HISTOPATHOLOGICAL EFFECT OF *Cymbopogon citratus* LEAF EXTRACT ON THE REPRODUCTIVE ORGANS OF ALBINO RATS

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN, NIGERIA

SEPTEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN MEDICAL LABORATORY SCIENCE

SEPTEMBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **ONUMBA ADAORA JOAN** (MRS) with matriculation number: **BMS2005048** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DATE

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DEDICATION

This project is dedicated to Almighty God.

ACKNOWLEDGEMENTS

The completion of this project work is a testament to the invaluable support and guidance I've received from many individuals. First and foremost, my deepest gratitude goes to God! for his grace and mercy in my life. Then my family, Onumba Charles and Bunmi Gloria, for your unwavering love, encouragement, and the sacrifices you've made. Your belief in me has been a constant source of strength and motivation.

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ABSTRACT

Reproductive health disorders are increasingly prevalent due to environmental and dietary factors, necessitating investigation into natural therapeutic alternatives. This study aimed to examine the histopathological effects of *Cymbopogon citratus* (lemongrass) leaf extract on the reproductive organs of albino rats. Twenty-four healthy albino rats weighing 180-200g were divided into four groups: Group A (control, n=2) received pellets and distilled water; Groups B, C, and D (n=4 each) were administered 250mg/kg, 500mg/kg, and 1000mg/kg of *Cymbopogon citratus* leaf extract respectively via oral gavage for one month. After treatment, animals were euthanized, blood samples collected for hormonal analysis using ELISA kits, and reproductive organs harvested for histopathological examination using hematoxylin and eosin staining. Results revealed normal ovarian histomorphology across all groups, with follicles containing oocytes surrounded by theca and granulosa cells at different maturation stages. Most testicular sections showed normal seminiferous tubules with Sertoli cells and sperm cells at various maturation stages. However, Group C1 exhibited testicular atrophy characterized by shrunken seminiferous tubules with thickened basement membranes and reduced germ cell populations. Significant hormonal changes were observed in Group B, showing significant differences in both testosterone and progesterone levels compared to controls ($p < 0.05$). No significant changes were observed in organ weights or full blood count parameters across all groups. The findings suggest that *Cymbopogon citratus* leaf extract exhibits dose-dependent effects on reproductive tissues, with moderate doses potentially causing testicular atrophy and hormonal alterations. While the extract demonstrated minimal effects at low and high doses, the intermediate dose showed adverse effects, highlighting the importance of dosage optimization in herbal medicine applications for reproductive health.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background of Study

Lemongrass (*Cymbopogon citratus*) is a tall, perennial grass indigenous to tropical regions, widely utilized in culinary applications, traditional medicine, and as a source of essential oils (Wadhwa *et al*., 2023). In recent years, the pharmacological properties of lemongrass have been increasingly documented, indicating various health benefits, including antimicrobial, antioxidant, and anti-inflammatory effects (Nambiar and Matela, 2022; Nagy *et al*., 2023; Wadhwa *et al*., 2023). These attributes are largely attributed to the presence of active phytochemical compounds, such as citral, geranial, and neral, which are known for their pharmacological activities (Iamba and Yaubi, 2021; Wadhwa *et al*., 2023).

Research focusing on the reproductive health effects of herbal extracts is of significant interest, particularly due to the increasing prevalence of reproductive disorders linked to environmental and dietary factors. Previous studies have posited that natural compounds may mitigate the adverse effects of such factors on reproductive health (Igwe *et al*., 2024). Studies examining the influence of lemongrass on general health metrics demonstrate a need for investigation into its specific effects on reproductive organs in animal models. Preliminary studies have indicated that lemongrass extracts can exert protective effects against nephrotoxicity and possess various biological activities such as enhancing immune function and reducing oxidative stress (Igwe and Okelue, 2024).

Research has shown that the physiological and biochemical mechanisms underlying reproductive health can be adversely affected by oxidative stress, a condition exacerbated by high levels of free radicals in the body (Nambiar and Matela, 2022).

1.2 Statement of Problem

The existing body of literature reveals a gap regarding the specific impacts of lemongrass on reproductive systems, particularly in male and female albino rats. While there has been significant research into the general health benefits of lemongrass (Wadhwa *et al*., 2023*)*, studies focusing on its impact on reproductive organs have been sparse. Previous studies have either investigated its antibacterial properties or its protective effects against different types of toxicity without delving into its specific effects on reproductive health parameters (Nambiar and Matela, 2022).

For instance, while Haggag's study highlights the nephroprotective effects in male rats, implications for reproductive health have not been exhaustively explored (Haggag, 2015). Further complications arise due to variations in dosage, preparation methods (fresh versus extract*)*, and species differences, which may influence bioactive compound efficacy (Olukunle and Adenola, 2019). This research proposal endeavors to bridge this knowledge gap by systematically evaluating the effects of lemongrass on the reproductive organs of male and female albino rats, thereby contributing to a better understanding of potential therapeutic applications.

1.3 Justification of the study

Cymbopogon citratus is widely utilized in various cultures for its culinary and medicinal properties. The plant is particularly valued for its antioxidant properties derived from its high content of flavonoids and other phytochemical compounds, which can exert protective effects against oxidative stress—an established contributor to reproductive toxicity (Igwe and Okelue, 2024). While preliminary studies have indicated the potential for lemongrass extracts to preserve reproductive function and mitigate damage in male and female albino rats (Igwe *et al*., 2024*)*, there are still significant gaps in systematic evaluations of its direct effects on reproductive organs. Understanding these effects is critical, especially in light of the increasing exposure to environmental pollutants that can compromise reproductive health (Al-Husseini *et al*., 2022). This research aims to elucidate the cytoprotective effects of lemongrass on reproductive organs, providing essential data that could contribute to the development of plant-based therapeutic strategies in reproductive health management. Moreover, it reinforces the growing body of knowledge advocating for the use of herbal remedies in modern medicinal practices, highlighting the potential of lemongrass as a viable, natural intervention to enhance reproductive wellbeing (Igwe and Okelue, 2024).

