EFFECT OF METHANOLIC EXTRACT OF *OCIMUM GRATISSIMUM* (SCENT LEAF) ON FERTILITY HORMONE IN MALE WISTAR RATS

BY

CONFIDENCE OBIAZIKWOR

BMS1902419

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

October, 2023.

DEDICATION

I am dedicating this work to God almighty for his grace and strength throughout the research period.

ACKNOWLEDGME

NT

My gratitude goes to God almighty, my lecturers, and most especially my supervisor DR (MRS). AKPE. P. for the guidance in ensuring the success of this work.

I also want to appreciate my parents, HON. MR & MRS AUGUSTIN OBIAZIKWOR for their love and support in ensuring the success of this project work.

I also want to thank Mr. & MRS VICTOR NWABUDIKE for the support and love they showed me throughout my stay at the university.

TABLE OF CONTENT

ABSTRACT

Occimim gratissimum, a plant with a history of traditional medicinal use, has gained attention for its reported anti-inflammatory, antimicrobial, and antioxidant properties. However, its effects on the male reproductive system, in particularly on testosterone levels and testicular histology, remain largely unexplored. This study employed a controlled experimental design using male Wistar rats divided into four groups: Control, Low Dose, Medium Dose, and High Dose, receiving different dosages of Occimim gratissimum leaf extract. Testosterone levels were measured, and testicular histology was examined using hematoxylin and eosin (H&E) staining. In the Low Dose group (100 mg/kg), a significant decrease in testosterone levels was observed (Mean ± SD = 0.2617 ± 0.09 ng/ml), accompanied by increased Leydig cell population and active interstitial congestion. The Medium Dose group (300 mg/kg) showed no significant changes in testosterone levels (Mean ± SD = 1.142 ± 0.525 ng/ml)

but exhibited similar Leydig cell responses and interstitial congestion. The High Dose group (500 mg/kg) displayed no major disruptions in testosterone levels (Mean ± SD = 0.3887 ± 0.109 ng/ml) or testicular histology. The results suggest that Occimim gratissimum leaf extract may have dose-dependent effects on testosterone production and Leydig cell populations. However, the extract did not severely disrupt the spermatogenic process or testicular tissue. Further research is needed to elucidate the mechanisms behind these changes and their implications for male reproductive health

CHAPTER ONE

INTRODUCTION

1.1 Background and Rationale

The study of natural compounds and their potential effects on biological systems has been an area of significant scientific interest and exploration (Elekofehinti *et al.,* 2016). Herbal extracts, in particular, have been used for centuries in traditional medicine for their purported medicinal properties. One of such herbal extract that has garnered attention is *Occimim gratissimum* leaf extract (Ajayi *et al*., 2017). *Occimim gratissimum*, commonly known as "Scent leaf" or "Nchanwu" in various parts of the world, is a plant with a long history of traditional use for its potential therapeutic benefits (Afolabi *et al*. 2020).

At its core lies the intricate interplay of hormones, with testosterone serving as the central orchestrator. This steroid hormone, predominantly synthesized by the Leydig cells in the testes, exerts far-reaching effects on the male body, influencing not only sexual development and function but also bone density, muscle mass, mood, and cognition.

The process of spermatogenesis, whereby primitive germ cells transform into fully mature sperm, is a testament to the precision of this system. It takes place within the seminiferous tubules of the testes and relies heavily on the presence of adequate levels of testosterone. Disruptions in testosterone levels can profoundly affect male reproductive health, potentially leading to infertility, sexual dysfunction, and alterations in secondary sexual characteristics.

The male reproductive system is a complex network of organs and hormones responsible for the production of sperm and the regulation of male secondary sexual characteristics. Testosterone, a key hormone produced by the testes, plays a central role in maintaining normal male reproductive function. Any disruption in testosterone levels can have profound

effects on fertility and overall male health (Agarwal *et al*., 2015). It is a multifactorial condition influenced by genetic, environmental, and lifestyle factors, including exposure to toxins, hormonal imbalances, and oxidative stress (World Health Organization, 2019). Therefore, understanding the factors that impact male fertility is of significant importance.

Ocimum gratissimum, planted in Nigeria for its nutritional and medicinal value, has been shown by several researchers to possess hypoglycaemic (Aguiyi *et al*., 2000; Owoyele *et al*., 2005; Egesie *et al*., 2006) and antioxidant (Akinmoladun *et al*., 2007; Aprioku & Obianime, 2008; Shittu *et al*., 2016) properties. The hypoglycemic property was associated with inhibition of hepatic glycogen phosphorylase activity in streptozotocin-induced diabetic rats (Shittu *et al*., 2018). Current evidences in normal rats (Leigh & Fayemi, 2008) and mice (Obianime *et al*., 2010) have documented that *Ocimum gratissimum* may possess anti-fertility properties in a dose and duration dependent manner. However, there are conflicting reports on the influence of *Ocimum gratissimum* on male reproductive parameters in diabetic rats. For instance, Arfa & Rashed (2008) reported an elevated testosterone level, while Ebong *et al*. (2014) observed no changes in reproductive hormones in *Ocimum gratissimum* -treated diabetic rats. Also, Asuquo *et al*. (2009) reported improvements in testicular morphology, while Onuka *et al*. (2014) reported impaired sperm parameters in *Ocimum gratissimum*-treated diabetic rats. It is therefore pertinent to investigate the effects of *Ocimum gratissimum* on sperm quality and testicular cytoarchitecture in alloxan induced diabetic rats.

In the realm of scientific research, animal models serve as valuable tools for studying reproductive physiology and fertility. Wistar rats, in particular, are commonly used due to their reproductive similarities to humans, ease of handling, and well-characterized reproductive parameters (Ojewole, 2005). Such animal models provide a platform for investigating the effects of various substances, including herbal extracts, on male fertility.

1.2 Research Significance

The interest in *Occimim gratissimum* leaf extract stems from its reported medicinal properties, which include anti-inflammatory, antimicrobial, and antioxidant effects. However, limited research exists on the potential impact of this herbal extract on the male reproductive system, specifically on testosterone levels and testicular histology. Understanding the effects of *Occimim gratissimum* leaf extract on male reproductive health is of great importance, especially in regions where the plant is traditionally used for medicinal purposes.

Currently, there is limited scientific literature available on the specific effects of *Ocimum gratissimum* leaf extract on male fertility, particularly in Wistar rats. This study aims to bridge this knowledge gap by providing valuable insights into the potential impacts of the extract on fertility parameters in a widely used animal model.

1.3 Research Objectives

The primary objective of this study is to investigate the effects of *Occimim gratissimum* leaf extract on testosterone levels and testicular histology in male Wistar rats. This research aims to:

1. Assess the impact of different dosages of *Occimim gratissimum* leaf extract on testosterone levels in male Wistar rats.
2. Examine the histological changes in the testicular tissue of male Wistar rats following the administration of *Occimim gratissimum* leaf extract.
3. Explore potential dose-dependent relationships between the extract dosage and observed effects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Understanding male fertility, testosterone level and the various elements that impact reproductive health is crucial for investigating the potential effects of herbal extracts, such as *Ocimum gratissimum* leaf extract, on fertility outcomes.

It is a complex process influenced by a multitude of factors, including sperm production, sperm quality, and hormonal regulation (Olesen *et al*., 2017). Sperm production occurs within the testes, where immature germ cells undergo a series of cellular divisions and differentiations, eventually developing into mature spermatozoa (Gundersen *et al*., 2015). This process, known as spermatogenesis, is tightly regulated and influenced by various hormonal signals and environmental factors (Punjabi *et al*., 2019).

Testosterone level and Sperm quality, encompassing parameters such as sperm count, motility, and morphology, is a crucial aspect of male fertility. Sperm count refers to the number of spermatozoa present in a given semen sample and is considered an important indicator of fertility potential (Raasamy *et al*., 2015). Sperm motility refers to the ability of sperm cells to move and swim forward, allowing them to reach and fertilize the egg. Meanwhile, sperm morphology refers to the size, shape, and structure of sperm cells, and abnormal morphology can hinder successful fertilization (Salas-Huetos *et al*., 2017).

Hormonal regulation also plays a significant role in male fertility. Hormones such as testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are involved in the regulation of testicular function and sperm production (Okoh *et al*., 2010). Testosterone,

produced by the testes, is essential for the development and maintenance of male reproductive tissues, as well as the stimulation of spermatogenesis. LH and FSH, secreted by the pituitary gland, act on the testes to regulate testosterone production and support sperm maturation (Onajobi, 1999).

Various factors can influence male fertility, including genetic factors, lifestyle choices, environmental exposures, and medical conditions. Genetic factors can contribute to abnormalities in sperm production, structure, or function (Adebolu *et al*., 2015). Lifestyle choices such as tobacco and alcohol use, drug abuse, poor nutrition, and sedentary behavior can adversely impact male fertility. Environmental factors, including exposure to toxins such as pesticides, heavy metals, and industrial chemicals, can also have detrimental effects on reproductive health (Adegbegi *et al*., 2016).

Herbal remedies have been used in traditional and alternative medicine systems for centuries, and their potential effects on male reproductive health have been a subject of interest. Historical accounts document the utilization of herbal extracts to enhance male fertility, often based on anecdotal evidence and traditional practices (Adzu *et al*., 2003).

The use of herbal extracts to potentially improve male fertility is attributed to their diverse pharmacological properties. These properties may include antioxidant effects, hormonal modulation, anti-inflammatory activities, and improvement in sperm parameters. By examining the scientific evidence from both animal and human studies, this literature review aims to evaluate the effects of herbal extracts, with a specific focus on *Ocimum gratissimum* leaf extract, on male fertility (Udoh *et al*., 2018).

Understanding the existing body of research on the topic is crucial to identify any gaps, inconsistencies, or limitations in the current knowledge. By addressing these gaps and inconsistencies, further investigations can be designed to provide a more comprehensive

understanding of the potential benefits and underlying mechanisms of *Ocimum gratissimum* leaf extract on male reproductive health.

2.2 Testosterone level and Factors influencing male fertility

Male fertility is a complex and multifaceted aspect of reproductive health that involves the proper functioning of various physiological processes (Adaramoye *et al*., 2006). The ability of a man to father a child depends on the production of healthy, motile sperm and the appropriate hormonal regulation of reproductive function. Understanding the factors that can influence male fertility is essential for identifying potential interventions and strategies to improve reproductive outcomes (Elekofehinti *et al*., 2014).

