THE EFFECT OF *OCIMUM GRATISSIMUM* EXTRACT ON SOME FERTILITY HORMONES AND TESTICULAR FUNCTION ON ADULT MALE WISTAR RATS

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CERTIFICATION

This is to certify this project on **"THE EFFECT OF OCIMUM GRATISSIMUM ON FERTILITY HORMONE AND TESTICULAR FUNCTION ON MALE WISTAR RATS"** was carried out by **UMORU MUNIRA EMMANUELLA**, with the matriculation number **BMS2101679** in partial fulfillment of the requirement of the award of Bachelor of Science Degree (B.Sc) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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ACKNOWLEDGMENT

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I am especially grateful to my supervisor, **Prof. Arthur Omorogiuwa**, for his outstanding mentorship and unwavering support throughout the course of this study. His insightful guidance, constructive feedback, and dedication to academic excellence were instrumental in refining both the focus and methodology of this research. Her commitment has been a continuous source of motivation and inspiration.

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I also extend sincere appreciation to all those who, in one way or another, contributed

DEDICATION

This work is dedicated to God Almighty, who is my Creator, my unwavering source of strength, and the source of my inspiration, knowledge, and insight. I have been able to start and finish this adventure only by His grace. I've found direction with His help, and I've flown on His wings.

This work is also devotedly dedicated to my family, whose constant belief in me, support, and encouragement have been the cornerstone of my tenacity. Their unwavering presence has strengthened my resolve and helped me overcome every obstacle. May they always be blessed abundantly by God.

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ABSTRACT

Ocimum gratissimum (scent leaf) is a medicinal plant widely used in traditional medicine, with certain ethnobotanical claims stating it promotes male virility. However, available scientific research shows mixed findings about its implications on male reproductive health, with some research revealing testicular injury and hormonal imbalance. *Ocimum gratissimum* is traditionally used to promote male vitality, although research information on its reproductive safety is inconsistent. This study studied the effects of a 500 mg/kg aqueous leaf extract of O. gratissimum on fertility hormones and testicular function in adult male Wistar rats for 28 days. Serum Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and testosterone were evaluated, with sperm count, motility, and morphology. The findings showed that while testosterone levels were normal, the extract caused a condition of compensated primary testicular dysfunction, as shown by significantly raised blood LH and FSH ($p<0.05$). A considerable decrease in the overall number of sperm was linked to this hormonal imbalance, suggesting compromised spermatogenesis. Ironically, by boosting motility and lowering the proportion of morphological defects, the extract also markedly enhanced sperm quality. These results point to a dualistic, site-specific action: the extract is gonadotoxic in the testes but may have a post-testicular, cytoprotective, antioxidant impact in the epididymis. Since the quantitative decrease in sperm production and hormonal indicators of testicular failure outweigh the qualitative gains, the overall effect at this dosage appears to be harmful to male fertility. These findings call into question the plant's conventional application as a fertility booster and call for care.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Ocimum gratissimum (L.), often known as smell leaf, is a perennial herbaceous plant that is a member of the Labiatae family. The plant is native to West Africa and tropical regions, particularly India. The Savannah and coastal regions of Nigeria are home to it. In addition to Nepal, Bengal, Chittagong, and the Deccan, it is grown in Ceylon and the South Sea Islands. The Yoruba-speaking tribe in southern Nigeria refers to the plant as "effinrin-nla." The Hausas in northern Nigeria refer to it as "Daidoya," whereas the Igbo refer to it as "Ahuji." (Effraim *et al*., 2003).O. gratissimum has been used extensively in many countries' traditional medical systems; its flowers and leaves are rich in essential oils, which are used to make teas and infusions (Rabelo *et al*., 2003). In Nigeria's coastal regions, the plant is used to treat epilepsy, high fever, and diarrhea (Effraim *et al*., 2003). In the Savannah regions, decoctions of the leaves are used to treat mental illness (Akinmoladu *et al*., 2007). The Ibos of Southeastern Nigeria use O. gratissimum to manage the baby's cord, to keep the wound surfaces sterile; and to treat fungal infections, fever, colds, and catarrh (Ijeh *et al*., 2005). Children in Brazilian tropical forests are sedated with a decoction of O. gratissimum roots (Cristiana *et al*., 2006). Nigerian tribal people utilize leaf extract to cure diarrhea and cold leaf infusions to treat hemorrhoids and upset stomachs (Kabir *et al*., 2005). According to Adebolu and Salau (2005), the plant is frequently used in traditional medicine to treat a variety of illnesses, including upper respiratory tract infections, diarrhea, headaches, eye and skin conditions, pneumonia, cough, fever, and conjunctivitis.

Testicular integrity and hormone control are two interconnected systems that are critical to male reproductive health. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and other central hormones are essential for the initiation and maintenance of spermatogenesis, desire, and secondary sexual features. The hypothalamic-pituitary-gonadal (HPG) axis, which maintains reproductive homeostasis, is how these hormones function. Reproductive potential may be hampered by any changes in hormone levels or testicular structure brought on by environmental pollutants, lifestyle choices, or medications. This is crucial since approximately half of all cases of infertility in couples worldwide are caused by male infertility (Dutta *et al*., 2019).

One of the biggest problems facing global health is male infertility. Male infertility is defined by the World Health Organization (WHO) as a male's failure to conceive a fertile woman following at least a year of consistent, unprotected sexual activity. According to statistical data, the male factor contributes to the overall burden in over 50% of all infertile couples worldwide and is the only cause in about 30% of instances (Leslie *et al*., 2024). The important role of the male factor in clinical infertility was also highlighted by earlier research, such as the extensive population study by Hull *et al*. (1985).

Semen analysis is the main method used to detect male fertility (Orieke *et al*., 2019). Male fertility can occur for a number of reasons, including both reversible and irreversible disorders. Each spouse may be impacted by other factors like as age, drugs, surgical history, exposure to chemicals in the environment, genetic issues, and systemic disorders. Finding a male's contributing factors, treating those that are reversible, determining whether he is a candidate for

assisted reproductive techniques (ART), and providing counseling for conditions that are irreversible and incurable are the main goals of evaluating him for infertility (Shih *et al*., 2019).

1.2 STATEMENT OF PROBLEM

Despite Ocimum gratissimum's widespread usage in traditional medicine and anecdotal assertions that it improves male vitality, the scientific literature currently in publication offers wildly conflicting results about its safety for reproduction. While some theories point to a possible improvement in testosterone levels and sperm quality, other thorough laboratory studies employing animal models have shown a marked drop in serum testosterone as well as severe histological impairments in the testes, such as reduced seminiferous tubule diameter and disorganized germ cells. A thorough, empirical investigation into the precise mechanisms by which the aqueous extract affects the regulatory hormones (LH, FSH, and T) and the integrity of testicular architecture in a standard animal model, the Wistar rat, is required due to this discrepancy between ethnobotanical reliance and contradictory laboratory reports.

This commonly consumed plant's unclear reproductive toxicity is a serious public health issue that has to be resolved with verified data that connects hormonal status to structural alterations.

1.3 JUSTIFICATION OF STUDY

Due to its antibacterial and anti-inflammatory qualities, *Ocimum gratissimum*, or smell leaf, is frequently employed in traditional medicine, particularly in areas with little access to traditional medical care. There is little scientific data about its impact on male reproductive health, despite its widespread use. Concerns over the potential impact of its phytochemicals on hormone balance and testicular function underscore the need for additional investigation. This study assesses the effects of *Ocimum gratissimum* on testicular structure and reproductive hormones in male Wistar rats in order to give empirical data that will direct the safe and informed use of this widely used herbal remedy.

1.4 AIM OF STUDY

The aim of this study is to evaluate the effect of *ocimum gratissimum* extract on some fertility hormones and testicular function and architecture in adult male Wistar rats.

1.5 RESEARCH QUESTIONS

1. What is the dose-dependent effect of *Ocimum gratissimum* extract on the serum levels of key fertility hormones (Luteinizing Hormone, Follicle-Stimulating Hormone, and Testosterone) in adult male Wistar rats?
2. How does treatment with Ocimum gratissimum extract influence testicular function, as measured by sperm count, motility, and morphology?
3. What impact does the extract have on the histo-architecture of the testes, specifically on the structure and integrity of the seminiferous tubules and spermatogenic cells?