1.4 Significance of Study

The significance of this study lies in its potential to inform both scientific and public health domains regarding the role of natural remedies in enhancing reproductive health. Given the increasing reliance on chemical interventions, exploring herbal alternatives is crucial for developing holistic health strategies (Olukunle and Adenola, 2019). Furthermore, by identifying the protective effects of lemongrass, the study could pave the way for subsequent research into safe, effective, and affordable treatments for reproductive health issues.

This study aligns with similar research that emphasizes the importance of traditional herbal medicine in addressing contemporary health problems. Research has shown that certain plant extracts can improve fertility parameters in various animal models (Taghyan *et al*., 2020). For example, recent studies demonstrated significant positive outcomes related to reproductive health through the use of natural compounds (Wodi *et al*., 2023). Understanding lemongrass's impact could yield promising natural solutions and therapeutic options for addressing reproductive system dysfunction.

1.5 Aim of study

This study aims to examine the histopathological effect of *Cymbopogon citratus* plant extract on the reproductive organs of albino rats.

1.6 Specific Objectives of the study

The specific objectives of this study are to:

to carry out extraction of *Cymbopogon citratus* leaves

1. Determine the histopathological effect of *Cymbopogon citratus* leaves extract on the histomorphology of the testis, prostate, ovaries, and uterus of albino rats.
2. Investigate the effect of *Cymbopogon citratus* leaves extract on the reproductive hormones of albino rats.

1.7 Research Questions

1. Does the consumption of *Cymbopogon citratus* leaves extract affect the morphology of the testis, prostate, ovaries, and uterus of albino rats?
2. Does the consumption of *Cymbopogon citratus* leaves extract impact the reproductive hormones of albino rats?

1.8 Research Hypothesis

Cymbopogon citratus plant extract affects the reproductive organs and hormones of albino rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Cymbopogon citratus* (Overview)

Cymbopogon citratus, commonly referred to as lemongrass, belongs to the Kingdom Plantae, Family Poaceae, and Genus Cymbopogon. This perennial plant is characterized by its tall, green, grass-like structure with a distinct lemon fragrance, primarily attributed to the essential oil it contains. Taxonomically, it is classified under the Latin name *Cymbopogon citratus Stapf*. Native to tropical regions of Asia, particularly India and Southeast Asia, lemongrass has been introduced and cultivated globally across tropical and subtropical climates for both culinary and medicinal purposes. In addition to its native regions, it has spread to parts of Africa, the Caribbean, and South America due to its popularity as a flavoring agent and herbal remedy (Wadhwa *et al*., 2023).

The environmental adaptability of *Cymbopogon citratus* contributes significantly to its widespread cultivation. It thrives in well-drained soils and prefers full sunlight, demonstrating resilience to varying moisture conditions. The ability to grow in diverse climates has made it a common sight in home gardens and commercial plantations alike, reinforcing its role in traditional medicine and culinary arts (Omotayo *et al*., 2024). This geographical distribution forms the basis for understanding the ethno-botanical significance of lemongrass, enhancing its relevance in both regional and international contexts.

Figure 2.1: ***Cymbopogon citratus*** **Plant (Tatiana, 2024).**

2.2 Nutritional and Phytochemical Profile

Cymbopogon citratus is not only praised for its flavor but also for its nutritional profile, which includes an array of macronutrients such as proteins, carbohydrates, and fibers. Various studies have identified specific phytochemical constituents that contribute to the medicinal properties of this plant. Notably, it contains flavonoids, which serve as free radical scavengers, and essential oils rich in citral, which possesses antimicrobial and anti-inflammatory properties (Wadhwa *et al*., 2023).

Research shows that the leaves of *Cymbopogon citratus* are particularly rich in flavonoids and other phenolic compounds, which play significant roles in antioxidant activities and contribute to the plant's therapeutic potential. For example, compounds like quercetin and kaempferol have been documented, highlighting their antioxidant capabilities and potential health implications (Shah *et al*., 2011). The essential oil extracted from lemongrass has garnered attention due to its unique chemical profile, which includes a substantial concentration of citral, making it a subject of interest for its antioxidant, antibacterial, and potential anticancer activities (Omotayo *et al*., 2024).

Furthermore, research by Mohammed *et al*. confirmed the antimicrobial activities of lemongrass, which support its incorporation in dietary supplements and traditional medicine, suggesting a preventive approach to various health issues, including infections (Mohammed *et al*., 2020). These existing findings provide a backdrop for further exploration of its benefits, showcasing the multifaceted roles of *Cymbopogon citratus* as both a nutritional and therapeutic plant.

2.3 Traditional Uses and Biological Activities

Historically, *Cymbopogon citratus* has been utilized in traditional medicine across various cultures, where it has been esteemed not only as a culinary herb but also as a remedy for various health issues. Its reported biological activities are extensive, ranging from antioxidant, anti-inflammatory, and antimicrobial properties to its emerging role in health improvement. Various studies have documented the plant's applications in managing digestive issues and respiratory conditions, and its usage as a calming agent (Shah *et al*., 2011).

Figure 2.2: ***Cymbopogon citratus*** **Plant (Tatiana, 2024).**

Significantly, recent investigations indicate potential benefits for reproductive health, such as improving spermatogenesis and modulating hormonal balances in male subjects, potentially mitigating reproductive toxicity from external agents (Wadhwa *et al*., 2023). Research employing animal models has shown that extracts of *Cymbopogon citratus* can ameliorate reproductive impairments induced by toxic substances, revealing a protective effect attributed to its antioxidant properties (El-din, 2023). Such protective roles are critical for addressing concerns related to health in toxicological contexts, particularly with increasing exposure to environmental and pharmaceutical toxins.

As the body of literature evolves, the traditional uses of *Cymbopogon citratus* are increasingly being supported by scientific evidence, underscoring the necessity for further investigations. These studies will clarify the biochemical mechanisms at play and enhance understanding of lemongrass's role as a therapeutic agent in various health applications.

2.4 Phytochemical Constituents

2.4.1 Active Compounds in *Cymbopogon citratus* Extract

Cymbopogon citratus, commonly known as lemongrass, is rich in a variety of phytochemicals that contribute to its therapeutic properties. Key constituents include flavonoids, alkaloids, terpenoids, and tannins, each of which plays a significant role in the plant's biological activities.