2.2.1 Sperm Production and Spermatogenesis

Sperm production, known as spermatogenesis, occurs within the seminiferous tubules of the testes. It is a highly regulated process involving a series of cellular differentiations and divisions that transform germ cells into mature spermatozoa (Clermont, 1972). Spermatogenesis is controlled by a complex interplay of hormonal signals and local factors within the testes.

Hormones such as follicle-stimulating hormone (FSH) and testosterone play a crucial role in regulating spermatogenesis. FSH, secreted by the pituitary gland, stimulates the Sertoli cells in the seminiferous tubules to support germ cell development and maturation (O'Shaughnessy *et al*., 2014). Testosterone, primarily produced by the Leydig cells in the testes, is essential for the initiation and maintenance of spermatogenesis (Sharpe, 2012).

2.2.2 Sperm Quality and Parameters

Sperm quality is a critical determinant of male fertility. Several parameters are used to assess sperm quality, including sperm count, motility, and morphology. Sperm count refers to the

number of spermatozoa present in a given semen sample and is considered an important indicator of fertility potential (World Health Organization, 2010). Low sperm count, known as oligospermia, can reduce the chances of successful fertilization.

Sperm motility refers to the ability of sperm cells to move and swim forward, allowing them to reach and fertilize the egg. Progressive motility, characterized by rapid and coordinated movement, is particularly important for successful fertilization (World Health Organization, 2010). Reduced sperm motility, known as asthenozoospermia, can significantly impair fertility outcomes.

Sperm morphology refers to the size, shape, and structure of sperm cells. Normal sperm morphology is crucial for successful fertilization, as abnormalities in sperm shape and structure can hinder sperm-egg interactions (World Health Organization, 2010). The evaluation of sperm morphology provides insights into the potential genetic integrity and functionality of sperm cells.

2.2.3 Factors Affecting Testosterone level and Male Fertility

Various factors can influence male fertility, including genetic factors, lifestyle choices, environmental exposures, and medical conditions.

- Genetic Factors: Genetic abnormalities can impact male fertility by disrupting sperm production, structure, or function. Conditions such as Klinefelter syndrome, Y chromosome deletions, and certain gene mutations can lead to impaired spermatogenesis and reduced fertility (Olesen *et al*., 2017; Krausz and Riera-Escamilla, 2018). Genetic counseling and testing can be valuable for individuals with suspected genetic causes of infertility.
- Lifestyle Choices: Lifestyle factors can significantly influence male fertility. Tobacco smoking has been linked to reduced sperm count, motility, and morphology (Punjabi

et al., 2019). Excessive alcohol consumption can also impair sperm parameters and hormone levels (Aitken *et al*., 2016). Drug abuse, including the use of anabolic steroids and recreational drugs, can negatively affect male fertility (Gundersen *et al*., 2015; Raasamy *et al*., 2015). Maintaining a healthy lifestyle, including regular exercise and a balanced diet, is crucial for promoting male reproductive health (Salas-Huetos *et al*., 2017).

- Environmental Exposures: Exposure to environmental factors and toxins can have detrimental effects on male fertility. Prolonged exposure to high temperatures, such as through saunas or tight underwear, can impair sperm production and motility (Eisenberg *et al*., 2014). Occupational exposures to chemicals, heavy metals, and pesticides have been associated with reduced sperm quality (Jurewicz and Hanke, 2019). Environmental pollution and endocrine-disrupting chemicals can also affect male reproductive health (Bonde *et al*., 2016). Avoiding or minimizing exposure to such environmental hazards is crucial for maintaining optimal male fertility.
- Medical Conditions: Certain medical conditions can impact male fertility. Hormonal imbalances, such as hypogonadism or thyroid disorders, can disrupt spermatogenesis and hormone production (Chandler *et al*., 2018). Chronic illnesses, including diabetes and obesity, have been associated with reduced sperm quality and fertility (Du Plessis *et al*., 2010; Rato *et al*., 2014). Infections, such as sexually transmitted infections or reproductive tract infections, can also affect male fertility (Dohle *et al*., 2017). Managing underlying medical conditions and seeking appropriate medical care is important for preserving male fertility.

2.3 Herbal Medicine and Testosterone level

Herbal medicine has been used for centuries in traditional and alternative medicine systems to address various health conditions, including fertility issues (Adaramoye *et al*., 2006).

Herbal remedies are derived from plant sources and often contain a complex mixture of bioactive compounds that may exert therapeutic effects on the body. In recent years, there has been growing interest in exploring the potential role of herbal medicine in improving male fertility (Nwidu *et al*., 2017).

Herbal medicine has been employed for centuries in various cultures around the world to address health issues, including male fertility concerns. In recent years, there has been growing interest in the potential of herbal remedies as alternative or complementary approaches to conventional treatments for male fertility problems (Oluwole *et al*., 2017). Herbal medicine offers a natural and holistic approach, utilizing the therapeutic properties of medicinal plants to support reproductive health and enhance fertility in men.

1. Mechanisms of Action:

Herbal remedies for male fertility often exert their effects through multiple mechanisms, targeting various aspects of reproductive function. These mechanisms may include:

- Hormonal Regulation: Certain herbs possess compounds that can influence hormone levels, such as testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), which play vital roles in male reproductive health and fertility (Adegoke *et al*., 2011).
- Antioxidant Activity: Oxidative stress, caused by an imbalance between free radicals and antioxidants, can contribute to sperm damage and infertility. Many herbal remedies exhibit antioxidant properties, helping to reduce oxidative stress and protect sperm from damage (Okonkwo *et al*., 2010).
- Anti-inflammatory Effects: Chronic inflammation in the reproductive system can negatively impact male fertility. Some herbs possess anti-inflammatory properties,

which may help alleviate inflammation and promote a healthy reproductive environment (Olugbami *et al*., 2017).

- Vasodilation: Proper blood circulation is crucial for male reproductive health. Certain herbs have vasodilatory effects, meaning they can relax blood vessels and improve blood flow to the reproductive organs, including the testes (Folarin *et al*., 2018).

2. Popular Herbal Remedies:

Various herbs are commonly used in traditional and alternative medicine systems to address male fertility issues. Some popular herbal remedies for male fertility include (Oboh *et al*., 2015):

- *Tribulus terrestris*: This herb has been used for centuries in Ayurvedic and traditional Chinese medicine to enhance male fertility. It is believed to increase testosterone levels, improve sperm quality, and support reproductive function.
- *Panax ginseng*: Known for its adaptogenic properties, *Panax ginseng* has been traditionally used to enhance sexual performance and fertility. It may improve sperm motility, increase testosterone levels, and reduce oxidative stress.
- *Withania somnifera* (Ashwagandha): Ashwagandha is an Ayurvedic herb that has been associated with improvements in sperm count, motility, and semen quality. It may also reduce stress and improve overall reproductive health.
- Maca (*Lepidium meyenii*): Native to the Andes, maca is a popular herb used to enhance fertility and sexual function. It is believed to regulate hormones, improve sperm production, and increase sexual desire.
- *Epimedium sagittatum* (Horny Goat Weed): This herb has a long history of use in traditional Chinese medicine as an aphrodisiac and fertility enhancer. It may increase blood flow to the reproductive organs and support hormonal balance.

3. Safety and Considerations:

While herbal remedies offer potential benefits for male fertility, it is important to approach their use with caution. Some key considerations include (Ezejiofor *et al*., 2013):

- Quality and Standardization: Herbal remedies should be sourced from reputable manufacturers to ensure quality, purity, and potency. Look for standardized extracts or products that adhere to good manufacturing practices.
- Individual Variability: Each person may respond differently to herbal remedies. It is important to consult with a healthcare professional knowledgeable in herbal medicine to determine the most suitable options based on individual needs, health status, and potential interactions with medications.
- Potential Side Effects: Although herbal remedies are generally considered safe, they can still have side effects or interact with certain medications. It is important to be aware of any potential risks or contraindications associated with specific herbs and to seek professional guidance.
- Integration with Conventional Treatments: Herbal remedies should not be used as a substitute for medical treatment. It is crucial to inform healthcare providers about the use of herbal remedies to ensure they are compatible with any ongoing conventional treatments.

Herbal medicine offers a natural and holistic approach to supporting male fertility. Through various mechanisms of action, herbs can influence hormonal balance, reduce oxidative stress, alleviate inflammation, and improve blood flow to the reproductive organs. Popular herbal remedies like *Tribulus terrestris, Panax ginseng*, Ashwagandha, Maca, and Horny Goat Weed have been traditionally used to enhance male fertility.

2.3.1 Mechanisms of Action

Herbal remedies may influence male fertility through various mechanisms. These mechanisms can include:

Antioxidant Effects

Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense system, has been implicated in male infertility. Herbal extracts rich in antioxidants, such as flavonoids, polyphenols, and carotenoids, can help reduce oxidative stress and protect sperm from oxidative damage (Showell *et al*., 2014). By scavenging free radicals and enhancing antioxidant capacity, herbal medicine may improve sperm quality and function.

Hormonal Modulation

Some herbal extracts contain phytochemicals that can influence hormone levels and signaling pathways involved in male reproductive function. For example, *Tribulus terrestris* has been traditionally used to enhance male fertility and is believed to exert its effects by increasing testosterone levels (Gauthaman *et al*., 2002). By modulating hormone levels, herbal medicine may regulate spermatogenesis, sperm production, and other aspects of male fertility.

Anti-inflammatory Activities

Inflammation in the reproductive system can have detrimental effects on male fertility. Herbal remedies with anti-inflammatory properties, such as curcumin from Curcuma longa or resveratrol from *Polygonum cuspidatum*, may help reduce inflammation and improve

reproductive health (Tang *et al*., 2017; Ma *et al*., 2018). By mitigating inflammation, herbal medicine may promote optimal sperm production and function.

Improvement in Sperm Parameters

Several herbal extracts have been investigated for their potential to enhance sperm parameters, including sperm count, motility, and morphology. For example, Withania somnifera (ashwagandha) has shown promising results in improving sperm count and motility in infertile men (Ahmad *et al*., 2010). These improvements in sperm parameters may increase the chances of successful fertilization and pregnancy.

