate were counted.

3.13 Sperm Motility

The sperm cell motility was determined with the correlation between progressively motile sperm cells after ejaculation and the fertility. The motility was evaluated with regards to three variables: Progressively motile, Non-progressive motility and immotile spermatozoa and it is usually expressed in percentage. Spermatozoa can show good motility in the seminal plasma 24 hours after ejaculation but in some semen samples the motility may decline much faster (WHO, 2010).

3.13.1 Procedure

A drop of the sperm cell was taken from the petri dish and dispensed on a clean grease free slide and further covered with a transparent cover slip. The slide was placed on the microscope focused at x10 and viewed at x40 objective magnification lens. The motility was scored in percentage according to their nature of motility as, Progressive, Non progressive and immotile sperm cells.

3.14 Sperm Morphology

The sperm cell morphology was assessed by staining the slide Papanicolaou stain

3.14.1 Reagent Composition

Acidic ethanol: 1.0 ml of concentrated hydrochloric acid to 200 ml of 70% (v/v) ethanol.

Xylene:ethanol, 1 + 1 (1:2): mix equal parts of 100% ethanol and xylene³²

3.14.2 Procedure

A drop of the sperm cells was dispensed on a grease free clean slide and a smear was made, the slide was left to air dry. The slide was immersed in 95% (v/v) ethanol for at least 15 minutes and sequentially in ethanol 80% (v/v) for 30 seconds, ethanol 50% (v/v) 30 seconds, distilled water for 30 seconds, Harris haematoxylin for 4 minutes, distilled water for 30 seconds, dipped four times in acidic ethanol, rinsed in running cold tap water for 5 minutes, ethanol 50% (v/v) for 30 seconds, ethanol 80% (v/v) for 30 seconds, ethanol 95% (v/v) for 15 minute, G-6 orange stain for 1 minute, ethanol 95% (v/v) for 30 second, ethanol 95% (v/v) for 30 second, ethanol 95% (v/v) for 30 seconds, EA-50 green stain for 1 minute, ethanol 95% (v/v) for 30 seconds, ethanol 95% (v/v) for 30 seconds, ethanol 100% for 15 seconds, mounted, covered with a coverslip and viewed with a microscope.

3.15 Quality Control

Quality control measures include ensuring proper calibration of the microscope and staining reagents for accurate results.

3.16 Micrograph

The sample is placed on the microscope, focused and the image of the morphology is captured using a digital camera.

3.17 Statistical Analysis

Graph Pad Prism version. 5.01 (Graph Pad Software Inc. San Diego, CA, USA) was used to analyze the data obtained. Normally distributed data were analyzed with one-way analysis of variance (ANOVA) and Turkey's post-test. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Table 4.1 The data presented depicts a comparison between initial and final body weights among the control group and experimental groups. There was statistically significant difference ($p < 0.05$) in the initial and final body weight was observed when comparing all groups to the control group.

Table 4.1 Initial and Final Body Weight of Study Subjects across all experimental groups in comparison with the control group

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Pvalue
	Control	10mg/kg	20mg/kg	40mg/kg	80mg/kg	
Initial Weight (g)	167.4±3.24	175.8 ±5.96	172.4±5.51	165.2 ±4.28	167.6 ±2.51	0.0016*
Final Weight (g)	186±5.96	216.2±4.06	217±6.99	196.2±3.54	203.8±5.09	

Values are shown in Mean ± SEM (*) indicates $p < 0.05$ which was considered statistically significant.

Table 4.2 The data presented depicts a comparison of sperm indices in Wistar Rats supplemented with different Concentrations of Long Jack XXXL Sperm Enhancer. The Sperm count increased from the control group 1 to group 2, group 3, group 4, to group 5. The differences in the means were however not statistically significant ($p > 0.05$) when compared with control. There was no difference ($p > 0.05$) in in the mean progressive motility, non-progressive motility, immotile form, normal morphology and abnormal morphology when compared with control.

Table 4.2 Comparison of Sperm indices in Wistar Rats supplemented with Different Concentrations of Long Jack XXXL Sperm Enhancer

Parameter	Group1 Control	Group 2 10mg/kg	Group3 20mg/kg	Group4 40mg/kg	Group5 80mg/kg	Pvalue	Fvalue
Sperm Count (x10 ⁶ cell/mL)	8.57±2.0	9.6±1.9	9.2±2.5	9.9±2.2	10.1±1.2	0.3877	1.111
Progressive Motility (%)	72±4.02	71.5±5.23	71.2±3.11	73.5±4.73	72.0±5.25	0.2427	1.534
Non-progressive Motility (%)	15.5±2.02	16.7±3.09	18.2±1.30	18.0±4.0	9.5±3.7	0.2731	1.427
Immotile (%)	12.50±2.1	12.25±2.3	11±1.47	8.5±.5	6.0±1.68	0.1100	2.283
Normal Morphology	83±2.04	79.25±2.2	79.75±0.48	81.75±0.75	83.00±1.291	0.2863	1.384
Abnormal Morphology	17±2.04	20.75±2.18	20.25±0.48	18.25±0.75	17.00±1.291	0.2863	1.384

Values are shown in Mean ± SEM p<0.05 is significant

List of Plate

Plate A1: Numerous normal mature sperm cells with tail head and neck.