1. Flavonoids

Flavonoids are renowned for their role as antioxidants and free radical scavengers. They possess water-soluble properties that allow them to combat oxidative stress within reproductive tissues. Oxidative stress is known to initiate cellular damage, which can lead to compromised reproductive functions. The antioxidative activity of flavonoids helps protect against lipid peroxidation and DNA damage, thereby preserving the integrity of reproductive cells (Sousa *et al*., 2021).

Moreover, flavonoids have been demonstrated to exert anti-inflammatory effects, which is particularly beneficial for reproductive health. Inflammation can adversely affect testicular and ovarian function, potentially leading to fertility issues. By modulating inflammatory pathways, flavonoids contribute to the stabilization of reproductive tissues, offering a protective mechanism against potential stressors such as toxins and oxidative damage (Mishra *et al*., 2024).

2. Alkaloids and Terpenoids

Alkaloids and terpenoids present in *Cymbopogon citratus* also play critical roles in its medicinal properties. Alkaloids have been linked to various biological activities, including modulation of neurotransmitter systems and potential anti-inflammatory effects. However, the specific influence of *Cymbopogon citratus* alkaloids on reproductive endocrine functions is less well-established and may require further investigation (Johnson *et al*., 2021).

Additionally, terpenoids, the primary constituents of lemongrass essential oil, have shown valuable pharmacological properties. These compounds are recognized for their ability to modulate inflammatory responses and mitigate oxidative damage. For instance, essential oils derived from Cymbopogon species have been reported to reduce pro-inflammatory cytokine production, thus highlighting their potential use in managing inflammation-related reproductive disorders (Manh *et al*., 2020).

3. Tannins

Tannins, another significant group of phytochemicals found in *Cymbopogon citratus*, contribute to the plant's protective effects on reproductive tissues. These polyphenolic compounds are known for their astringent properties and ability to stabilize cell membranes, which can help preserve cellular integrity and function (Sousa *et al*., 2021).

Tannins have also been shown to counteract oxidative stress by scavenging free radicals and reducing inflammation. By mitigating these damaging factors, tannins play a protective role in maintaining the health of reproductive cells (Sousa *et al*., 2021). Their antioxidant capabilities can significantly reduce the risk of cellular degeneration, thereby promoting better reproductive health outcomes.

2.4.2 Interactions and Synergistic Effects

The combined presence of these phytochemicals in *Cymbopogon citratus* leads to cumulative effects that enhance its overall impact on reproductive health. Research suggests that the synergistic interactions among flavonoids, alkaloids, terpenoids, and tannins can produce more potent biological activities than when these compounds are taken individually (Zhang *et al*., 2019).

For example, a study confirmed that synergistic actions of phytochemicals could enhance their anti-inflammatory properties beyond individual capacities, emphasizing the significance of natural mixtures in therapeutic applications (Zhang *et al*., 2019). Such interactions are essential for understanding the biochemical rationale behind the protective effects of *Cymbopogon citratus* against oxidative stress and inflammation, particularly in the context of reproductive health.

The diverse array of phytochemicals present in *Cymbopogon citratus* contributes to its health benefits, particularly through antioxidative and anti-inflammatory mechanisms. Future research should continue to explore these interactions and their implications for enhancing reproductive health.

2.5 Overview of the Reproductive System

2.5.1 Gross Anatomy of the Reproductive Organs

The reproductive system of mammals consists of distinct yet interconnected structures in both male and female organisms, which contribute to the complex processes of reproduction. In males, the primary organs include the testes, epididymis, vas deferens, seminal vesicles, prostate gland, and penis. The testes, responsible for spermatogenesis, are typically located in the scrotum, which provides a cooler environment essential for sperm development (Obukhowo *et al*., 2021). Each testis is composed of numerous seminiferous tubules where spermatozoa are produced. The epididymis is a coiled duct that facilitates the maturation and storage of sperm (Obukhowo *et al*., 2021).