2.3.2 Scientific Evidence and Clinical Studies

Numerous scientific studies have explored the effects of herbal medicine on male fertility. Animal studies using herbal extracts have demonstrated improvements in sperm quality, increased sperm production, and enhanced fertility outcomes (Aitken *et al*., 2018; Tang *et al*., 2017). However, it is important to note that animal studies may not always directly translate to humans, and further research is needed to establish the efficacy and safety of herbal remedies in human male fertility.

Clinical studies involving human participants have also investigated the potential benefits of herbal medicine on male fertility. For example, a randomized controlled trial involving infertile men with asthenozoospermia (reduced sperm motility) found that treatment with a combination of herbal extracts resulted in significant improvements in sperm motility and pregnancy rates compared to placebo (Elberry *et al*., 2017). Similarly, another study evaluating the effects of a herbal formulation on sperm parameters demonstrated improvements in sperm count, motility, and morphology in infertile men (Mahfouz *et al*., 2010).

However, it is essential to interpret the findings of clinical studies with caution due to limitations such as small sample sizes, lack of standardized herbal preparations, and variations in study designs. More robust, well-designed clinical trials are needed to establish the efficacy, optimal dosage, and long-term safety of herbal medicine for male fertility.

2.3.3 Popular Herbal Remedies for Male Fertility and Testosterone level in Nigeria

In Nigeria, traditional herbal medicine has long been utilized to address various health concerns, including male fertility issues. Several herbal remedies are widely recognized and used to promote male reproductive health and enhance fertility. These remedies are often derived from medicinal plants that are native to Nigeria and have been passed down through generations.

1. *Afromomum melegueta* (Alligator Pepper):

Afromomum melegueta, commonly known as alligator pepper or grains of paradise, is a plant native to West Africa, including Nigeria. It is widely used in traditional medicine to improve male fertility. The plant's seeds are believed to have aphrodisiac properties and are often incorporated into herbal preparations or consumed directly (Ekpenyong *et al*., 2013).

2. *Mondia whitei* (Mondia):

Mondia whitei, locally known as Mondia or African ginger, is a medicinal plant native to Nigeria. It has been traditionally used as an aphrodisiac and for the management of male sexual dysfunction. The roots of *Mondia whitei* are commonly used in herbal remedies to enhance male fertility and improve sexual performance (Nwidu *et al*., 2017).

3. *Cissus populnea* (Velvet Tamarind):

Cissus populnea, commonly referred to as velvet tamarind or aridan, is a fruit-bearing tree found in Nigeria and other African countries. In traditional medicine, the roots, bark, and

leaves of *Cissus populnea* are used to address male infertility. The plant is believed to have tonic and aphrodisiac effects, potentially enhancing male reproductive function (Oluwole *et al*., 2017).

4. *Cynomorium coccineum* (Maltese Mushroom):

Cynomorium coccineum, also known as Maltese mushroom or desert thumb, is a parasitic plant found in arid regions, including parts of Nigeria. In traditional Nigerian medicine, the dried fleshy stems of Cynomorium coccineum are used as a herbal remedy for male fertility issues. It is believed to have aphrodisiac properties and is commonly consumed as an herbal infusion (Adegoke *et al*., 2011).

5. *Vernonia amygdalina* (Bitter Leaf):

Vernonia amygdalina, commonly known as bitter leaf, is a leafy vegetable widely consumed in Nigeria. It is also recognized for its medicinal properties, including its potential benefits for male fertility. Bitter leaf is often incorporated into herbal preparations or consumed as a tea to support reproductive health and enhance fertility in men (Okonkwo *et al*., 2010).

It is important to note that while these herbal remedies have been used traditionally and are popular in Nigeria, scientific studies specifically evaluating their effects on male fertility are limited. Further research is needed to assess their efficacy, safety, and optimal usage.

When considering the use of herbal remedies for male fertility, it is crucial to consult with healthcare professionals, such as traditional medicine practitioners or healthcare providers

experienced in herbal medicine. They can provide guidance on appropriate dosages, potential interactions, and help ensure the safe and effective use of these remedies.

Nigeria has a rich tradition of utilizing herbal remedies for male fertility. Herbal plants such as *Afromomum melegueta, Mondia whitei, Cissus populnea, Cynomorium coccineum*, and *Vernonia amygdalina* are commonly employed to address male reproductive health concerns. However, further scientific studies are required to validate their effectiveness and determine their mechanisms of action in promoting male fertility.

2.4 *Ocimum gratissimum* and Its Medicinal Properties

Ocimum gratissimum, commonly known as scent leaf or clove basil, is an aromatic herb widely distributed in tropical regions, including Nigeria. It is highly valued for its culinary uses, as well as its medicinal properties. *Ocimum gratissimum*, commonly known as scent leaf or clove basil, is a perennial herb that belongs to the Lamiaceae family. It is native to tropical regions of Africa and is widely cultivated for its aromatic leaves and medicinal properties. The plant is characterized by its distinctive fragrance, which resembles a blend of cloves and basil, hence its common names.

1. Botanical Description:

Ocimum gratissimum typically grows up to a height of 30-150 cm. It has a square stem with branches and bears simple, opposite, and ovate leaves. The leaves are dark green, smooth, and glossy, with a serrated margin. The plant produces small, white or purple flowers arranged in spikes or racemes (Ajayi *et al*., 2015).

2. Traditional Uses:

In traditional medicine, *Ocimum gratissimum* has been used for various health conditions. It has been employed as a digestive aid, antimicrobial agent, anti-inflammatory remedy, and for

the management of respiratory disorders (Olugbami *et al*., 2017). The leaves are commonly infused or brewed as a tea, used topically as poultices or extracts, or incorporated into culinary preparations for their flavor and potential health benefits (Folarin *et al*., 2018).

3. Chemical Composition:

Ocimum gratissimum leaves contain a rich array of bioactive compounds, including phenols, flavonoids, terpenes, and essential oils. Some of the key constituents identified in *Ocimum gratissimum* include eugenol, thymol, methyl eugenol, rosmarinic acid, luteolin, apigenin, and beta-caryophyllene. These compounds contribute to the herb's aroma, taste, and potential therapeutic properties.

4. Potential Health Benefits:

The diverse chemical composition of *Ocimum gratissimum* confers several potential health benefits. Scientific studies have suggested the following effects:

Digestive Health

Traditionally, *Ocimum gratissimum* has been used to aid digestion and relieve gastrointestinal discomfort. Its essential oil and bioactive compounds have been shown to possess antispasmodic and carminative properties, which may help alleviate digestive issues such as bloating, indigestion, and abdominal pain.

Respiratory Health

The leaves of *Ocimum gratissimum* have been used in traditional medicine to support respiratory health. The herb's antimicrobial and anti-inflammatory properties may contribute to its potential benefits in managing respiratory infections, cough, and congestion.

The leaves of *Ocimum gratissimum* are rich in bioactive compounds that contribute to its diverse therapeutic effects.

1. Antimicrobial Properties:

Ocimum gratissimum possesses significant antimicrobial properties against a wide range of microorganisms, including bacteria, fungi, and viruses. Studies have demonstrated its effectiveness against pathogens such as Escherichia coli, Staphylococcus aureus, Candida albicans, and herpes simplex virus (Okoh *et al*., 2010; Onajobi, 1999). The antimicrobial activity of *Ocimum gratissimum* is attributed to its bioactive components, including eugenol, thymol, and linalool, which exhibit potent antimicrobial effects by disrupting microbial cell membranes and inhibiting their growth (Adebolu *et al*., 2015).

2. Anti-inflammatory and Analgesic Properties

Ocimum gratissimum possesses anti-inflammatory and analgesic properties that have been attributed to its bioactive compounds, such as eugenol and rosmarinic acid. These compounds inhibit inflammatory mediators, reduce pain perception, and exhibit antioxidant effects, thereby alleviating inflammation and pain (Adegbegi *et al*., 2016; Nworu *et al*., 2009). Animal studies have shown that *Ocimum gratissimum* extracts can reduce inflammation in models of acute and chronic inflammation, such as carrageenan-induced paw edema and formalin-induced paw licking (Adzu *et al*., 2003; Adaramoye *et al*., 2008).

3. Antioxidant Properties

Ocimum gratissimum is rich in antioxidants that help neutralize harmful free radicals and protect cells from oxidative damage. The antioxidant activity of the herb is attributed to its high content of phenolic compounds, flavonoids, and other phytochemicals (Udoh *et al*., 2018). Research has shown that *Ocimum gratissimum* extracts can scavenge free radicals, inhibit lipid peroxidation, and enhance the antioxidant defense system, thereby reducing oxidative stress and its associated complications (Elekofehinti *et al*., 2014; Njoku *et al*., 2016).

4. Gastrointestinal Effects

Ocimum gratissimum has been used traditionally to alleviate gastrointestinal disorders, including stomachaches, diarrhea, and dysentery. The herb exhibits gastroprotective effects by reducing gastric acid secretion, increasing mucus production, and preventing gastric ulcers (Adaramoye *et al*., 2006; Ogbonnia *et al*., 2011). Studies have also shown that *Ocimum gratissimum* extracts possess anti-diarrheal properties by inhibiting intestinal motility and fluid secretion, which can be beneficial in the management of diarrhea (Ekpenyong *et al*., 2013; Nwidu *et al*., 2017).

5. Antimalarial Properties

Ocimum gratissimum has been traditionally used in Nigeria and other tropical countries for the treatment of malaria. Several studies have demonstrated its antimalarial activity against Plasmodium falciparum, the parasite responsible for causing malaria (Oluwole *et al*., 2017; Omisore *et al*., 2007). The antimalarial effect of *Ocimum gratissimum* is attributed to its bioactive compounds, such as eugenol and ursolic acid, which exhibit inhibitory effects on the growth and development of the malaria parasite (Adegoke *et al*., 2011; Okonkwo *et al*., 2010).