4. How do the observed changes in hormonal profiles, sperm parameters, and testicular histology correlate to indicate the overall impact of *Ocimum gratissimum* on male reproductive potential?

1.6 SPECIFIC OBJECTIVES

1. To determine and compare the serum concentrations of Testosterone (T), Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) in the *Ocimum gratissimum* - treated group relative to the control group.
2. To evaluate the gross morphological changes, specifically organ weight and weight ratio, of *Ocimum gratissimum* the testes following the administration of the extract.
3. To assess and compare the histopathological alterations in the testicular micro-architecture, including measurements of the seminiferous tubule diameter, germinal layer thickness, and interstitial cell integrity, between the treatment and control groups.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OCIMUM GRATISIMUM

Ocimum gratissimum is one of the several species of Ocimum that is widely used in medicine worldwide. Formulations of O. gratissimum leaf essential oil, also known as ocimum oil, have been added to a number of bases as topical antiseptics and used to heal small wounds, boils, and pimples (Azuamah *et al*., 2024). Due in significant part to its volatile essential oil concentration, O. gratissimum has long been known for its strong antibacterial, anti-inflammatory, and antioxidant qualities. Its formulations have been used to topical applications as antiseptics for minor wounds in addition to treating common diseases. Additionally, O. gratissimum and Xylopia aethiopica are traditionally used together to make teas that women drink throughout their puerperium (Melo *et al*., 2019).

2.1.1 MORPHOLOGY AND MICROSCOPY

O. gratissimum is a shrub with branching branches that can reach a height of 1.9 meters. Up to 10 x 5 cm in size, the leaves are oblong to ovate-lanceolate, sub-acuminate to acuminate at the apex, cuneate and decurrent at the base, with a coarsely crenate, serrated border, pub-scent, and spotted on both sides. The leaves exhibit glandular and enveloping trichomes. On the bottom surface, stomata are present, while they are uncommon or nonexistent on the upper surface. There aren't many ordinary trichomes; the long, up to six-celled ones are usually found on the

margins, while the short, two-celled ones are mostly located on the lamina. Racemes can reach a length of 18 cm, while petioles can reach a length of 6 cm.

Dense pubescence is present in the peduncles. The calyx is greenish-white to greenish-yellow, campanulate, and up to 5 mm long. When moist, nutlets become mucilaginous (Bhat, 2003). Simple pluricellular hairs on the leaf veins also include diacytic stomata, the secretory glands most prevalent in the leaf, and the epidermal cells on the leaf's two surfaces have uneven shapes. The epidermis monoestratificada (beam), a layer of parenchyma fenced in sub-epidermal position, the parenchymal pond, and the epidermis monoestratificada lower are all visible in the cross section (Garcia *et al*., 1998).

2.2 MALE FERTILITY HORMONES

2.2.1 TESTOSTERONE

In addition to fostering secondary sexual traits including increased muscle and bone mass, body hair growth, vocal alterations, voice deepening, and anabolic effects, testosterone is essential for the development of male reproductive tissues such the prostate and testicles. Additionally, it is in charge of the basic sexual development, which includes spermatogenesis, testicular descent, penis and testicular hypertrophy, and increased libido.Around seven months of pregnancy, when the testes start secreting appropriate amounts of testosterone, the testes often start to descend into the scrotum. Testosterone can help a male kid's testes descend through the inguinal canals if the child is born with undescended but normal testes that do not descend by 4 to 6 months of age (Kalfa *et al.,* 2019).

Increased aggression, sex drive, dominance, courtship display, and a host of other behavioral traits are linked to it (Mooradian *et al*., 1987). Furthermore, testosterone plays a major role in overall mood, cognition, social and sexual behavior, energy and metabolic output, the cardiovascular system, and the prevention of osteoporosis in both sexes (Bassil *et al*., 2009). Male testicles and, to a lesser degree, female ovaries are the main organs that secrete testosterone in humans and the majority of other vertebrates. Testosterone levels in adult males are typically seven to eight times higher than those in adult females (Torjesen and Sandnes, 2004). There are three primary hormonal effects of testosterone. Direct action on certain androgen receptors, indirect action through intracellular conversion to dihydrotestosterone by 5-alpha reductase, as in prostate cells, and estrogen impact when aromatase converts to estradiol (Sizar *et al*., 2023).

Male pattern baldness, increased mortality in men with prostate cancer, hyperandrogenism, and a higher risk of heart failure are all linked to high testosterone levels in men (Gann *et al*., 1996). Through negative feedback, testosterone restricts its secretion. Elevated blood levels of testosterone provide feedback to the anterior pituitary, reducing its sensitivity to GnRH stimuli, and to the hypothalamus, which suppresses GnRH release (Plant and Marshall, 2001). Luteinizing hormone (LH) primarily controls testicular testosterone secretion by controlling the rate-limiting conversion of cholesterol to pregnenolone in Leydig cell mitochondria by the cytochrome P-450 cholesterol side-chain cleavage enzyme complex on the inner mitochondrial membrane. One of the proteins that controls the transfer of cholesterol to mitochondrial steroidogenic enzymes is sterol carrier protein 2 (Gallegos *et al*., 2001). Ocimum gratissimum may increase testosterone levels in animal models and improve sperm quality (motility, viability, and count), per a recent review (Ekakitie and Udi, 2025).