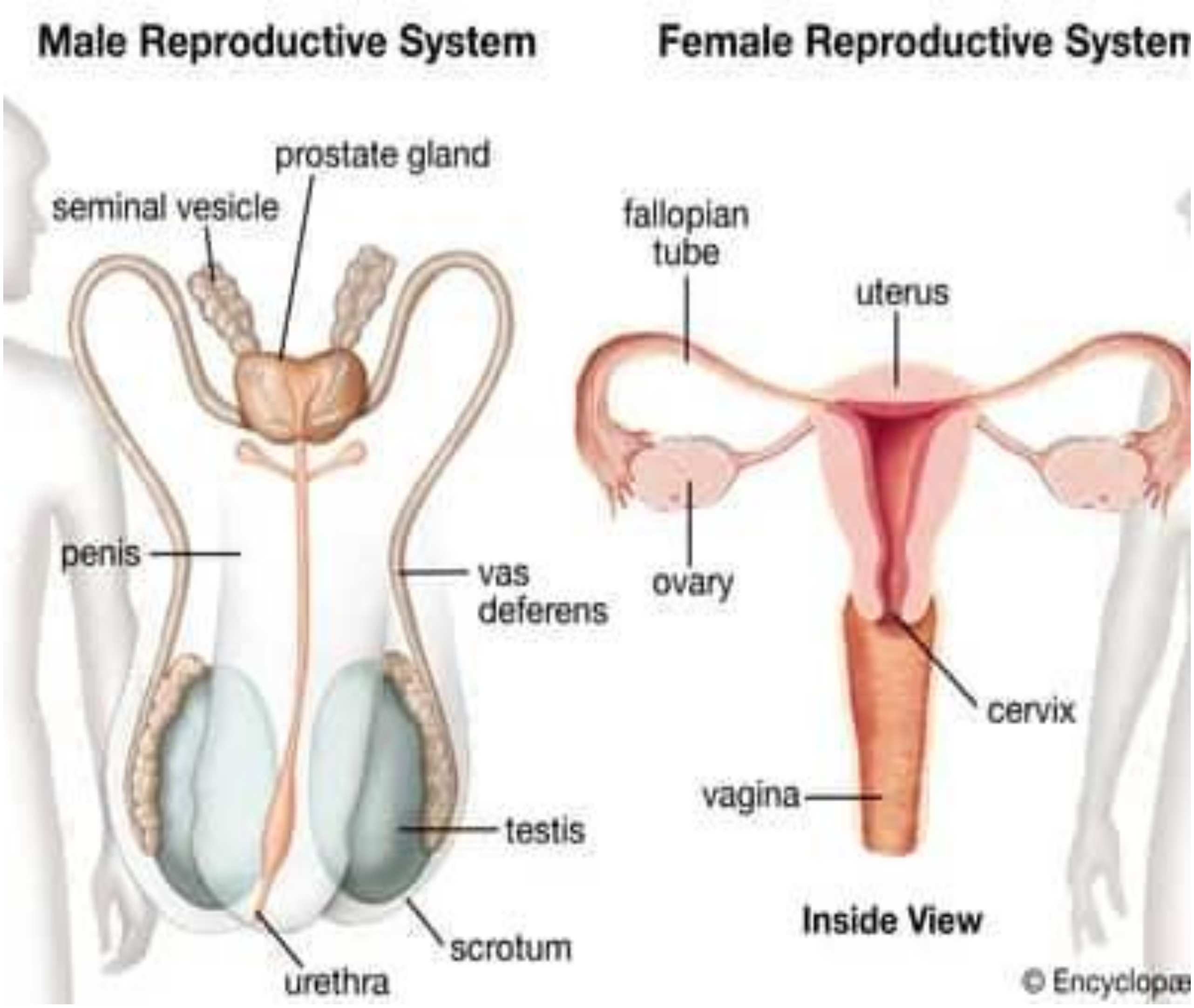

Figure 2.3**: Organs and structures of the male and female reproductive systems (Utiger, 2025).**

Accessory glands, such as the seminal vesicles and prostate, produce fluids that nourish and transport sperm. Seminal fluid, which constitutes a significant portion of semen, helps to neutralize the acidity of the female reproductive tract, enhancing sperm viability and motility (Obukhowo *et al*., 2021). The male reproductive system's anatomical organization underscores its evolved efficiency in ensuring successful fertilization.

In contrast, the female reproductive system comprises structures including the ovaries, fallopian tubes, uterus, and vagina. The ovaries serve multiple functions such as the production of oocytes and the secretion of hormones like estrogen and progesterone, which are crucial for regulating the menstrual cycle and maintaining pregnancy (Igwe *et al*., 2024). The uterus, whose inner lining thickens in preparation for possible implantation of a fertilized egg, contains muscular walls that contract during childbirth. The fallopian tubes facilitate the transport of oocytes from the ovaries to the uterus and are typically the site of fertilization (Igwe *et al*., 2024).

2.5.2 Histology of the Reproductive Organs

Histological studies of the male and female reproductive systems reveal the specialized structures essential for their functions. In the testis, the histological organization comprises seminiferous tubules lined with germ cells and Sertoli cells, which are crucial for sperm maturation (Obukhowo *et al*., 2021). Leydig cells, located in the interstitial space, secrete testosterone, an important androgen responsible for the development of male secondary sexual characteristics and optimal spermatogenesis (Obukhowo *et al*., 2021).

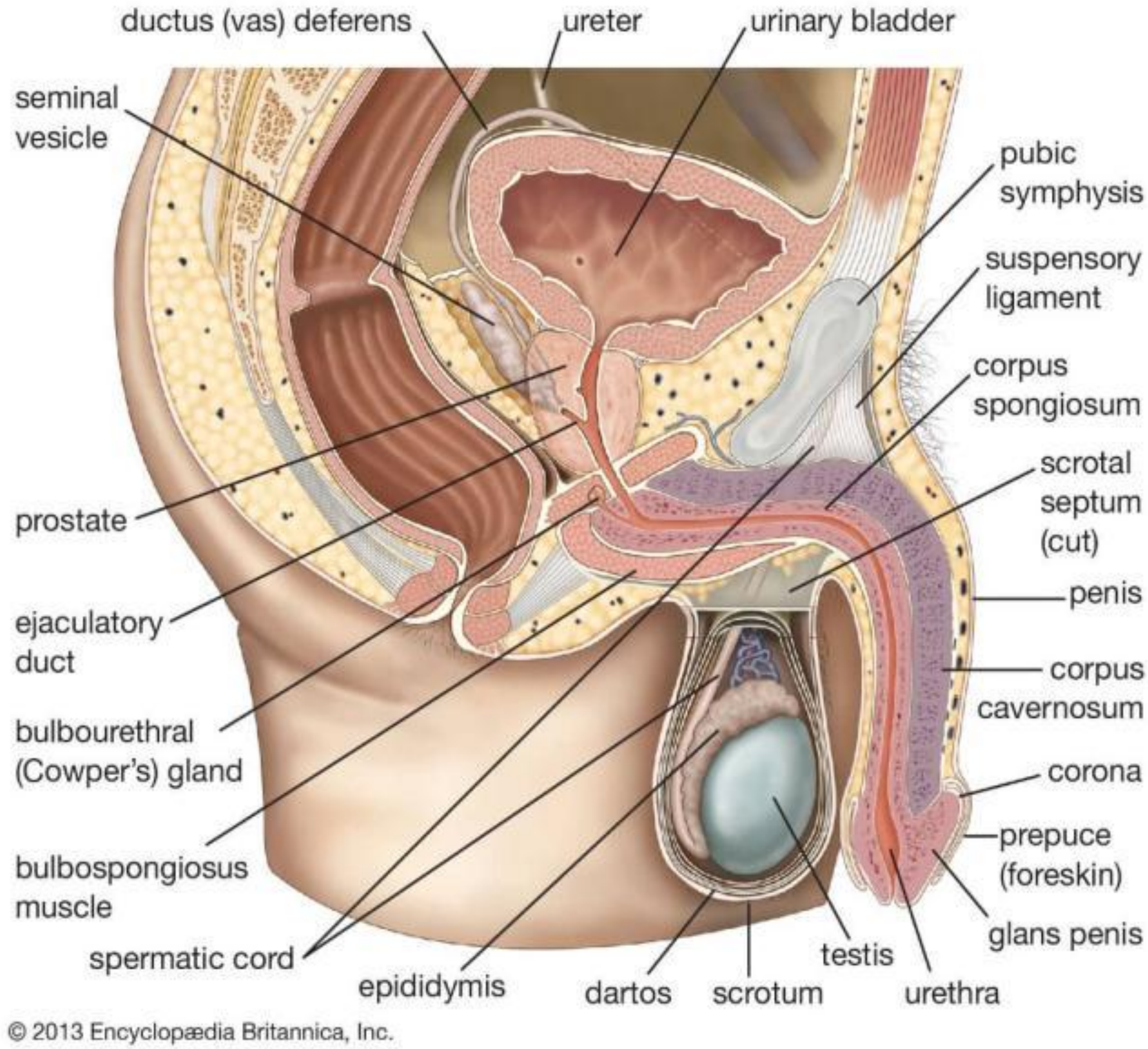

Figure 2.4: Organs of the male reproductive system (Utiger, 2025).