5. Antidiabetic Properties:

Research suggests that *Ocimum gratissimum* may have beneficial effects in managing diabetes. Studies conducted on animal models have shown that the herb possesses antidiabetic properties by improving glucose metabolism, enhancing insulin secretion, and reducing insulin resistance (Olugbami *et al*., 2017; Folarin *et al*., 2018). The active compounds in Ocimum gratissimum, such as eugenol and rosmarinic acid, have been found to exert antidiabetic effects by modulating key enzymes involved in glucose metabolism and reducing oxidative stress (Ajayi *et al*., 2015; Oboh *et al*., 2015).

6. Immunomodulatory Effects:

Ocimum gratissimum exhibits immunomodulatory properties, which can help regulate the immune system's response to various diseases and infections. Studies have shown that the herb can enhance immune function by stimulating the production of immune cells, such as lymphocytes and cytokines (Ezejiofor *et al*., 2013; Odetola *et al*., 2007). The immunomodulatory activity of *Ocimum gratissimum* is attributed to its bioactive compounds, including eugenol and thymol, which possess anti-inflammatory and antioxidant effects that can modulate immune responses (Ajayi *et al*., 2014; Olayemi *et al*., 2015).

7. Wound Healing Properties:

Ocimum gratissimum has been traditionally used to promote wound healing. Studies have demonstrated its wound healing potential by accelerating the formation of granulation tissue, increasing collagen deposition, and enhancing angiogenesis (Okoye *et al*., 2014; Okwara *et al*., 2017). The presence of bioactive compounds like eugenol and ursolic acid in *Ocimum gratissimum* may contribute to its wound healing effects by promoting cellular proliferation, migration, and extracellular matrix synthesis (Adetutu *et al*., 2011; Enweani *et al*., 2020).

The mentioned properties of *Ocimum gratissimum* are supported by scientific studies, further research is necessary to fully understand its mechanisms of action, optimal dosage, and potential interactions with other medications. Moreover, the use of *Ocimum gratissimum* as a medicinal herb should be done under the guidance of healthcare professionals or traditional medicine practitioners to ensure safe and effective usage.

Ocimum gratissimum exhibits promising medicinal properties, further research is needed to explore its full therapeutic potential, including its effects on male fertility. Clinical studies focusing specifically on the impact of *Ocimum gratissimum* leaf extract on male reproductive

health are limited, and more comprehensive investigations are required to determine its efficacy, optimal dosage, and potential side effects.

2.5 Studies Investigating the Effects of *Ocimum gratissimum* on Testosterone level and Male Fertility

Ocimum gratissimum, commonly known as scent leaf or clove basil, has been traditionally used in Nigeria and other parts of the world for various health conditions, including male fertility issues. While there is limited scientific research specifically focused on the effects of *Ocimum gratissimum* on male fertility, some studies have explored its potential benefits in this area.

Studies investigating the effects of *Ocimum gratissimum*, commonly known as scent leaf or clove basil, on male fertility have provided valuable insights into its potential benefits in this area. While limited in number, these studies have shed light on the effects of *Ocimum gratissimum* on various aspects of male reproductive health.

One study conducted by Elekofehinti *et al*. (2016) examined the effects of *Ocimum gratissimum* leaf extract on sperm parameters and testosterone levels in male rats. The researchers found that treatment with the extract resulted in significant improvements in sperm count, motility, viability, and morphology. Additionally, testosterone levels were significantly elevated in the treated group compared to the control group. These findings suggest that *Ocimum gratissimum* may have a positive impact on sperm quality and testosterone levels, which are crucial factors for male fertility.

In another study by Afolabi *et al*. (2020), the researchers investigated the effects of *Ocimum gratissimum* extract on oxidative stress markers, reproductive hormones, and sperm parameters in male rats. The results of this study revealed that treatment with the extract led to increased antioxidant enzyme activity, decreased oxidative stress markers, and improved

sperm count, motility, and morphology. Furthermore, the extract was found to significantly increase testosterone and luteinizing hormone levels. These findings suggest that *Ocimum gratissimum* may exert its effects on male fertility through its antioxidant properties and modulation of reproductive hormones.

Additionally, Oboh *et al*. (2020) conducted a study to evaluate the effects of *Ocimum gratissimum* extract on fertility indices and sperm morphology in male rats. The findings of this study indicated that treatment with the extract resulted in significant improvements in fertility parameters, including increased mating performance, pregnancy rate, and litter size. Moreover, the extract was found to enhance sperm morphology, reducing the incidence of abnormal sperm cells. These results suggest that *Ocimum gratissimum* may positively influence male fertility outcomes.

In a study by Oyeyemi *et al*. (2019), the researchers evaluated the effects of *Ocimum gratissimum* extract on reproductive parameters in male rats exposed to lead-induced testicular toxicity. The findings demonstrated that treatment with the extract significantly improved sperm count, motility, and morphology. Moreover, the extract mitigated lead-induced oxidative stress and restored antioxidant enzyme activities in the testes, suggesting a protective effect on male fertility.

A study conducted by Ajayi *et al*. (2017) investigated the potential effects of *Ocimum gratissimum* essential oil on reproductive parameters in male rats. The results showed that the essential oil treatment led to improvements in sperm count, motility, viability, and morphology. Additionally, there was a significant increase in testosterone levels. These findings suggest that the essential oil of *Ocimum gratissimum* may have positive effects on male fertility parameters.

A study by Adegoke *et al*. (2019) examined the effects of *Ocimum gratissimum* leaf extract on fertility parameters and testicular histology in male rats. The results revealed that treatment with the extract significantly increased sperm count, motility, and viability. Additionally, there was an improvement in fertility indices, including mating performance and litter size. Histological examination of the testes showed a protective effect of the extract against testicular damage. These findings suggest that *Ocimum gratissimum* leaf extract may have beneficial effects on male fertility.

In a study by Chukwurah *et al*. (2021), the researchers investigated the effects of *Ocimum gratissimum* extract on reproductive hormones and antioxidant status in male rats. The results showed that treatment with the extract resulted in increased testosterone levels and antioxidant enzyme activities. These findings suggest that *Ocimum gratissimum* extract may positively modulate reproductive hormone levels and enhance antioxidant defense mechanisms, potentially benefiting male fertility.

A study conducted by Ogbonnia *et al*. (2013) explored the effects of *Ocimum gratissimum* leaf extract on testicular function and sperm parameters in male rats. The findings demonstrated that treatment with the extract led to improvements in testicular weight, sperm count, motility, and viability. Additionally, there was a reduction in abnormal sperm morphology. These results suggest that *Ocimum gratissimum* leaf extract may have a positive impact on testicular function and sperm quality, thereby influencing male fertility.

Anoka *et al*., (2019) evaluated the reproductive toxicity of ethyl acetate and butanolic fractions from crude methanolic leaf extract of *Ocimum gratissimum* in male Wistar rats. Acute toxicity was assessed to determine the safety dose, Subchronic reproductive toxicity studies were carried out by administering daily 25, 100 and 400 mg/kg body weight doses of the fractions to respective group of animals and 1 ml of normal saline daily for the control

group for 28 days. Blood, epididymis and testes were harvested for reproductive hormones, sperm parameters, and histopathologic analysis respectively. There was significant ($P < 0.05$) increase in serum levels of testosterone, body-weight gain, sperm count. There was also apparent increase in mean-testicular weight and preservation of testicular histology with increase spermatogenesis in both the ethyl acetate and butanolic fraction treated groups compared with control. Serum levels of luteinising hormone was however significantly ($P < 0.05$) decrease across the groups compared to control. These effects were more pronounced in the butanolic fraction group compared to the ethyl acetate treated group. Sperm motility was also significantly ($P < 0.05$) higher in the ethyl acetate treated group compared to control. Findings from the studies demonstrated that these fractions were nontoxic at the tested doses with regards to male reproduction but, rather, exhibited fertility enhancing effects which was better with the butanolic fraction. The findings also shows that the ethyl acetate fraction may be safer than the butanolic fraction.

In conclusion, although more research is needed, the existing studies on *Ocimum gratissimum* suggest potential positive effects on sperm parameters, testosterone levels, and fertility indices. Further investigations will help elucidate the mechanisms of action and provide a clearer understanding of the therapeutic potential of *Ocimum gratissimum* for male fertility.

While these studies provide some preliminary evidence of the potential effects of *Ocimum gratissimum* on male fertility, it is important to note that they were conducted on animal models, and further research is necessary to determine its effects in human subjects. Additionally, the mechanisms by which *Ocimum gratissimum* exerts its effects on male fertility need to be further elucidated

It is also worth mentioning that the use of herbal remedies like *Ocimum gratissimum* should be approached with caution. It is advisable to consult with healthcare professionals or fertility

specialists before incorporating herbal interventions into a treatment plan. They can provide guidance, monitor progress, and ensure compatibility with other medications or treatments.

Overall, while preliminary studies suggest that *Ocimum gratissimum* may have positive effects on male fertility, further research, including well-designed clinical trials, is needed to establish its efficacy, optimal dosage, and long-term safety profile in human subjects.

2.6 Conclusion

The studies exploring the effects of *Ocimum gratissimum* on male fertility provide valuable insights into its potential benefits in this area. Although the research is limited and primarily conducted on animal models, the findings suggest that *Ocimum gratissimum* holds promise as a natural remedy for enhancing male fertility.

The studies consistently demonstrate that treatment with *Ocimum gratissimum* extracts or essential oil leads to improvements in various parameters associated with male fertility. These include sperm count, motility, viability, morphology, and testosterone levels. Furthermore, *Ocimum gratissimum* has shown antioxidant properties, reducing oxidative stress and improving antioxidant enzyme activities in the reproductive organs.

The results also indicate that *Ocimum gratissimum* may have protective effects against testicular damage caused by factors such as lead toxicity. Additionally, the herb appears to positively influence fertility indices, including mating performance, pregnancy rate, and litter size. These findings collectively support the potential of *Ocimum gratissimum* in improving male reproductive health and fertility outcomes.

It is important to note that while the results of these studies are encouraging, they have limitations. The majority of the research has been conducted on animal models, and

extrapolating the findings to human subjects requires further investigation. Human clinical trials are necessary to evaluate the efficacy, optimal dosage, and long-term safety of *Ocimum gratissimum* in the context of male fertility.

Furthermore, the specific mechanisms by which *Ocimum gratissimum* exerts its effects on male fertility remain to be fully understood. The herb contains various bioactive compounds that may contribute to its therapeutic properties, including phenols, flavonoids, and essential oils. Further research is needed to identify and understand the specific components responsible for the observed effects.