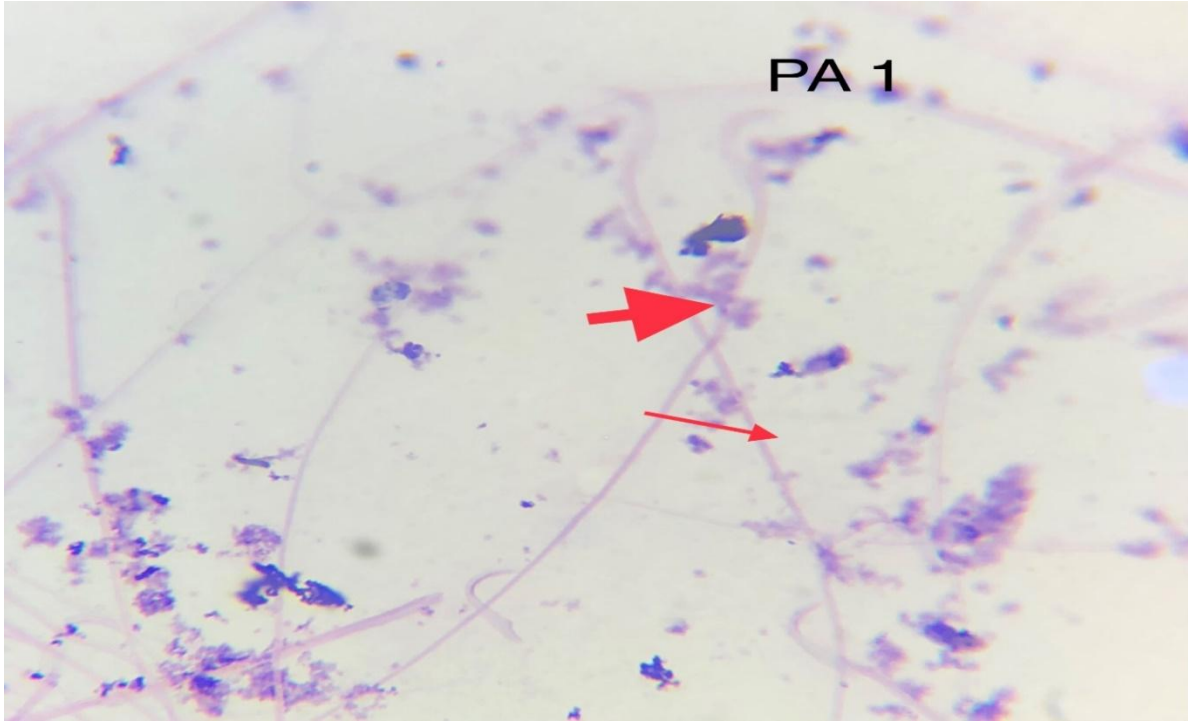


Plate A1: Numerous normal mature sperm cells with head, neck and tail

CHAPTER FIVE

DISCUSSION

The World Health Organization reported that over 186 million couples in developed countries suffered from infertility a condition that is more worrisome in the so-called infertility belt of sub-Saharan Africa and about 40-50 % of the problem is attributed to men. Long Jack XXXL Sperm Enhancer an herbal supplement containing long jack (*Eurycoma longifolia*) commonly referred to as Tongkat Ali, siberian ginseng, maca extract, fenugreek extract and horny goat weed. It has been revered for its aphrodisiac and fertility-enhancing properties. Traditionally, Long Jack preparations were consumed as decoctions or infusions, but modern formulations are available in various forms such as extracts, capsules, and powders, making it more accessible to consumers worldwide. Sperm quality and quantity parameters, notably sperm concentration, motility, and morphology, are indicative of male fertility and may shed light on potential testicular factors contributing to infertility (Esteves *et al.*, 2012). This study was designed to evaluate the effect of long jack sperm enhancer on the sperm quality and quantity of male Wistar rats. From data presented in this study, there was no significant difference in sperm count, sperm motility and sperm morphology among the groups. Although sperm count increased with increasing concentration of herbal product supplemented, the difference was not statistically significant. This study is at variance with previous study (Chan *et al.*, 2009), who reported elevated levels of sperm count, motility, morphology in rats treated with *Eurycoma longifolia* at doses of 50, 100, 200mg/kg in their research work. They attributed the increase to elevated levels of testosterone in the circulation. The discrepancy in the result may be due to the concentration used in this

study. Whereas the highest concentration used in this study was 80 mg/kg, the previous author used 200 mg/kg, a concentration that is 100% higher than the present study.

5.1 Conclusion

From this study, it can be deduced that the supplementation of Wistar rats with long jack sperm enhancer showed no marginal increase in sperm quality and quantity at this concentrations.

5.2 Recommendation

Based on the findings of this study, it is recommended that further research be conducted to determine the appropriate therapeutic dosage that shows positive effect on sperm quality and quantity.

5.3 Study Limitation

It is vital to pinpoint the limitation inherent to this study, of which the use of animal models stands out as they may not fully reflect human physiology.

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