In females, the ovaries' histological structure showcases several follicles at different maturation stages, from primordial follicles to antral and Graafian follicles. Each follicle contains an oocyte surrounded by a layer of granulosa cells that provide support and produce hormones contributing to the regulation of the menstrual cycle (Igwe *et al*., 2024). The uterus is lined with a dynamic endometrium that undergoes cyclical changes in response to hormonal stimulation, preparing for potential implantation. These variations in histological structure and cellular organization emphasize the functional zonation found within these respective organs.

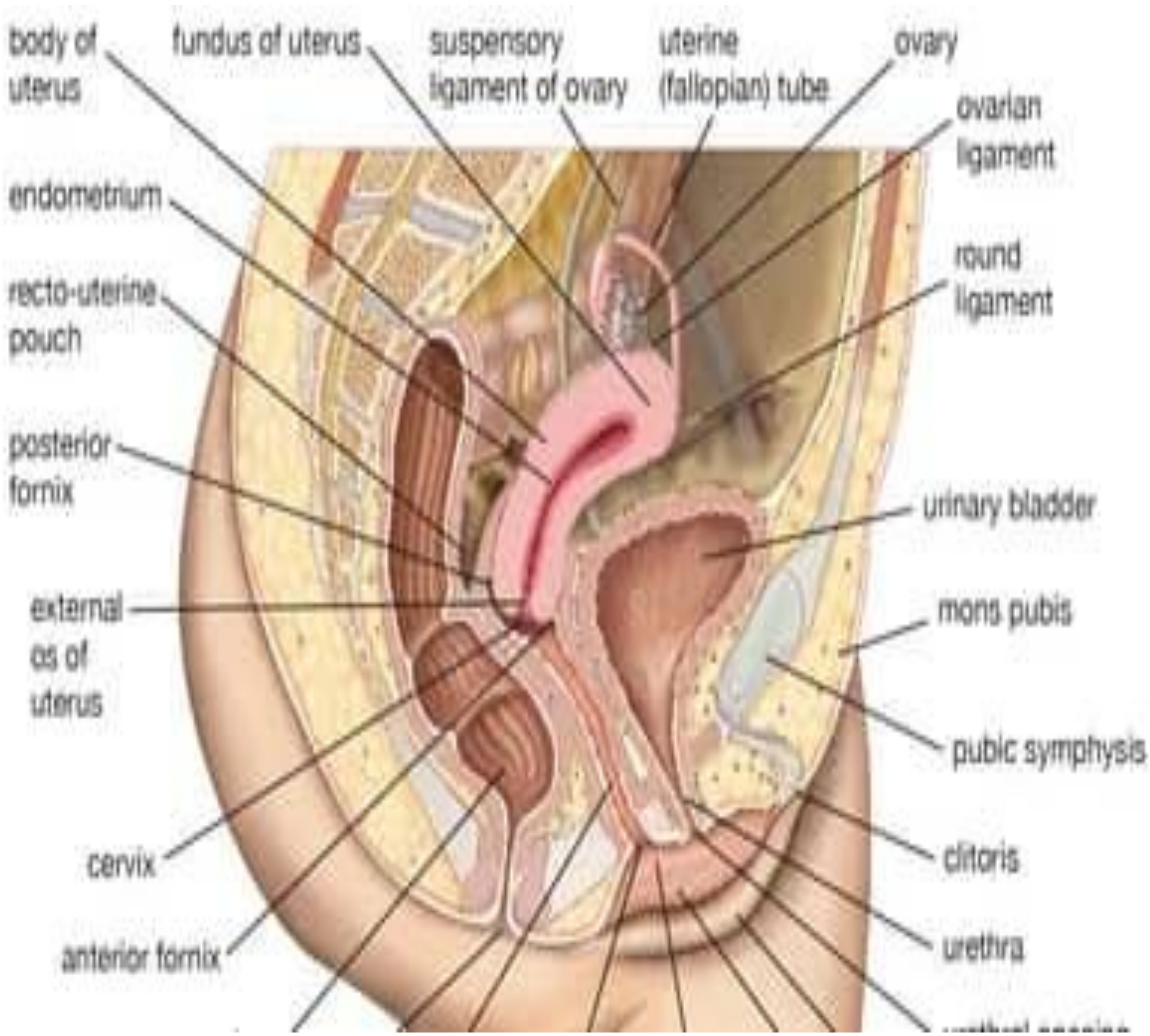

Figure 2.5: Organs of the Female reproductive systems (Utiger, 2025).