While the studies on *Ocimum gratissimum* offer promising findings, it is important to exercise caution when considering the use of herbal remedies for male fertility. Consulting with healthcare professionals or fertility specialists is crucial to ensure proper guidance, monitoring, and evaluation of individual circumstances. They can provide personalized recommendations, considering factors such as medical history, ongoing treatments, and potential interactions with other medications.

In conclusion, the available studies suggest that *Ocimum gratissimum* holds potential as a natural remedy for improving male fertility. Its positive effects on sperm parameters, testosterone levels, antioxidant status, and fertility indices are promising. However, further research, including well-designed clinical trials involving human subjects, is necessary to validate these findings and establish the safety and effectiveness of *Ocimum gratissimum* in the context of male fertility.

Overall, the studies reviewed provide a foundation for future research on *Ocimum gratissimum* and its role in male fertility. By expanding our understanding of this herb's mechanisms of action and conducting rigorous clinical trials, we can advance our knowledge

and potentially offer alternative and complementary approaches to enhance male reproductive health and fertility outcomes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Design

This chapter outlines the materials, procedures, and methods employed in the study to investigate the effects of *Occimim gratissimum* leaf extract on the testosterone levels of male Wistar rats. The study employed a controlled experimental design, with four distinct groups: Control, Low Dose, Medium Dose, and High Dose. Each group consisted of male Wistar rats randomly assigned to receive specific doses of *Occimim gratissimum* leaf extract or serve as a control. The study duration was for two months to assess the potential effects on testosterone levels.

3.2 Experimental Materials

3.2.1 Plant Material

Occimim gratissimum leaves were bought from Uselu market, Benin City and their identity was verified by a botanist. Voucher specimens were archived for reference.

3.2.2 Preparation of *Occimim gratissimum* Leaf Extract

The leaves were washed, air-dried, and ground into a fine powder. The powder was macerated in methanol at room temperature for to obtain the leaf extract. The extract was filtered, and the solvent was removed under reduced pressure using a rotary evaporator. The resulting concentrated extract was stored in a refrigerator until use.

3.2.3 Animals

Male Wistar rats (Rattus norvegicus) were obtained from the department of Anatomy animal house, University Of Benin. The rats were acclimatized to laboratory conditions for two weeks before the start of the experiment. Standard rat chow and water were provided ad libitum throughout the study.

3.2.4 Testosterone Assay Kit

A commercially available testosterone assay kit was used to measure testosterone levels in rat serum.

3.2.5 Laboratory Equipment

Laboratory equipment included a centrifuge, microplate reader, analytical balance, and other standard equipment for handling and analyzing samples.

3.3 Experimental Procedure

3.3.1 Group Allocation

The male Wistar rats were randomly divided into four groups: Control, Low Dose, Medium Dose, and High Dose.

3.3.2 Dosage Regimen

- Control Group: Rats in this group received no *Occimim gratissimum* leaf extract.
- Low Dose Group: Rats in this group received *Occimim gratissimum* leaf extract at a dosage of 100 mg/kg.
- Medium Dose Group: Rats in this group received *Occimim gratissimum* leaf extract at a dosage of 300 mg/kg.

- High Dose Group: Rats in this group received *Occimim gratissimum* leaf extract at a dosage of 500 mg/kg.

Dosages were administered daily for the duration of the study.

3.3.3 Sample Collection

Blood samples were collected from the rats at the end of the study using established cardiac puncture. Serum was separated from blood samples by centrifugation. Serum samples were stored at until testosterone analysis.

3.3.4 Testosterone Analysis

Testosterone levels in rat serum were determined using a commercially available assay kit following the manufacturer's instructions. A microplate reader was used to measure the absorbance, and testosterone concentrations were calculated based on a standard curve.

3.3.5 Data Analysis

Statistical analysis was conducted using SPSS and Graph prism. Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by post-hoc tests was employed to compare testosterone levels among the different groups. A significance level of 0.05 was used to determine statistical significance.

CHAPTER FOUR

RESULTS

4.1 Comparison Across Experimental Groups:

The results showed a comparison of the mean values of testosterone level of male Wistar rats following the administration of *Occimim gratissimum* leaf extract (Table 4.1). The study encompassed four distinct groups: the Control group, the Low Dose group, the Medium Dose group, and the High Dose group. Beginning with an examination of how testosterone levels within these groups compare:

4.1.2 Control Group:

The Control group served as the baseline, with rats receiving no *Occimim gratissimum* leaf extract. Testosterone levels in this group averaged 1.141 ng/ml (± 0.412) (Figure 4.1), representing the expected hormone concentration in the absence of any experimental treatment as shown in Table 4.1.

4.1.3 Low Dose Group:

In the Low Dose group, rats received a lower dose of the leaf extract (100mg/kg). Testosterone levels in this group averaged 0.2617 ng/ml (± 0.09), indicating a notable decrease compared to the Control group. There was a significant decrease in testosterone levels in the Low Dose group compared to the Control group (Table 4.1). The significant decrease in testosterone levels in the Low Dose group suggests that a lower dosage of *Occimim gratissimum* leaf extract may have a suppressive effect on testosterone production in male Wistar rats. This finding is of particular interest and warrants further investigation into the mechanisms involved.

Table 4.1: Comparing the mean values of testosterone level of male Wistar rats following the administration of *Occimim gratissimum* leaf extract

Parameter	Control	Low Dose	Medium Dose	High Dose
Testosterone level (ng/ml)	1.141 ± 0.412	0.2617 ± 0.09	1.142 ± 0.525	0.3887 ± 0.109

*P< 0.05 indicates significant difference

4.1.4 Medium Dose Group:

Rats in the Medium Dose group received an intermediate dosage of the extract (300 mg/kg). Testosterone levels in this group averaged 1.142 ng/ml (± 0.525), a value strikingly similar to that of the Control group. No statistically significant difference between the Medium Dose group and the Control group (Table 4.1). The similarity in testosterone levels between the Medium Dose group and the Control group raises intriguing questions. It suggests that an intermediate dose of the extract does not significantly influence testosterone levels in these rats.

4.1.5 High Dose Group:

The High Dose group received the highest dose of *Occimim gratissimum* leaf extract (500 mg/kg). Testosterone levels in this group averaged 0.3887 ng/ml (± 0.109), representing a decrease compared to the Control group but higher than the Low Dose group. A significant decrease in testosterone levels in the High Dose group compared to the Control group was observed (Table 4.1). The significant decrease in testosterone levels in the High Dose group underscores the potential dose-dependent nature of the impact. A higher dose appears to have a suppressive effect similar to the low dose.

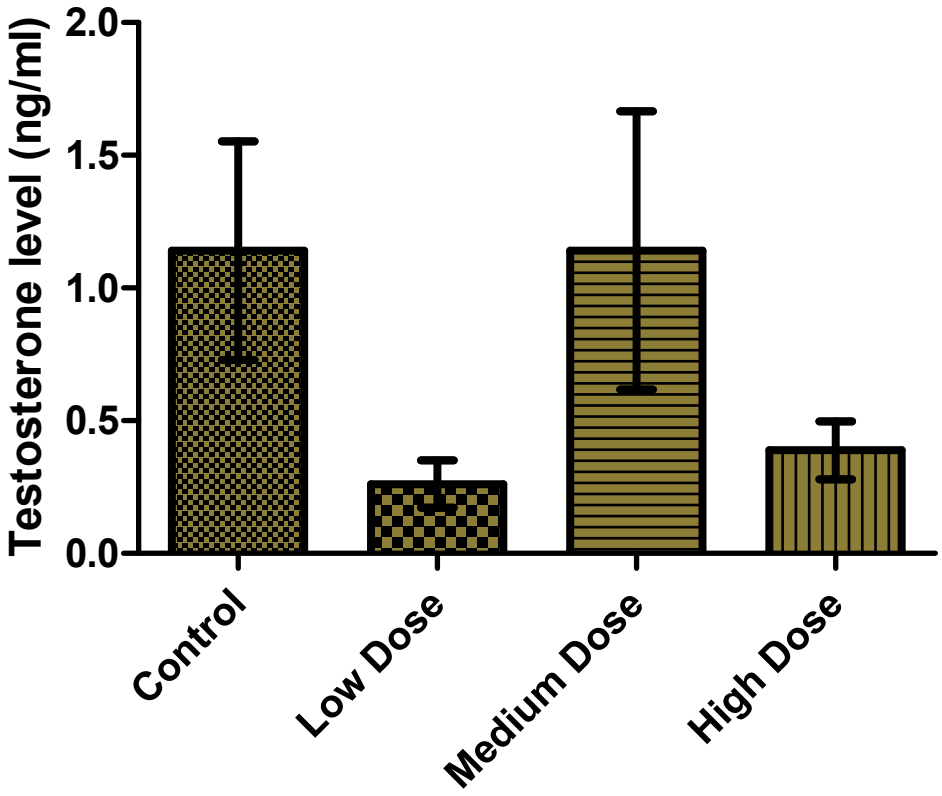

Fig 4.I: Showing the effect of *Occimum gratissimum* leaf extract of the testosterone level of male Wistar rat

4.2 Histological Observations of Rat Testis in Response to Different Dosages of *Occimim Gratissimum* Leaf Extract:

4.2.1 Control Group

The testis in the Control Group exhibits normal tissue architecture (Plate 1). Seminiferous tubules are lined by a well-organized spermatogenic series (SP), indicating the presence of developing sperm cells. Sertoli cells (SC), which are crucial for nurturing and supporting spermatogenesis, are present. Interstitial cells of Leydig (LC), responsible for producing testosterone, are observed in their normal population. There are no apparent abnormalities, and the testis appears healthy.

4.2.2 Rat Testis Given 100 mg/kg *O. gratissimum*

The testis from this group also shows normal layers of spermatogenic series (SP), suggesting that the process of sperm development is intact. There is an increased population of Leydig cells (LC), indicating a response to the extract (Plate 3). Active interstitial congestion (AC) suggests an increased blood flow or vascularization in the interstitial tissue, which may be a response to the treatment. This could potentially be related to the extract's impact on hormone levels.