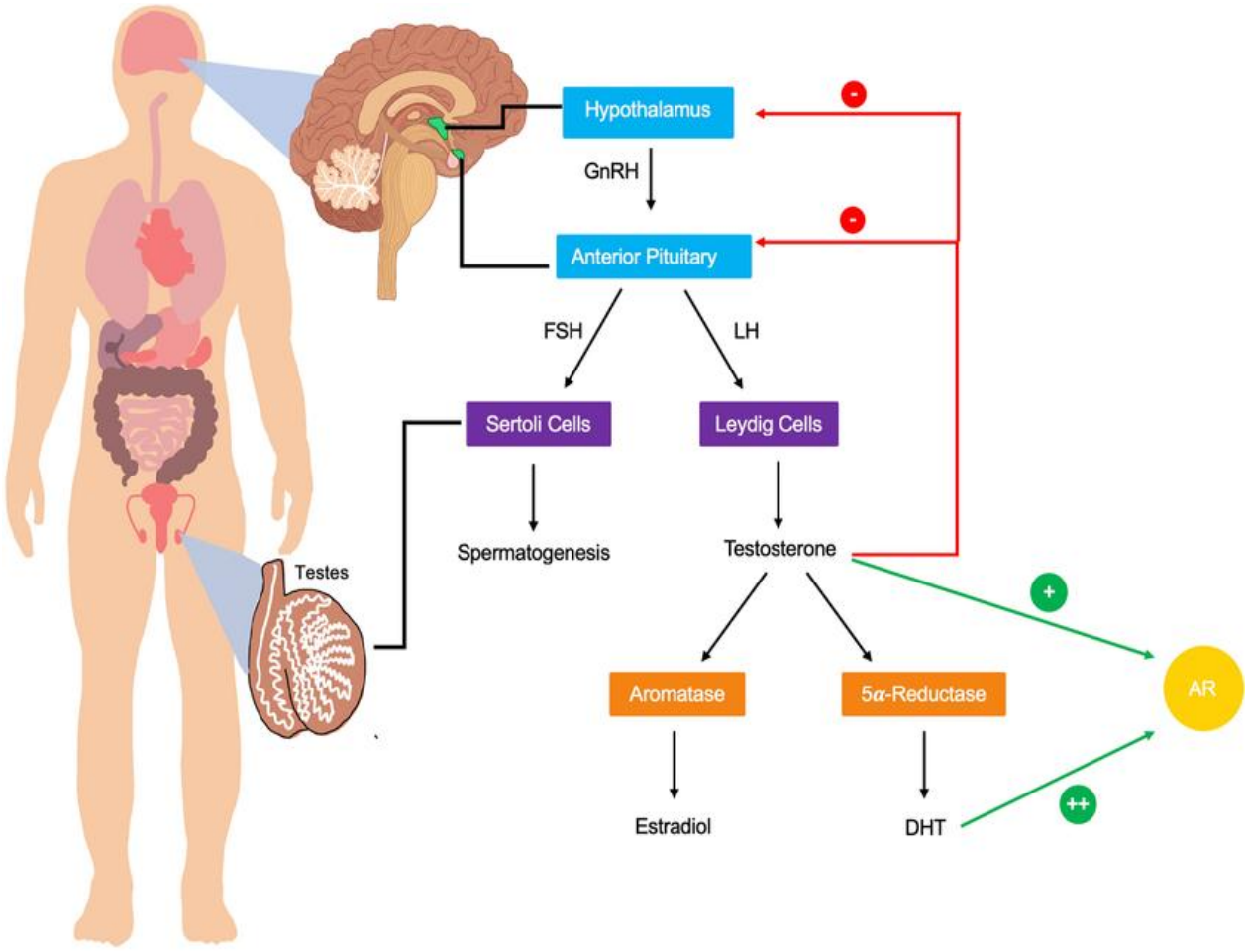

Figure 2. 1: Synthesis, secretion of LH, FSH and control of testosterone (Ketchem *et al*., 2023).

2.2.2 LUTEINIZING HORMONE (LH)

The gonadotrophin cells in the adenohypophysis (anterior pituitary) co-secrete follicle-stimulating hormone and luteinizing hormone (LH), a glycoprotein hormone.

The maturation of primordial germ cells is aided by LH. In men, LH triggers the production of testosterone by the Leydig cells in the testes. LH causes the ovaries in women to produce steroid hormones (Ilahi and Ilahi, 2022). Gonadotropin-releasing hormone (GnRH) stimulates the production of LH, while estrogen in females and testosterone in males block it.Alpha and beta subunits of glycoproteins make up LH (Strickland *et al*., 2019). In particular, there are 92 amino acids in the alpha subunit of LH and 121 amino acids in the beta subunit. The combined mass of these two subunits is 28 kDa (Ezcurra *et al.,* 2014). In men, LH causes the testes' Leydig cells to release more testosterone. In females, LH causes the ovaries to release steroids, ovulation, and the corpus luteum to release progesterone following ovulation (Kamel-Elsayed *et al*., 2023). Gonadotropin receptors in the testes are dependent on the pulsatile production of LH. Additionally, a loss of gonadotropin receptors in the testes might result from persistently high levels of LH in the blood and testes (Strickland *et al*., 2019).

2.2.3 FOLLICLE STIMULATING HORMONE (FSH)

Gonadotropin-releasing hormone (GnRH) from the hypothalamus causes the anterior pituitary to release follicle-stimulating hormone (FSH) (Stamatiades and Kaiser, 2018). Alpha and beta subunits make up the glycoprotein dimer known as FSH. While the alpha subunit is shared by TSH, hCG, and LH, the beta subunit is specific to FSH (Barbieri, 2014). FSH secretion is stimulated by GnRH. GnRH is produced by the hypothalamus and released into the hypophyseal portal circulation, where it acts on G-protein-coupled receptors at the anterior pituitary's gonadotropic cells. These gonadotropic cells release luteinizing hormone (LH) and FSH into the peripheral circulation. The most important factor in children's testicular volume is the proliferation of Sertoli cells, which is stimulated by FSH in males (Grinspon *et al*., 2018).

Spermatogenesis is induced and maintained by FSH receptors, which are present in the testes' Sertoli cells (Barbieri, 2014). Maintaining a normal sperm count and function requires both FSH and testosterone. Research has demonstrated that FSH deficiency affects both the quantity and quality of sperm (Nieschlag *et al*., 1999; Orlowski and Sarao, 2023). Hyperfunctioning pituitary adenomas or unresponsive gonads are linked to elevated FSH levels.Either anterior pituitary or hypothalamic dysfunction is linked to low FSH levels.

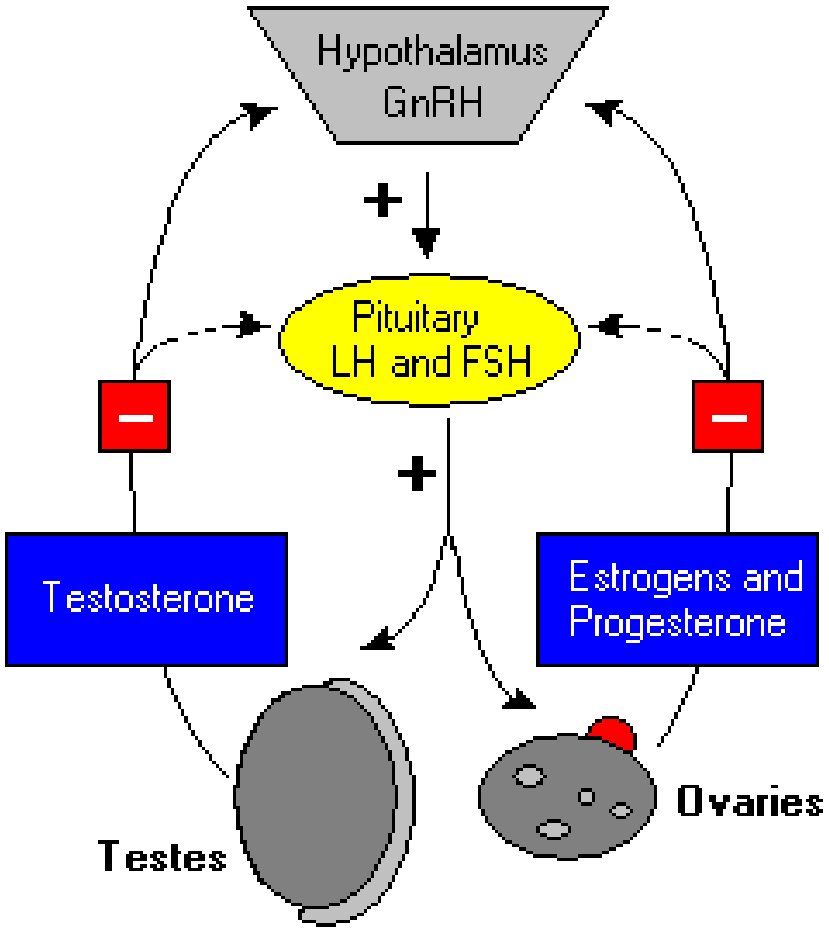

Figure 2. 2: Diagram showing the regulation of LH and FSH.

Source: (**https://vivo.colostate.edu/hbooks/pathphys/endocrine/hypopit/lhfsh.gif**)

Date accessed: 10/21/2025

2.3 TESTICULAR ARCHITECTURE AND FUNCTION

The seminiferous tubules, where spermatogenesis takes place, and the interstitial space, which contains the steroidogenic Leydig cells, are the two interrelated functional compartments that make up the testes (Aladamat and Tadi, 2022). The growing germ cells (spermatogonia, spermatocytes, and spermatids) and the supporting Sertoli cells are found in the seminiferous tubules (Shittu *et al*., 2019). A crucial morphometric indicator of the degree of spermatogenic activity and the integrity of the germinal epithelium is the seminiferous tubule's overall diameter. The Leydig cells, which are found in the interstitial space, are in charge of producing testosterone. In order to stimulate and sustain sperm production in conjunction with FSH signaling to Sertoli cells, high intratesticular concentrations of this testosterone must diffuse locally into the seminiferous tubules (Aladamat and Tadi, 2022). Defects in spermatogenesis are associated with lower levels of intratesticular testosterone (Walker, 2011).

Severe disruptions to this architecture, such as mild vacuolation inside the seminiferous tubules, disorganized germinal cell layers, arrest of sperm maturation, and an apparent decrease in seminiferous tubule diameter, were observed in earlier studies examining the effects of O. gratissimum extract (Shittu *et al*., 2019). These results show significant toxicity to the developing germ cells and Sertoli cells. Histological damage (Sertoli/germ cell function) and a drop in testosterone (Leydig cell function) indicate that the extract may have dual toxicity, jeopardizing both the structural support and the vital hormonal milieu needed for successful reproduction.