2.6 Endocrine Regulation and Functional Physiology

The complex interplay of hormones regulates the physiological functions of the reproductive system in both males and females. In males, testosterone plays a pivotal role, influencing behaviors, spermatogenesis, and the development of secondary sexual characteristics (Obukhowo *et al*., 2021). Hormonal regulation begins with gonadotropin-releasing hormone (GnRH) from the hypothalamus, which stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH promotes testosterone synthesis from Leydig cells, while FSH facilitates spermatogenesis through its action on Sertoli cells (Obukhowo *et al*., 2021)

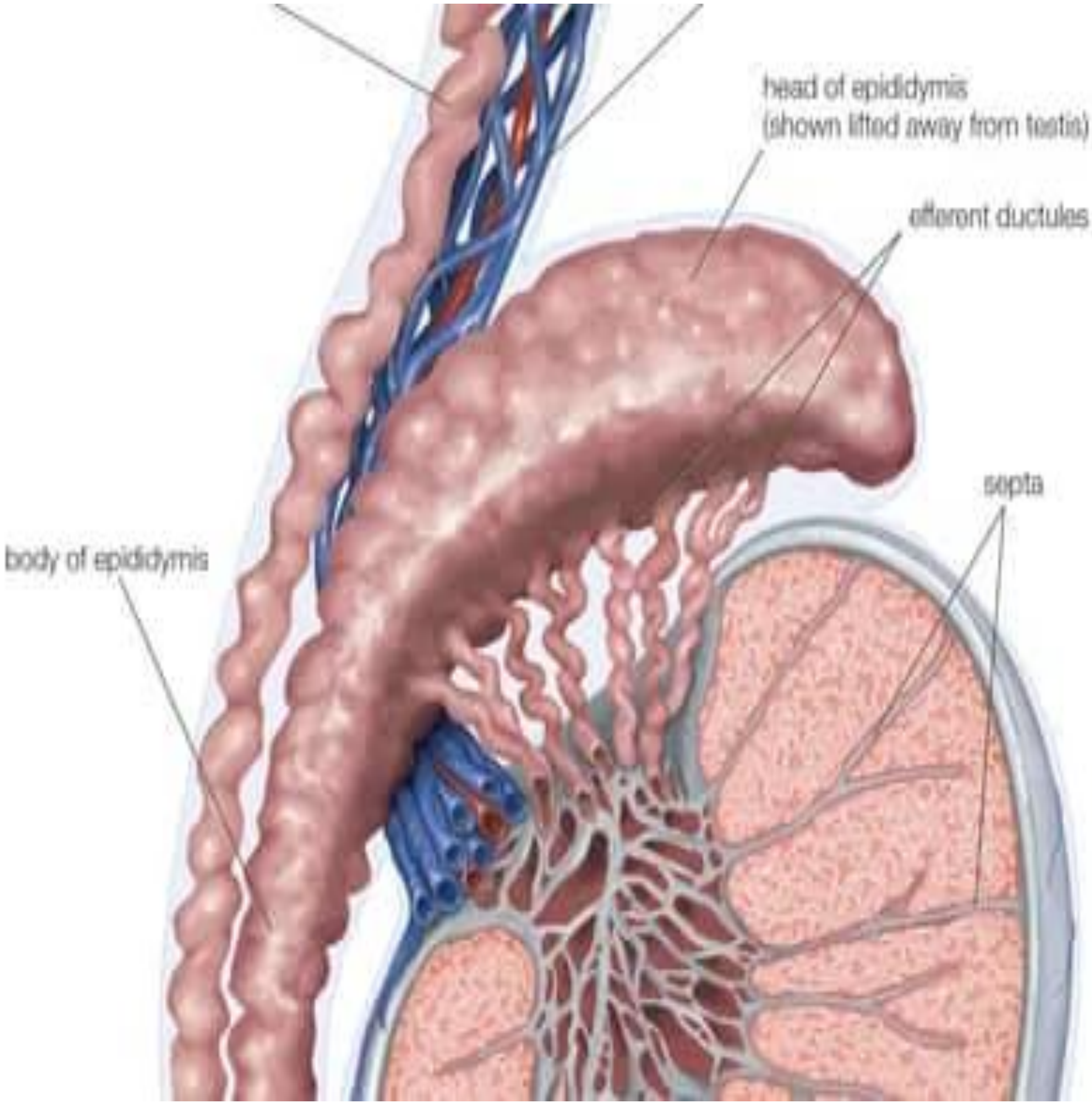

Figure 2.6: Human male testis, epididymis, and ductus deferens through the long seminiferous tubules and stored in the epididymis of the testes until it is ready to leave the male body. (Utiger, 2025).