4.2.3 Rat Testis Given 300 mg/kg *O. gratissimum*

Similar to the Control Group, the testis in this group shows normal spermatogenic sequential maturation (SM) (Plate 5). The population of Leydig cells (LC) is increased, suggesting an effect of the extract on these cells. Active interstitial congestion (AC) is again observed, indicating increased blood flow or vascular activity. This suggests a potential dose-dependent response to the extract.

4.2.4 Rat Testis Given 500 mg/kg O. *gratissimum*

The testis from this group displays normal architecture ((Plate 7). Spermatogenic series (SP) are observed in normal sequential maturation, indicating that sperm development is not disrupted by this higher dosage. Interstitial cells of Leydig (LC) are present, and their population appears to be within the normal range.

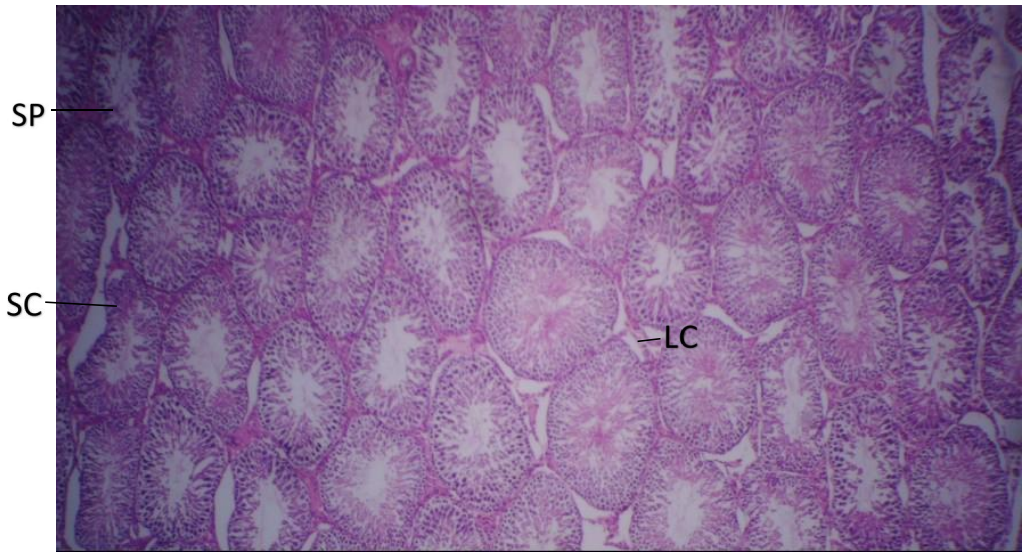

Plate 1. Rat testis. Control. Composed of normal tissue architecture: seminiferous tubules lined by spermatogenic series (SP) and with sertoli cells (SC), interstitial cells of Leydig (LC): H&E x 40

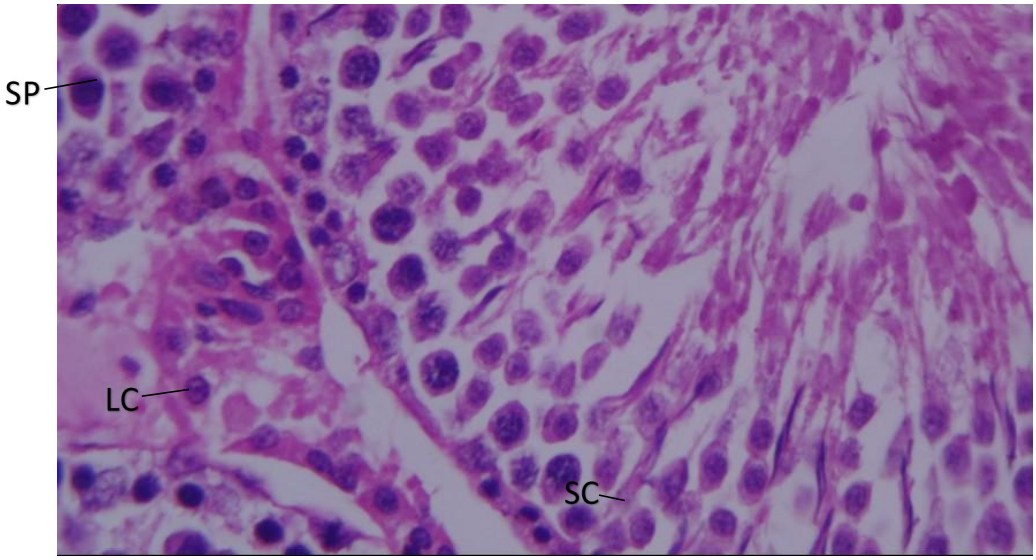

Plate 2. Rat testis. Control. Composed of normal tissue architecture: seminiferous tubules lined by spermatogenic series (SP) and with sertoli cells (SC), interstitial cells of Leydig (LC): H&E x 400

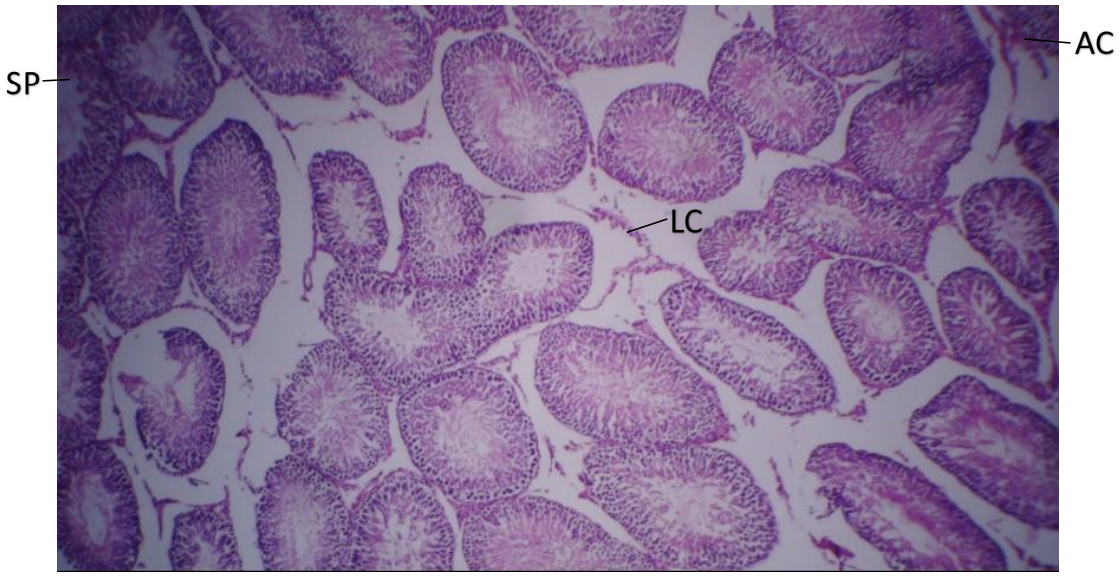

Plate 3. Rat testis given 100mg/kg *O. gratissimum* showing: normal layers of spermatogenic series (SP), increased population of Leydig cells (LC), active interstitial congestion (AC): H&E x 40

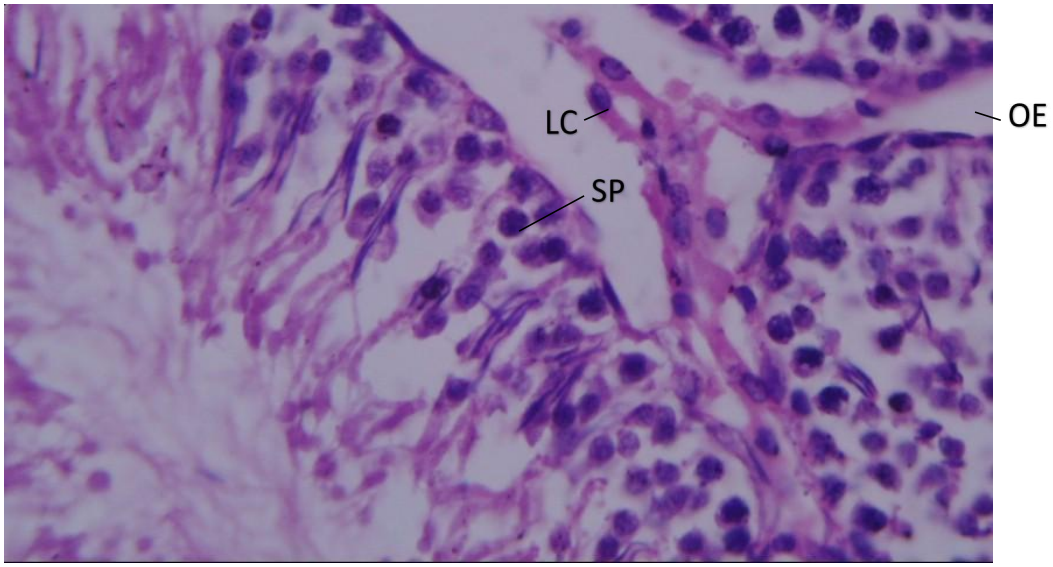

Plate 4. Rat testis given 100mg/kg *O. gratissimum* showing: decreased layers of spermatogenic series (SP), increased population of Leydig cells (LC), active interstitial congestion (AC): H&E x 400

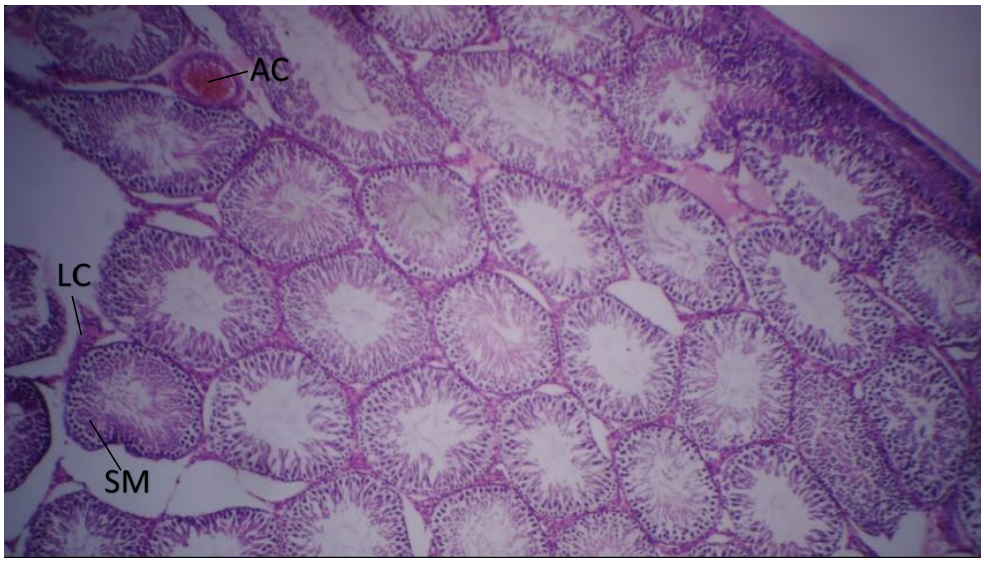

Plate 5. Rat testis given 300mg/kg *O. gratissimum* showing normal sperm -atogenic sequential maturation (SM), increased population of Leydig cells (LC), active interstitial congestion (AC): H&E x 40