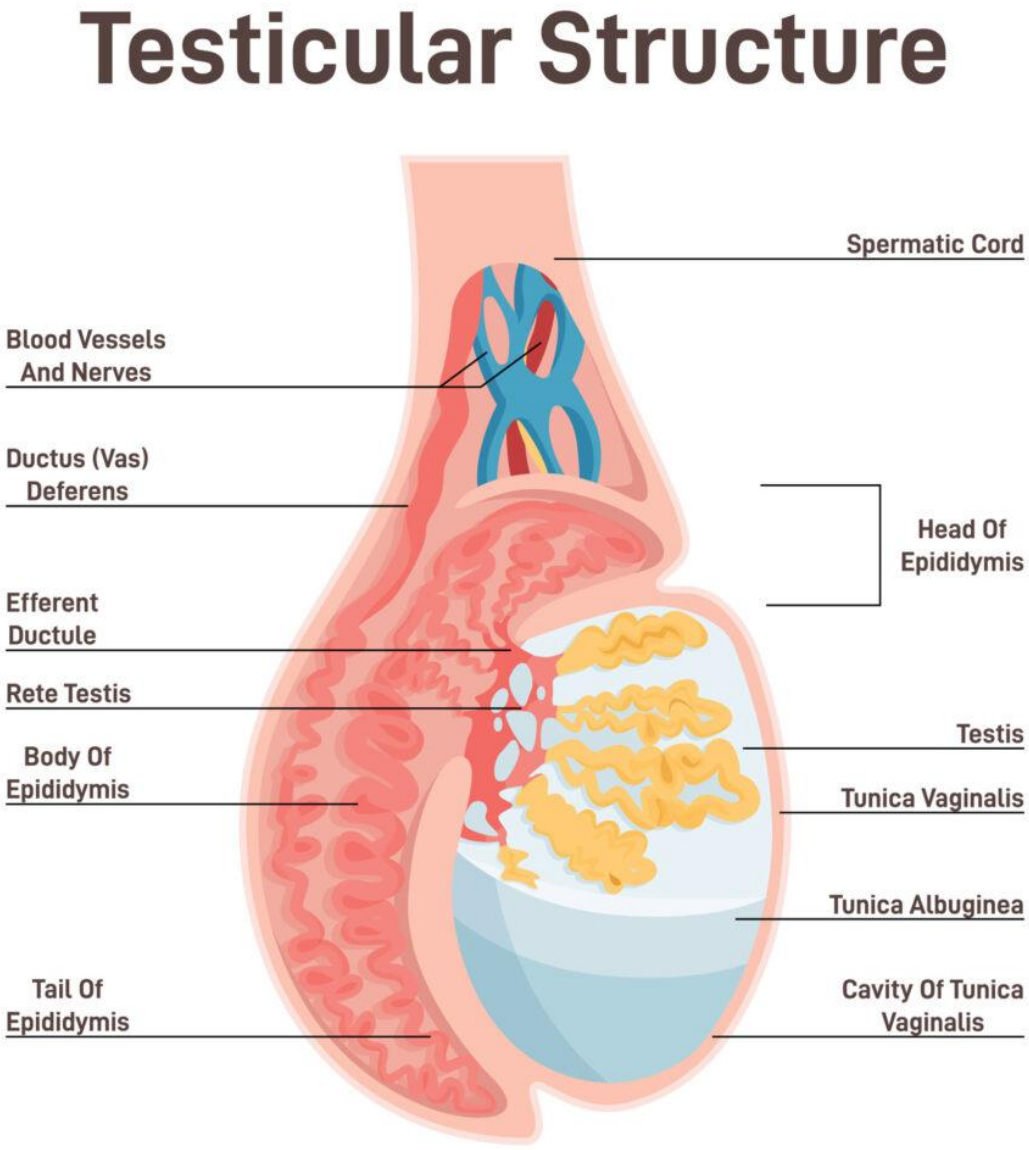

Figure 2. 3: Diagram of the Testes.

Source: (https://www.rcemlearning.co.uk/wp-content/uploads/Anatomy-of-the-testicle-1024x1024.jpg)

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2.4 SEMEN ANALYSIS

Semen analysis characteristics offer the final functional endpoint for evaluating reproductive toxicity and pharmacology in the Wistar rat model, although not being explicitly carried out in this hormonal and histological study (Adamkovicova *et al*., 2016).Parameters that directly link histological integrity to fertilization potential are assessed in semen analysis.

Key parameters include:

Sperm Concentration: The total number of spermatozoa, which is the direct output measure of spermatogenic efficiency (Orieke *et al*., 2019).

Sperm Motility: Assessed by the percentage of spermatozoa capable of movement, categorized by individual and progressive motility. Progressive motility is essential for achieving fertilization.

Sperm Viability: The percentage of live spermatozoa, typically assessed using vital staining techniques.

Sperm Morphology: Evaluation of the percentage of sperm cells displaying abnormal shapes (e.g., bent tails, detached heads), which often indicates disturbances during the complex process of spermatogenesis.

(Chawre *et al*., 2024; Sapozhkova and Eremin, 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS USED

• Feed

• Water

• Plates

• Test tubes

• Centrifuge

• Oral gavage needles

• Syringes (2ml and 5ml)

• Latex gloves

• Chloroform

• Cotton wool

• Weighing scale

• Serum Separator Tube (SST)

• Plain bottles

• Plastic cages

• Sawdust

3.2 STUDY AREA

This study was carried out in the animal house of the department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State.

3.3 SOURCES OF OCIMUM GRATISIMUM (SCENT LEAF)

Fresh leaves of *Ocimum gratissimum* (common name; scent leaf) were procured from a specific local market in Benin City, Edo State, Nigeria. To ensure accuracy and consistency in the phytochemical profile of the material, the plant was botanically identified and authenticated by a specialist in the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen (UBH-0209) was prepared and deposited in the departmental herbarium for future reference and verification.

3.4 PREPARATION OF EXTRACT AND DOSE CONCENTRATION

The fresh leaves were kept in the oven at 800c for ten minutes to stop any enzyme activity and then at 600c for 30minutes. They were collected from the oven, air dried and ground into coarse powder. 50g of the powdered leaves was stirred into 450ml of methanol. The mixture was then kept aside for 48hours to allow it to infuse. It was then filtered using a filter paper. The filtrate was concentrated to 200ml (1ml of the extract being equivalent to 0.25g of the starting material). The extract was kept in Petri dishes with tight fitting covers in a refrigerator until it was time to use.

The extract was dissolved into a solution with 2g of the extract into 20ml of distilled water. The dosage was calculated in this equation;

$$\frac{\text{Weight of experimental animals(in Kg)x dosage}}{\text{Concentration of solution (in ml)}}$$

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3.5 EXPERIMENTAL ANIMALS AND DESIGN

Thirteen (14) healthy male Wistar rats, weighing between 100–150 g, were obtained from the Animal House of the Department of Anatomy, University of Benin, and housed in the same facility. Upon procurement, the animals were subjected to a mandatory four-day period of acclimatization to the laboratory environment prior to the commencement of the study. During this time, they were maintained under standard animal husbandry conditions: ambient temperature of 25–29 °C, a natural light/dark cycle, and free access to standard rat chow and clean drinking water.

Following acclimatization, the animals were randomly divided into two groups. Group 1 (n = 7) served as the control group and received normal feed and water, while Group 2 (n = 7) served as the test group and was administered the plant extract.

Body weight: Body weights of all animals were precisely recorded using a calibrated digital weighing scale (precision 0.1g) immediately prior to the start of treatment.

Group	Name	Treatment	Number of rats
1	Control group	Distilled water	6
2	Test group	500mg/kg of extract	7

3.6 STUDY DURATION

The experiment lasted for a period of four (4) weeks, after which the animals were sacrificed and samples were collected.