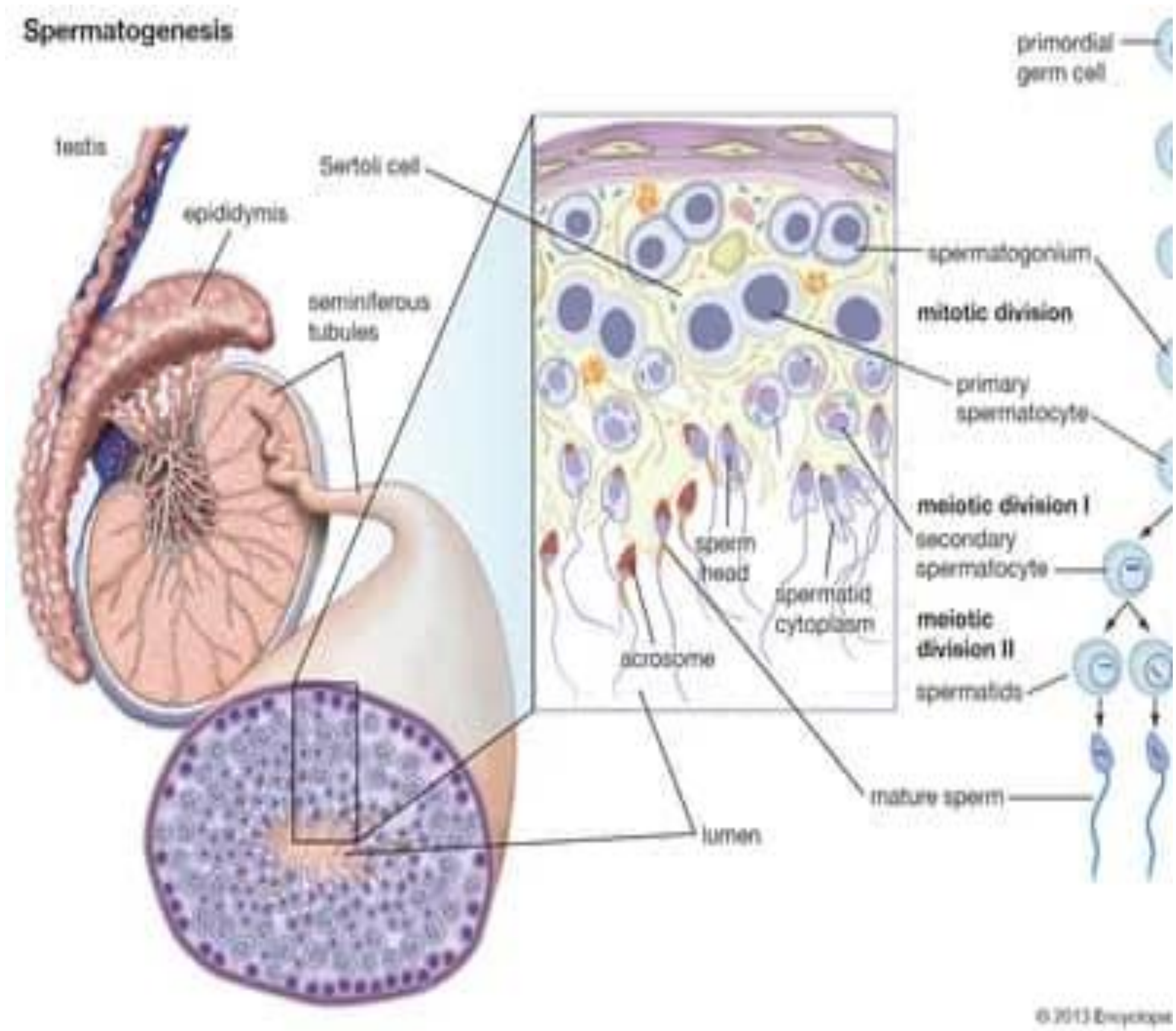

Figure 2.7: Human male testis, epididymis, and ductus deferens through the long seminiferous tubules and stored in the epididymis of the testes until it is ready to leave the male body. (Utiger, 2025).

In females, the cyclical release of hormones orchestrates the processes of ovulation and menstruation. FSH stimulates ovarian follicle development and estrogen production, while LH triggers ovulation (Igwe *et al*., 2024). The interplay between estrogen and progesterone is fundamental for preparing the endometrium for implantation and maintaining pregnancy. Furthermore, the effects of external agents, including phytochemicals such as those in *Cymbopogon citratus,* can modulate these hormonal pathways, influencing both spermatogenesis and ovulation. Some recent investigations have pointed to the potential impact of flavonoids and other compounds found in lemongrass on hormonal levels and reproductive function, indicating possible applications in reproductive health (Igwe *et al*., 2024).

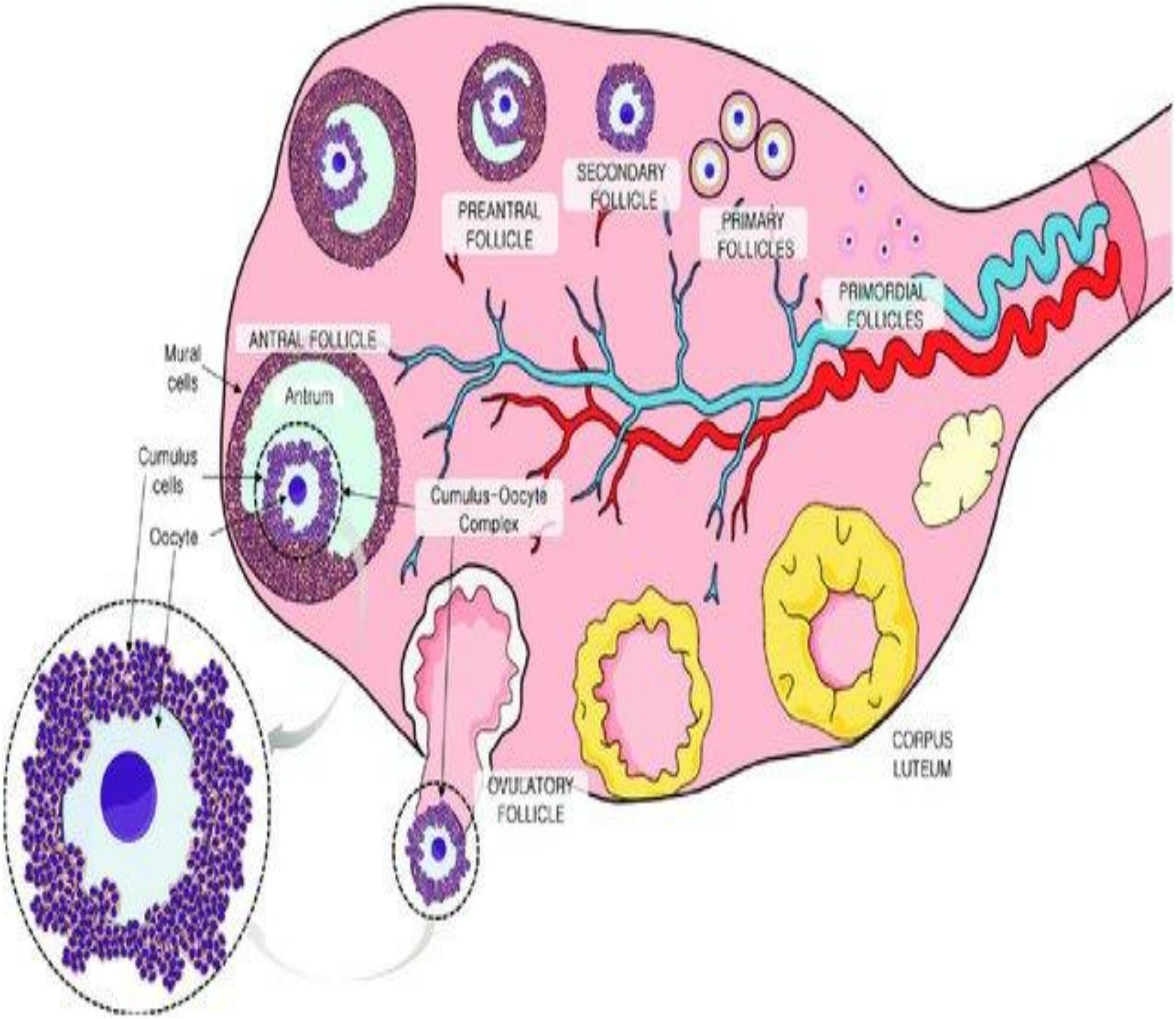

Figure 2.8: Ovulatory cycle (Utiger, 2025)

Ultimately, hormonal pathways are integral in governing the proliferation and differentiation of reproductive cells. Understanding these intricate hormonal interactions offers insight into the physiological effects of herbal remedies like *Cymbopogon citratus* on reproductive health, by investigating how these compounds can influence reproductive function and mitigate impairments caused by toxic substances or environmental stressors (Igwe *et al*., 2024).