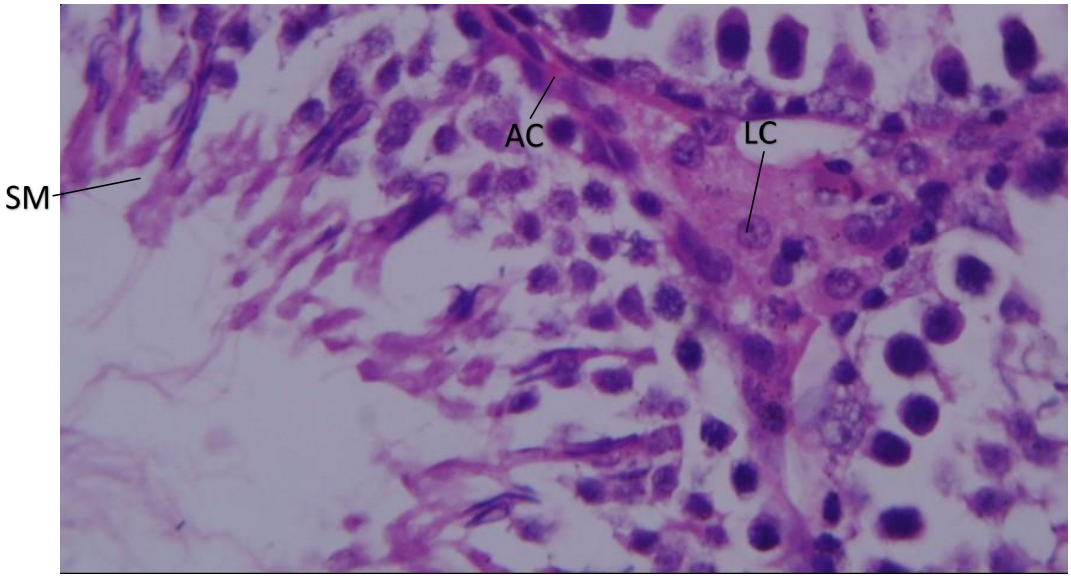

Plate 6. Rat testis given 300mg/kg *O. gratissimum* showing normal sperm -atogenic sequential maturation (SM), increased population of Leydig cells (LC), active interstitial congestion (AC): H&E x 400

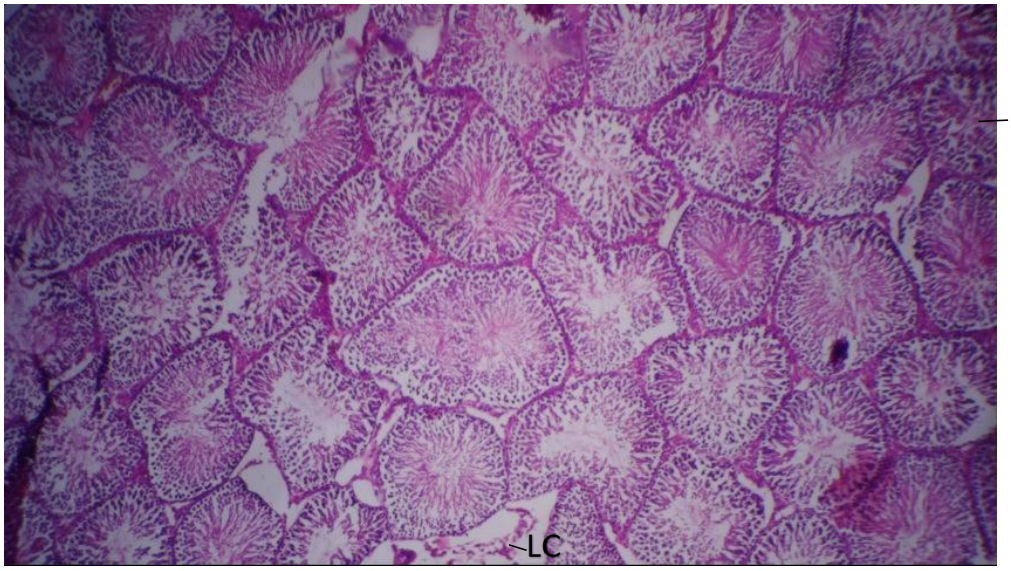

Plate 7. Rat testis given 500/kg *O. gratissimum* showing normal architecture: spermatogenic series in normal sequential maturation (SP), interstitial cells of Leydig (LC): H&E X40

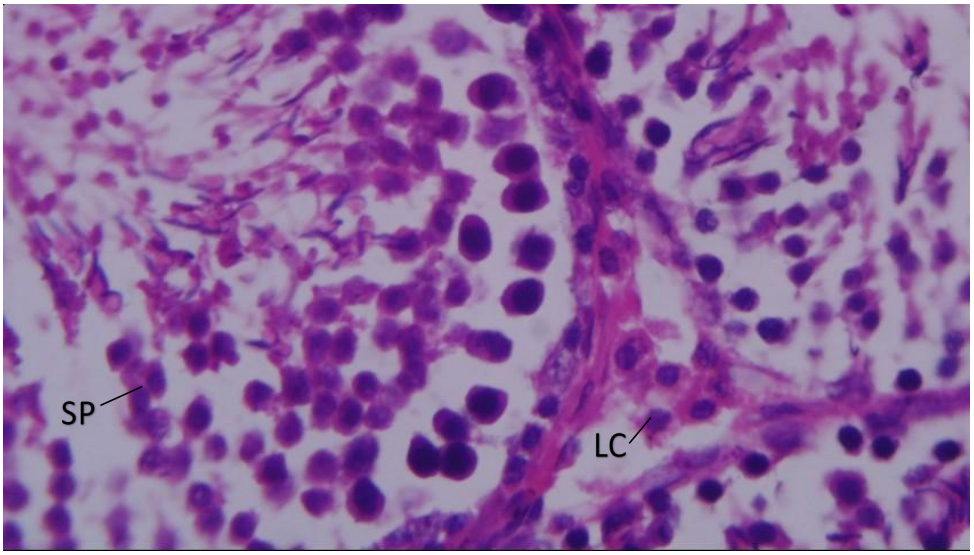

Plate 8. Rat testis given 500/kg *O. gratissimum* showing normal architecture: spermatogenic series in normal sequential maturation (SP), interstitial cells of Leydig (LC): H&E x 400

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study aimed to investigate the effects of *Occimim gratissimum* leaf extract on the testosterone levels of male Wistar rats. The results revealed interesting findings regarding the impact of different doses of the extract on testosterone production in these experimental groups. In this discussion section, the implications of these findings and provide insights into their potential mechanisms will be addressed.

5.2 Effects of *Occimim gratissimum* Leaf Extract on Testosterone Levels

The Control group served as the baseline, representing the normal testosterone levels in male Wistar rats. The average testosterone level in this group was 1.141 ng/ml (± 0.412), which aligns with the expected hormone concentration in the absence of any experimental treatment. This provides a reference point for evaluating the effects of the *Occimim gratissimum* leaf extract in line with the study carried out by Oguanobi *et al.,* (2012); Okoduwa *et al.,* (2017) and Shittu *et al.,* (2019).

In the Low Dose group, rats received a lower dose of the leaf extract (100 mg/kg). Notably, testosterone levels in this group significantly decreased to an average of 0.2617 ng/ml (± 0.09) compared to the Control group. This finding suggests that a lower dosage of *Occimim gratissimum* leaf extract may have a suppressive effect on testosterone production in male Wistar rats (Njan et al., 2019). While this result is intriguing, further investigations are required to elucidate the underlying mechanisms responsible for this decrease.

The Medium Dose group received an intermediate dosage of the extract (300 mg/kg), resulting in testosterone levels similar to those of the Control group (1.142 ng/ml ± 0.525).

This lack of a significant difference between the Medium Dose group and the Control group suggests that an intermediate dose of the extract does not markedly influence testosterone levels in male Wistar rats. This observation raises questions regarding the dose-response relationship and warrants additional exploration (Câmara et al., 2014).

In the High Dose group, rats received the highest dose of *Occimim gratissimum* leaf extract (500 mg/kg). Testosterone levels in this group averaged 0.3887 ng/ml (± 0.109), which was lower than in the Control group but higher than in the Low Dose group. This decrease in testosterone levels in the High Dose group, along with the significant difference observed compared to the Control group, suggests a dose-dependent effect. It appears that a higher dose of the extract also has a suppressive effect on testosterone production, similar to the low dose.

5.3 Histological Findings

Control Group

The testes in the Control Group exhibited a typical and healthy histological appearance. Seminiferous tubules were lined by a well-organized spermatogenic series (SP), representing the normal process of sperm development. Sertoli cells (SC) and interstitial cells of Leydig (LC) were present in their usual populations, indicating normal testicular function.

Rat Testis Given 100 mg/kg *O. gratissimum*

In this group, the testicular tissue still showed normal spermatogenic series (SP), suggesting that the process of sperm development was not severely affected by the extract. However, there was a notable increase in the population of Leydig cells (LC), and active interstitial congestion (AC) was observed. This suggests that the administration of 100 mg/kg of *Occimim gratissimum* leaf extract may have stimulated the Leydig cells and led to increased

blood flow within the interstitial tissue. These changes could potentially be related to alterations in testosterone levels, warranting further investigation.