3.7 SAMPLE COLLECTION

At the conclusion of the experiment, the final body weights of the rats were recorded using a calibrated digital weighing scale. The animals were then anaesthetized by exposure to chloroform vapour within a sealed chamber containing cotton wool soaked in chloroform. Once complete anaesthesia was confirmed, each rat was placed in a dorsal position on a dissection table. A midline incision was made through the abdominal and thoracic cavities using surgical scissors to expose the heart. Blood was then collected by cardiac puncture and transferred into Serum Separator Tubes (SST) for subsequent biochemical analysis.

The caudal epididymis was carefully identified and dissected from the testis. It was then cleaned of adhering adipose tissue and transferred to a petri dish containing 2 mL of pre-warmed (37°C) normal saline. Several incisions were made into the caudal epididymis with a sharp sterile surgical blade to release spermatozoa into the saline solution. The solution was gently swirled

and incubated at 37°C for 10 minutes to allow for adequate sperm dispersion. This sperm suspension was then used for the assessment of sperm motility, count, and morphology.

Immediately after blood collection, both testes were carefully dissected out from each rat. The testes were freed from the epididymides and cleared of any adhering tissues. The right testis from each animal was weighed on a digital balance to determine the gonadosomatic index. It was then fixed by complete immersion in a large volume (approximately 10 times the tissue volume) of Bouin's fluid for 48 hours.

3.8 HORMONAL ANALYSIS

Serum concentrations of Testosterone (T), Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits specifically validated for the Wistar rat model. All assays were conducted strictly according to the manufacturers' instructions, adhering to rigorous quality control standards regarding sensitivity and variability. This analysis is critical for determining whether the extract's effects are mediated by direct gonadal toxicity or by central regulation via the hypothalamus/pituitary.

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3.9 TESTICULAR HISTOLOGY

The excised testicular tissue reserved for structural analysis was promptly immersed in 10% Neutral Buffered Formalin (NBF) for fixation for at least 48 hours. The fixed tissues underwent routine histological processing, including dehydration through ascending grades of alcohol, clearing in xylene, and embedding in paraffin wax. Sections of 5mu thickness were cut using a

rotary microtome, mounted on glass slides, and stained using the standard Hematoxylin and Eosin (HandE) technique for morphological evaluation. Microscopic examination was conducted under light microscopy. Qualitative analysis assessed the overall micro-architecture, focusing on the integrity of the basement membrane, the organization of the germinal epithelium, the presence of cellular vacuolation, and any signs of germ cell maturation arrest. Quantitative analysis (morphometry) involved measuring the diameter of at least 20 randomly selected seminiferous tubules per rat using a calibrated ocular micrometer.

3.10 STATISTICAL ANALYSIS

All data were expressed as Mean ± Standard Error of the Mean (SEM). The results were analyzed using a one-way analysis of variance (ANOVA) followed by a post-hoc test (Tukey's test) to determine significant differences between the treatment groups and the control. The statistical analysis will be performed using a software package such as GraphPad Prism (version 9.0). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

Table 4. 1: Comparing the mean values of reproductive hormones in male Wistar rats following the administration of *ocimum gratissimum* extract.

Parameters	Control	*ocimum gratissimum*	p-values
Prolactin	10.73 ± 0.317	12.93 ± 1.036	0.0885
LH	5.975 ± 1.217	11.38 ± 0.296	0.0050
FSH	9.075 ± 2.04	16.23 ± 0.792	0.0171
Testosterone	3.225 ± 0.312	2.850 ± 0.065	0.2837
Testicular Weight	1.025 ± 0.063	1.133 ± 0.120	0.4261

P< 0.05 indicates significant difference

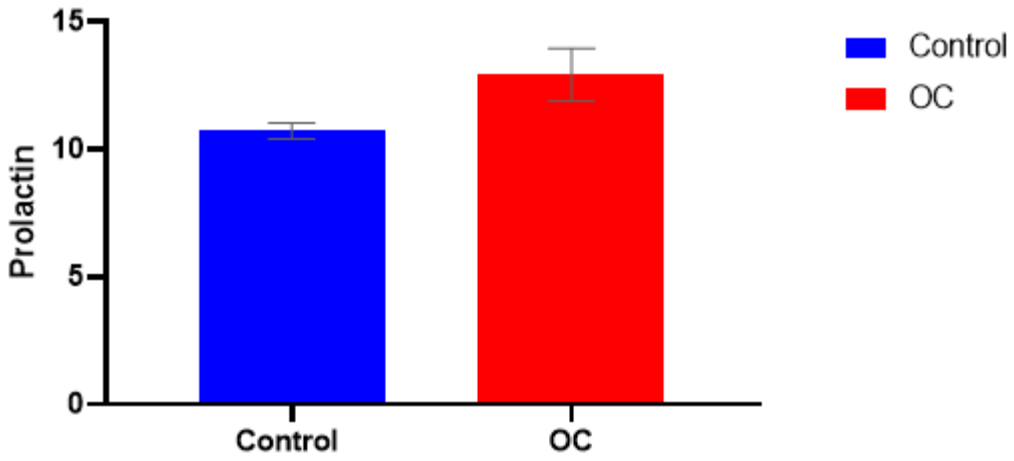

Figure 4. 1: Chart showing the effect of *ocimum gratissimum* on prolactin hormone of male Wistar rats. There were no significant differences in *ocimum gratissimum* treated group compared with control. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

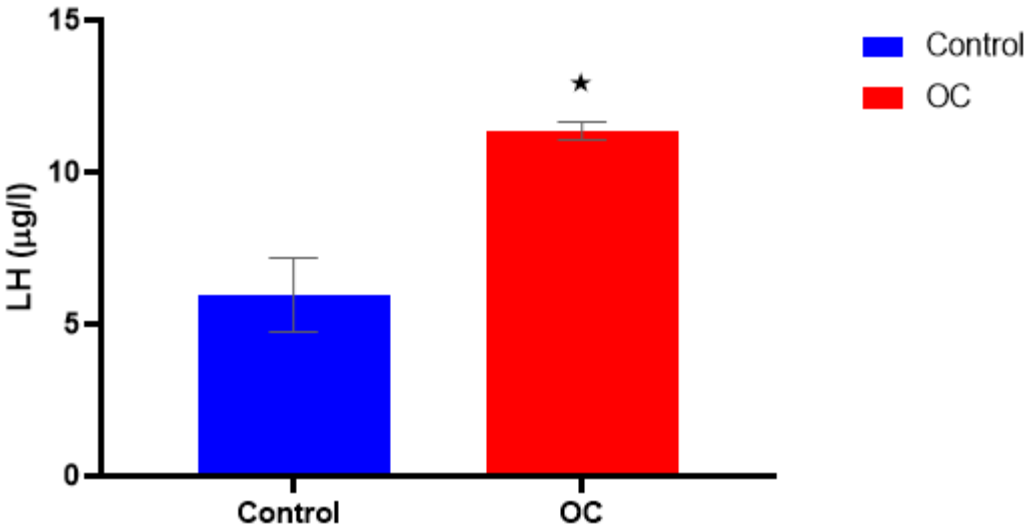

Figure 4. 2: Chart showing the effect of *ocimum gratissimum* on Luteinizing hormone of male Wistar rats. There was a significant increase in *ocimum gratissimum* treated group compared with control. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

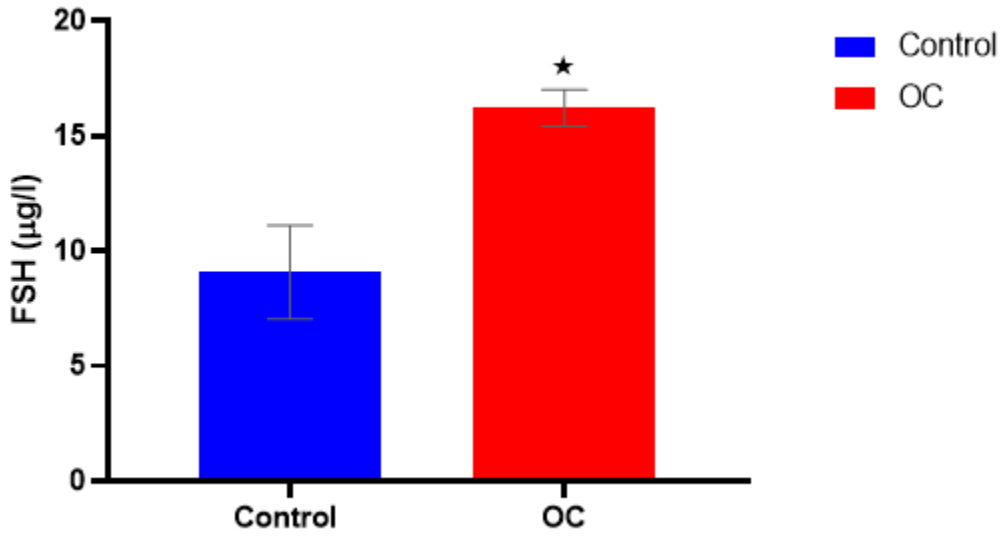

Figure 4. 3: Chart showing the effect of *ocimum gratissimum* on follicle stimulating hormone of male Wistar rats. There was a significant increase in *ocimum gratissimum* treated group compared with control. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

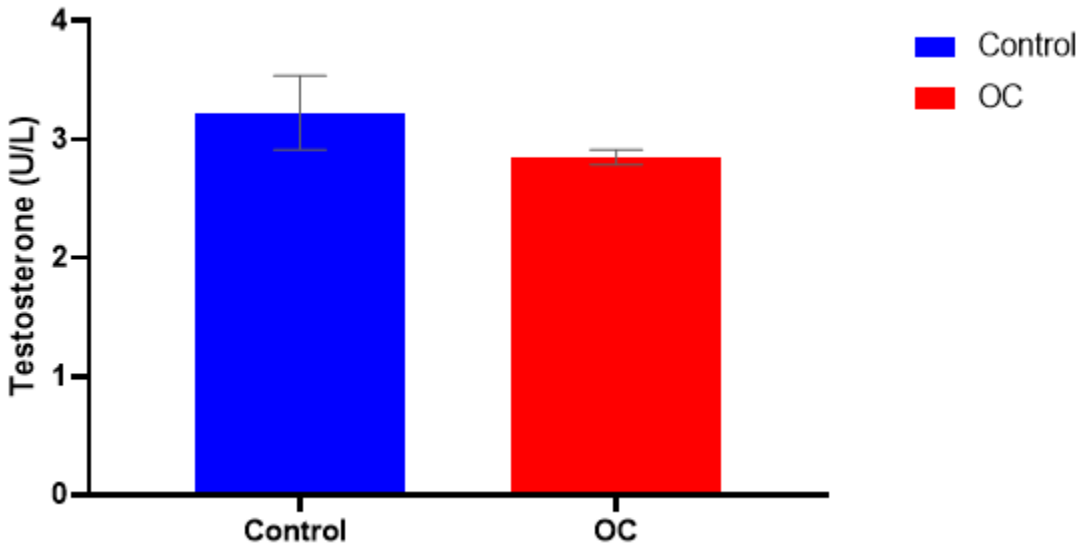

Figure 4. 4: Chart showing the effect of *ocimum gratissimum* on testosterone hormone of male Wistar rats. There was no significant difference in *ocimum gratissimum* treated group compared with control. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

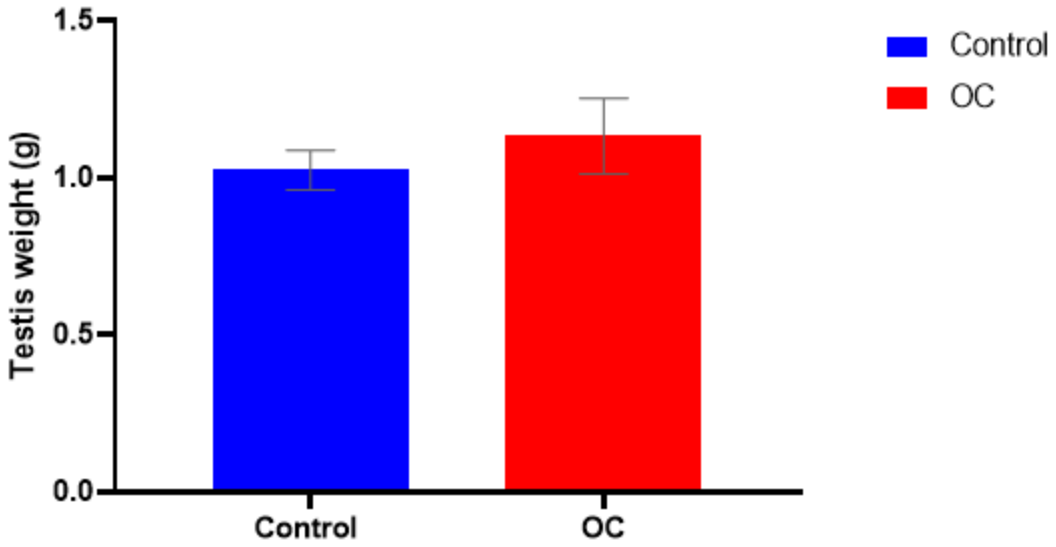

Figure 4. 5: Chart showing the effect of *ocimum gratissimum* on testis weight of male Wistar rats. There was no significant difference in *ocimum gratissimum* treated group compared with control. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

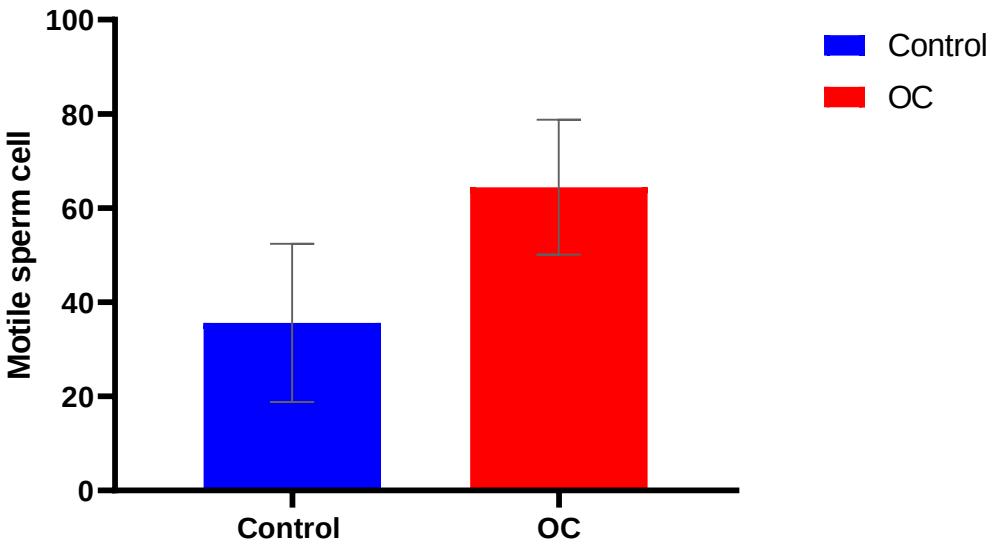

Figure 4. 6: The effect of *ocimum gratissimum* on motile sperm cells of male Wistar rats.

There was a statistically significant increase in *ocimum gratissimum* treated group compared with compared control.

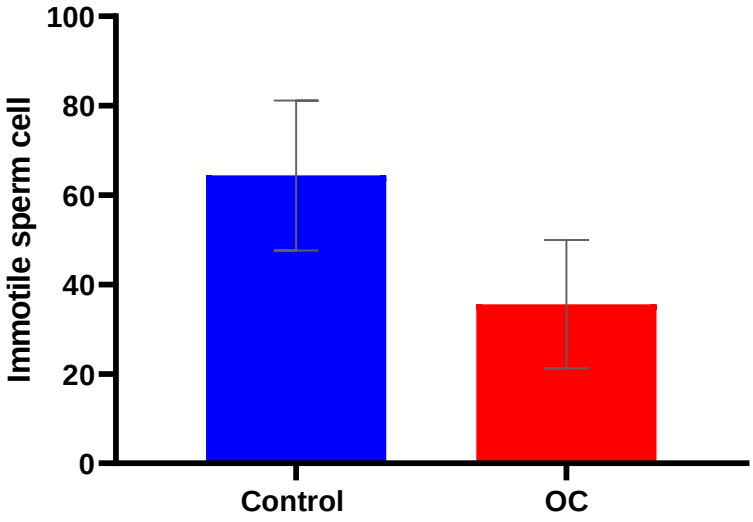

Figure 4. 7: The effect of *ocimum gratissimum* on non-motile sperm cells of male Wistar rats.