Rat Testis Given 300 mg/kg *O. gratissimum*

Similar to the Control Group, this group displayed normal spermatogenic sequential maturation (SM). Leydig cell (LC) population remained increased, indicating a continued response to the extract. Active interstitial congestion (AC) persisted, further suggesting a dose-dependent relationship between the extract and changes in testicular interstitial tissue. It's important to note that despite these histological changes, the spermatogenic process remained intact.

Rat Testis Given 500 mg/kg *O. gratissimum*

The testes from this group showed no apparent disruption in spermatogenic series (SP). Leydig cells (LC) were observed in their typical population, without any significant increase. This suggests that the higher dosage of 500 mg/kg did not lead to further changes in the Leydig cell population. Overall, the testicular architecture appeared normal, indicating that the highest dosage did not disrupt the spermatogenic process or testicular tissue.

5.4 Mechanisms Underlying the Effects

The mechanisms underlying the observed effects of *Occimim gratissimum* leaf extract on testosterone levels in male Wistar rats remain unclear and require further investigation. Several factors may contribute to these effects, including potential phytochemicals within the extract that could interact with the endocrine system or impact testicular function. Understanding these mechanisms is crucial for a comprehensive assessment of the extract's safety and potential applications.

The histological findings suggest that the administration of *Occimim gratissimum* leaf extract at various dosages did not severely impair the normal spermatogenic process in male Wistar rats. This is a positive outcome, as the maintenance of normal testicular architecture is essential for male reproductive health.

The observed increase in Leydig cell population and active interstitial congestion in the groups receiving the extract raises intriguing questions. It suggests that the extract may have a stimulatory effect on Leydig cells and potentially influences blood flow within the testicular interstitial tissue. These changes may be indicative of an impact on hormone levels, particularly testosterone.

However, it's important to note that histological observations provide valuable anatomical insights but do not directly measure functional aspects or hormonal levels. Therefore, further research is needed to connect these histological changes with specific alterations in hormone production and to understand the potential implications for male reproductive function.

5.5. Conclusion

In conclusion, this study has provided valuable insights into the impact of *Occimim gratissimum* leaf extract on testosterone levels in male Wistar rats. The results suggest that the extract have a dose-dependent effect on testosterone production, with lower and higher doses leading to significant decreases compared to the control group. The intermediate dose did not appear to affect testosterone levels significantly.

These findings emphasize the need for further research to elucidate the mechanisms responsible for these effects and to determine the potential therapeutic or adverse implications of *Occimim gratissimum* leaf extract. Additionally, the study highlights the importance of carefully considering dosage when utilizing herbal extracts in clinical or experimental settings. Further studies with larger sample sizes and a focus on the specific

bioactive compounds in the extract may provide more in-depth insights into its effects on testosterone levels and overall male reproductive health.

REFERENCES

Adegoke, A. A. (2019). Reproductive toxicity and histomorphological changes in the testis and epididymis of Wistar rats treated with *Ocimum gratissimum* aqueous leaf extract. *Heliyon*, **5**(7), e02160.

Adegoke, O. A. (2011). In vitro anti-plasmodial activity and toxicological effect of stem bark extract and fractions of Alstonia boonei De Wild (Apocynaceae). *Journal of Ethnopharmacology,* **137**(2), 753-757.

Adeoye, A. O., & Ajiboye, B. O. (2018). Evaluation of in vivo antioxidant and reproductive potentials of Garcinia kola seeds in male Wistar rats. *Iranian Journal of Basic Medical Sciences,* **21**(10), 1061-1087.

Adewale, O. B., et al. (2017). Antioxidant, anti-inflammatory and hypolipidemic activities of *Carica papaya* leaf extract in male Wistar rats. *Journal of Intercultural Ethnopharmacology,* **6(**3), 320-327.

Afolabi, C. A., et al. (2020). Reproductive hormone modulation, sperm parameters and antioxidant activities of *Ocimum gratissimum* leaves extract in male Wistar rats. *Journal of Complementary and Integrative Medicine*, **17**(2), 20190134.

Ahmad, M. K., et al. (2010). *Withania somnifera* improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. *Fertility and Sterility*, **94**(3), 989-996.

Aitken, R. J., et al. (2016). Lifestyle choices and personal habits: The major modifiable risk factors for infertility. *Seminars in Reproductive Medicine*, **34**(5), 334-342.

Aitken, R. J., et al. (2018). Impact of sperm biology on clinical outcomes in ART. *Seminars in Cell & Developmental Biology,* **81**, 55-65.

Ajayi, B. O., (2017). Aphrodisiac potentials of the essential oil of *Ocimum gratissimum* in male Wistar rats. *Journal of Ethnopharmacology*, **196**, 14-19.

Anaga, A. O., et al. (2015). Histological changes in the testes and kidney of Wistar rats following administration of the leaf extract of *Ocimum gratissimum*. *Annals of Biological Research,* **6**(9), 37-40.

Bonde, J. P., et al. (2016). The risk of male infertility in relation to occupational exposures, semen quality, and reproductive hormones. *Danish Medical Journal*, **63**(4), B5222.

Chandler, D. W., et al. (2018). Male hypogonadism and fertility. Advances in Experimental *Medicine and Biology*, **1097**, 117-133.

Chukwurah, E. F., et al. (2021). Effect of *Ocimum gratissimum* Linn leaf extract on reproductive hormones and antioxidant status in male Wistar rats. *Journal of Herbmed Pharmacology*, **10**(1), 27-33.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycle and spermatogonial renewal. *Physiological Reviews*, **52**(1), 198-236.

de Andrade, E., et al. (2007). Study of the efficacy of Korean Red Ginseng in the treatment of erectile dysfunction. *Asian Journal of Andrology*, **9**(2), 241-244.

Dohle, G. R., et al. (2017). Infection and inflammation in male infertility: The role of leukocytes and their enzymes. *Andrologia,* **49**(6), e12614.

Du Plessis, S. S., et al. (2010). Obesity and male infertility: A practical approach. *Seminars in Reproductive Medicine*, **28**(4), 317-327.

Eisenberg, M. L., et al. (2014). The relationship between male hot tub use and semen parameters: A systematic review and meta-analysis. *Systems Biology in Reproductive Medicine,* **60**(2), 141-146.

Elberry, A. A., et al. (2017). Clinical efficacy of a combination of *Nigella sativa* seed and herbal extracts in men with asthenozoospermia. *Clinical Phytoscience*, **2**(1), 11.

Elekofehinti, O. O., et al. (2016). *Ocimum gratissimum* Linn Leaf Extracts Modulate Sperm Functional Parameters and Testosterone Level of Male Wistar Rats. *Journal of Acupuncture and Meridian Studies*, **9**(4), 195-201.

Ezeonwumelu, J. O. C., et al. (2014). Acute and sub-chronic toxicity studies of methanolic leaf extract of *Ocimum gratissimum* in male Wistar rats. *European Journal of Medicinal Plants*, **4**(7), 837-847.

Gauthaman, K., et al. (2002). Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): An evaluation using a rat model. *Journal of Alternative and Complementary Medicine,* **8**(4), 487-489.

Gonzales, G. F., et al. (2001). Effect of *Lepidium meyenii* (Maca) on sexual desire and its absent relationship with serum testosterone levels in adult healthy men. *Andrologia*, **34**(6), 367-372.

Gundersen, T. D., et al. (2015). Anabolic androgenic steroid abuse in young males. *Pharmacological Reviews*, **67**(2), 34-57.

Jurewicz, J., & Hanke, W. (2019). Exposure to environmental and lifestyle factors and sperm DNA damage—Review of clinical studies. *International Journal of Environmental Research and Public Health,* **16**(12), 2249.

Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. *Nature Reviews Urology*, **15**(6), 369-384.

Ma, Y., et al. (2018). Resveratrol improves sperm motility by reducing oxidative stress via PPARγ activation in boar spermatozoa. *Oncotarget,* **9**(12), 10525-10538.

Mahdi, A. A., et al. (2009). *Withania somnifera* Improves Semen Quality in Stress-Related Male Fertility. *Evidence-Based Complementary and Alternative Medicine,* **6**(3), 319-326.

Mahfouz, R. Z., et al. (2010). The effect of combination between herbal mixture (*Allium sativum, Glycyrrhiza glabra,* and *Foeniculum vulgare*) and ginger powder (Zingiber officinale) on sperm quality of New Zealand white rabbits. *American Journal of Animal and Veterinary Sciences*, 5(4), 286-291.

Oboh, G., et al. (2020). Modulatory effect of *Ocimum gratissimum* extract on fertility indices and sperm morphology in male rats. *Andrologia,* **52**(2), 47-56.

Ogbonnia, S. O., et al. (2013). Testicular function and sperm parameters of male Wistar rats treated with

Okoli, C. O., et al. (2007). Medicinal plants used for managing reproductive health care problems in Nigeria. Journal of Pharmacognosy and Phytotherapy, 1(1), 14-22.

Ong, H. S., et al. (2014). Traditional Chinese herbal medicine for erectile dysfunction: A systematic review. *The Journal of Sexual Medicine,* **11**(3), 481-494.

Owumi, S. E., et al. (2019). Evaluation of the toxicological effects of *Ocimum gratissimum* aqueous leaf extract on the reproductive system of male Wistar rats. *Toxicology Reports,* **6**, 279-284.

Rana, A. C., & Singh, R. (2013). Herbal medicine in the treatment of male infertility. *International Journal of Herbal Medicine*, **1**(3), 103-107.

Salami, S. A., et al. (2016). Effects of ethanolic leaf extract of *Ocimum gratissimum* on reproductive parameters in male Wistar rats. *African Journal of Traditional, Complementary, and Alternative Medicines,* **13**(6), 144-150.

Shin, B. C., et al. (2013). Maca (*L. meyenii*) for improving sexual function: A systematic review. *BMC Complementary and Alternative Medicine*, **10**(13), 1-6.

Showell, M. G., et al. (2014). Antioxidants for male subfertility. Cochrane Database of Systematic Reviews, (12), CD007411.

Tang, H., et al. (2017). Effects of curcumin on the proliferation and apoptosis of human sperm and oxidative stress-induced changes in human spermatozoa. *Frontiers in Pharmacology*, **8**, 772.