There was a statistically significant decrease in *ocimum gratissimum* treated group compared with control group.

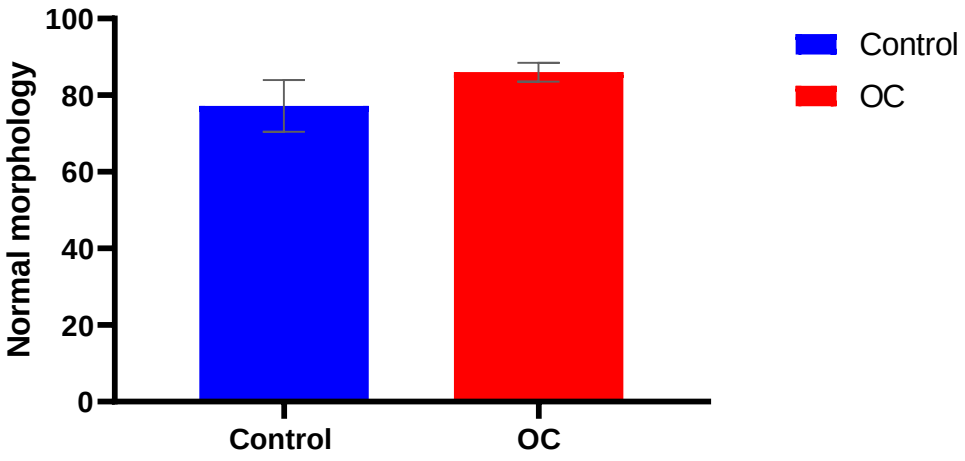

Figure 4. 8: The effect of *ocimum gratissimum* on normal sperm mophology of male Wistar rats.

There was no statistically significant difference in *ocimum gratissimum* treated group compared with control group.

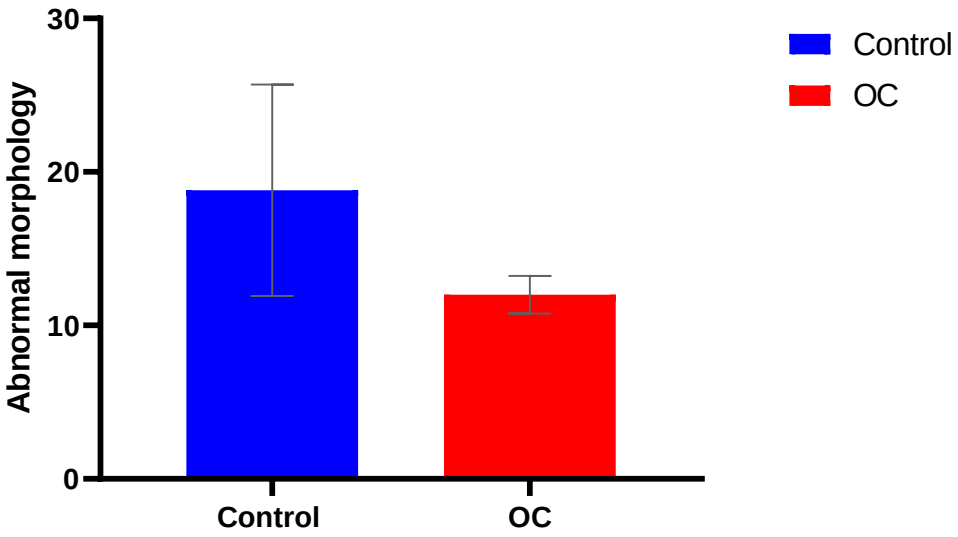

Figure 4. 9: The effect of *ocimum gratissimum* on abnormal sperm mophology of male Wistar rats.

There was a statistically significant decrease in *ocimum gratissimum* treated group compared with control group.

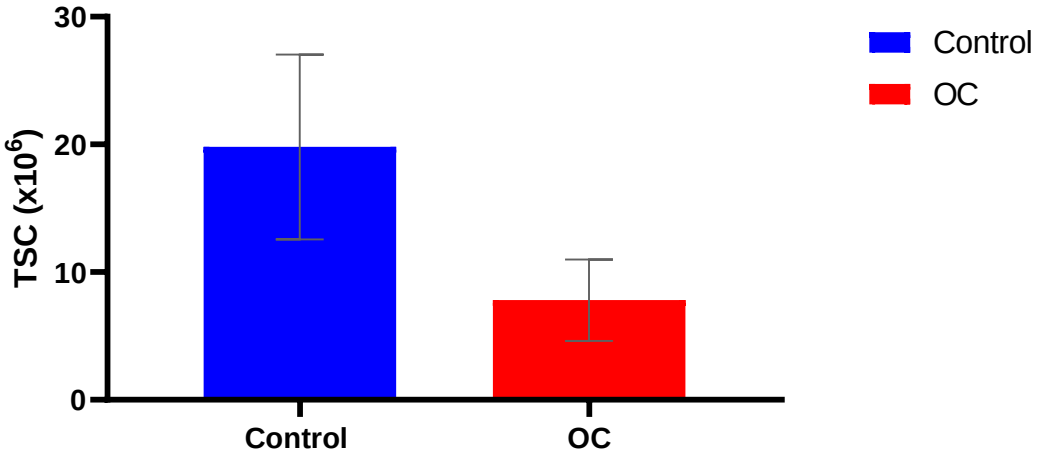

Figure 4. 10: The effect of *ocimum gratissimum* on total sperm count of male Wistar rats.

There was a statistically significant decrease in *ocimum gratissimum* treated group compared with control group.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study addressed the specific research objectives listed in Chapter 1 by evaluating the impact of an aqueous extract of Ocimum gratissimum on important reproductive hormones and testicular function in adult male Wistar rats.

5.1.1 Compensated Testicular Dysfunction

The most physiologically significant finding of this investigation is the hormonal profile observed in the rats treated with *Ocimum gratissimum* extract. The data reveals a statistically significant increase in the serum concentrations of both Luteinizing Hormone (LH) (11.38 ± 0.296 mIU/mL vs. 5.975 ± 1.217 mIU/mL in controls; p=0.0050) and Follicle-Stimulating Hormone (FSH) (16.23 ± 0.792 mIU/mL vs. 9.075 ± 2.04 mIU/mL in controls; p=0.0171). Concurrently, serum testosterone levels and absolute testicular weight remained statistically unchanged compared to the control group. This specific hormonal signature is highly informative and suggests a state of physiological stress rather than enhancement.

The male reproductive system is governed by the hypothalamic-pituitary-gonadal (HPG) axis, a finely tuned endocrine circuit regulated by negative feedback. The hypothalamus releases Gonadotropin-Releasing Hormone (GnRH), which stimulates the anterior pituitary to secrete LH and FSH. LH acts on the Leydig cells in the testicular interstitium to stimulate testosterone production, while FSH acts on the Sertoli cells within the seminiferous tubules to support spermatogenesis. Crucially, testosterone exerts negative feedback on both the hypothalamus and

the pituitary, suppressing the release of GnRH, LH, and FSH to maintain hormonal homeostasis (Jimbo, 2025).

In a healthy state, an increase in testosterone would lead to a decrease in LH and FSH. Conversely, a primary failure of the testes to produce testosterone would remove this negative feedback, causing a compensatory rise in LH and FSH levels (Jimbo, 2025). The hormonal pattern observed in this study, a significantly elevated LH and FSH in the presence of normal testosterone, is therefore anomalous and deviates from the expected feedback response. This profile is clinically recognized as compensated primary testicular dysfunction, also known as compensated hypogonadism (Fanis *et al*., 2023; Jimbo, 2025). This condition signifies that the testes are beginning to fail and require a much stronger stimulatory signal from the pituitary gland to maintain a normal output of testosterone.

The elevated LH level suggests that the Leydig cells have been rendered less sensitive or efficient by one or more components within the *O. gratissimum* extract. To maintain a serum testosterone concentration of 2.850 ± 0.065 ng/mL (statistically similar to the control's 3.225 ± 0.312 ng/mL), the pituitary gland had to nearly double its output of LH. This indicates that the extract is likely exerting a direct, mildly toxic effect on the Leydig cells, impairing their steroidogenic capacity. This finding is consistent with studies on other testicular toxicants that cause a reduction in Leydig cell number or function, prompting a compensatory pituitary response (Adelakun *et al*., 2022).

Similarly, the significant elevation in FSH points to a disruption within the seminiferous tubules. FSH is the primary driver of Sertoli cell function, which is essential for nurturing developing germ cells and maintaining the structural integrity of the tubules. An elevated FSH level,

particularly when spermatogenesis is compromised, is a classic indicator of Sertoli cell dysfunction or direct germ cell damage (Jimbo, 2025). The pituitary gland is attempting to overcome a deficit in testicular sperm production by increasing its stimulatory signal to the Sertoli cells.

Taken together, this hormonal evidence strongly suggests that the aqueous extract of *O. gratissimum* at a dose of 500 mg/kg induces a subclinical state of testicular injury. The reproductive system is successfully compensating for this insult by upregulating gonadotropin secretion, thereby masking a decline in testicular efficiency. However, this compensated state reflects an ongoing pathological process and a system under significant strain. Prolonged exposure could potentially lead to Leydig and Sertoli cell exhaustion, culminating in decompensated testicular failure with a subsequent crash in both testosterone production and spermatogenesis, which highlights a potential reproductive health risk.

5.1.2 Spermatogenesis: Decreased Production And Improved Quality

The analysis of sperm parameters revealed a seemingly contradictory set of outcomes that further illuminates the complex mechanism of action of the *O. gratissimum* extract. The study found a statistically significant decrease in the total sperm count in the treated group (Figure 4.10), a clear indicator of impaired testicular function. However, this was accompanied by a statistically significant increase in the percentage of motile sperm (Figure 4.6), a decrease in non-motile sperm (Figure 4.7), and a significant decrease in the percentage of morphologically abnormal sperm (Figure 4.9). This paradox; a reduction in sperm quantity coexisting with an improvement

in sperm quality, suggests that the extract exerts distinct, and opposing, effects at different sites within the male reproductive tract.

The observed decrease in total sperm count is the functional consequence of the testicular stress indicated by the hormonal profile. Spermatogenesis, the process of sperm production within the seminiferous tubules, is clearly being inhibited. This finding aligns with the elevated FSH levels, which represent a failed compensatory attempt to stimulate sperm production. This result is strongly supported by a significant body of literature demonstrating that various extracts of *O. gratissimum* can be gonadotoxic. Previous studies have reported severe histopathological damage to the testes, including erosion of the germinal epithelium, disorganization of germ cell layers, arrested sperm maturation, and a reduction in the diameter of the seminiferous tubules following administration of the extract (Ojo, 2008). This suggests a direct cytotoxic or anti-proliferative effect of the extract's phytochemicals on the rapidly dividing spermatogonia or the supportive Sertoli cells.

In contrast, the concurrent improvement in sperm motility and morphology is highly unlikely to be a testicular phenomenon. A more plausible explanation is that these beneficial effects occur post-testicularly, within the epididymis. After being produced in the testes, spermatozoa are transported to the epididymis, where they undergo a critical maturation process to acquire motility and fertilizing capacity. This environment, however, also exposes them to high levels of reactive oxygen species (ROS), which can cause oxidative damage on the sperm plasma membrane, impairing motility and causing structural defects (Nguyen *et al*., 2023). *Ocimum gratissimum* is known to be rich in potent antioxidant compounds, such as flavonoids, phenols, and tannins (Joseph *et al*., 2025). It is hypothesized that these water-soluble antioxidants, absorbed into the bloodstream, concentrate in the epididymal fluid. There, they could act to

neutralize ROS, creating a less hostile environment and protecting the maturing spermatozoa from oxidative damage. This would result in a higher proportion of the surviving sperm population emerging from the epididymis with intact membranes, robust motility, and normal morphology. This hypothesis is substantiated by *in vitro* research where low concentrations of *O. gratissimum* essential oils, when added as a supplement to semen extenders, significantly improved the quality and viability of stored canine and boar sperm by exerting an antioxidant effect (Ganguly & Kumar, 2019).

This shows that the extract exhibits site-specific, dualistic effects. It is gonadotoxic within the seminiferous tubules, leading to reduced sperm production, while simultaneously being cytoprotective within the epididymal lumen, enhancing the quality of the sperm that survive the initial production deficit. This dual-action hypothesis successfully resolves the paradoxical findings of the spermiogram. However, from a fertility perspective, the net effect is almost certainly negative. The reduction in the total number of available spermatozoa is a far more critical limiting factor for fertility than a modest qualitative improvement in the surviving population. This underscores the importance of a comprehensive evaluation of reproductive parameters, as focusing on a single metric like motility could lead to dangerously misleading conclusions about a substance's safety.

5.1.3 The Role of Phytochemistry, Dose, and Preparation

The statement of the problem for this study highlighted the profoundly contradictory findings in the scientific literature regarding the reproductive safety of *Ocimum gratissimum*. The results of the present investigation, demonstrates both negative and positive effects. The conflicting outcomes in the study are likely due to the complex interplay of the plant's phytochemistry,

which is heavily influenced by the extraction method, the administered dose, and the duration of treatment.

A substantial portion of the literature reports similar anti-fertility effects. For instance, an oil extract of *O. gratissimum* administered at 300 mg/kg for 60 days caused a significant decrease in serum testosterone, sperm motility, sperm viability, and overall fertility in male rats (Parandın & Rohanı, 2010). Similarly, other studies using aqueous and benzene extracts have documented significant histopathological damage to the testes and an increase in sperm abnormalities (Rahmat Parandin & Rohani, 2015). A likely molecular basis for this toxicity is eugenol, a primary lipophilic constituent of *Ocimum* species (Ojo, 2008). Independent studies on pure eugenol have confirmed its negative reproductive impact, demonstrating that its administration reduces serum testosterone levels and impairs sperm viability and motility in Wistar rats (Ganguly & Kumar, 2019).

Conversely, another set of studies presents evidence for pro-fertility or protective effects. A study utilizing ethyl acetate and butanolic fractions of a crude methanolic extract reported a significant increase in serum testosterone and sperm count, alongside preserved testicular histology (Agbonu & Ichaba, 2025). Another investigation found that an aqueous fraction of *O. gratissimum* could protect against plumbagin-induced testicular damage, reversing the decline in testosterone and improving sperm parameters by bolstering the testes' antioxidant defenses (Njan *et al*., 2019). These beneficial effects are attributed to the plant's rich content of antioxidant polyphenols, flavonoids, and tannins, which can mitigate oxidative stress, a key driver of male infertility (Salemcity *et al*., 2021).

5.2 CONCLUSION

In conclusion, the aqueous extract of *Ocimum gratissimum* at the 500 mg/kg oral dose administered exhibits a dualistic and site-specific action: it is detrimental to the process of sperm production within the testes while being protective to the quality of mature sperm within the epididymis. The overall impact on male fertility is likely negative, as the quantitative loss in sperm production and the hormonal signs of testicular failure far outweigh the qualitative improvements. These findings raise significant concerns about the safety and efficacy of *Ocimum gratissimum* for enhancing male reproductive health. Its widespread use in traditional medicine for this purpose should be approached with considerable caution, pending further research to elucidate a safe dose and preparation method that might successfully separate its beneficial antioxidant properties from its inherent testicular toxicity.

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