2.7 Histomorphological Effects on the Reproductive System

2.7.1 Normal Histomorphology versus Toxic Alterations

The normal histological architecture of the reproductive system is vital for its proper functionality. In the male reproductive system, the testes are comprised of seminiferous tubules, where spermatogenesis occurs. Within these tubules, Sertoli cells support and nourish developing sperm, while Leydig cells located in the interstitial spaces produce testosterone, which regulates the growth and development of male reproductive tissues (Anna and Tony, 2020). The organization of these cells and the specific structure of the testis are crucial for fertility.

In contrast, the female reproductive system features ovarian follicles, where oocytes develop and mature. Each follicle is surrounded by granulosa cells that facilitate hormonal regulation within the female reproductive cycle. The uterus has a distinct endometrial lining, which undergoes cyclical changes in preparation for possible implantation of a fertilized embryo (Igwe and Okelue, 2024).

However, exposure to various toxic agents can lead to significant histopathological changes in these organs. In studies involving male rat models, exposure to high doses of *Cymbopogon citratus* extracts resulted in degeneration of spermatogenic cells, leading to impaired spermatogenesis and consequently reduced sperm quality (Anna and Tony, 2020). Such alterations can also be characterized by the presence of fibrosis, inflammatory infiltrates, and necrotic changes within the cellular architecture. Degenerative lesions in the seminiferous tubules disrupt both the structural and functional integrity of testicular tissues, thus negatively impacting fertility.

2.7.2 Role of Oxidative Stress and Inflammatory Mediators

Oxidative stress, driven by the excess production of reactive oxygen species (ROS*)*, poses a considerable threat to the cellular integrity of reproductive tissues. Elevated ROS levels can lead to lipid peroxidation, protein oxidation, and DNA damage, all of which compromise cellular function and viability (Aitken *et al*., 2022). The male reproductive system is particularly vulnerable, where oxidative stress can initiate apoptotic pathways in germ cells and Leydig cells, further exacerbating infertility issues (Aitken *et al*., 2022).

Research has shown that antioxidant-rich extracts, such as those derived from *Cymbopogon citratus*, can reverse oxidative damage. In one study, treatment with this extract demonstrated a significant reduction in oxidative damage parameters and preserved spermatogenic activity in male rats exposed to oxidative stress via hydrogen peroxide (Anna and Tony, 2020). This emphasizes the potential for using natural antioxidants to ameliorate reproductive toxicity and restore normal histological architecture.

Moreover, the inflammatory response often correlates with oxidative stress as cytokines and other mediators are released, leading to further cellular damage and dysfunction. Therefore, mitigating oxidative stress through antioxidant therapies not only preserves normal histomorphological characteristics but can also reduce inflammatory responses, promoting overall reproductive health (Jovanović *et al*., 2023).

2.7.3 Dose-Dependent Effects and Thresholds of Toxicity

The impact of phytoextracts, such as those from *Cymbopogon citratus*, on reproductive tissues can exhibit dose-dependent effects. Studies indicate that low to moderate doses may offer protective benefits, enhancing reproductive function through antioxidant mechanisms (Igwe and Okelue, 2024). Conversely, higher doses can lead to adverse outcomes characterized by significant histopathological alterations in testicular and ovarian tissues.

For instance, the administration of high doses (e.g., 1000 mg/kg body weight) of *Cymbopogon citratus* extracts resulted in degenerative changes and loss of spermatogenic cells in the testes of studies involving male rats (Anna and Tony, 2020). This accentuates the critical importance of understanding thresholds of toxicity, as overstimulation or damage from excessive doses can outweigh the potential therapeutic benefits.

2.8 Effects of *Cymbopogon citratus* on the Reproductive System

2.8.1 Experimental Studies in Animal Models

Cymbopogon citratus, commonly known as lemongrass, has been studied for its effects on reproductive health in various animal models, particularly in rats. Research has indicated that the application of *Cymbopogon citratus* extracts can significantly impact reproductive indices such as organ weights, hormone levels, and histological parameters of reproductive tissues. In experiments designed to evaluate these effects, male albino rats exposed to graded doses of lemongrass demonstrated altered serum testosterone levels and reproductive organ weights when compared to control groups (Igwe *et al*., 2024).

In a study conducted by El-Din in 2023, male rats treated with lemongrass extracts exhibited a notable decrease in serum total cholesterol and an increase in high-density lipoprotein (HDL-C) levels. These changes correlated with improved reproductive performance, as reflected in histological analyses showing an increase in the number of viable spermatogenic cells and a decrease in histopathological abnormalities, such as degeneration and necrosis in the testes (El-Din, 2023). This protective effect includes the histological preservation of seminiferous tubules, which are essential for normal spermatogenesis (El-Din, 2023).

Conversely, higher doses of lemongrass extracts have been associated with signs of toxicity and adverse histopathological changes, such as significant histopathological deterioration and a marked reduction in sperm count (Manh *et al*., 2020). These findings illustrate the necessity of balancing efficacy and safety when administering herbal extracts, which can exhibit varying effects depending on the dosage range.

2.8.2 Mechanisms of Action

The underlying mechanisms of *Cymbopogon citratus*'s reproductive effects involve multiple biological pathways primarily associated with its antioxidant properties. The extract is rich in flavonoids and phenolic compounds, which have been shown to enhance antioxidative defenses in reproductive tissues (Igwe *et al*., 2024). These compounds act by scavenging free radicals and reducing oxidative stress, thereby protecting reproductive cells from damage induced by toxic agents.

El-Din in 2023 highlighted that the antioxidant activity of lemongrass might significantly be attributed to its ability to modulate key signaling pathways associated with oxidative stress. The research indicates that co-administration of *Cymbopogon citratus* with other protective agents, such as coenzyme Q10, results in synergistic effects that further enhance the organ's resilience against reproductive toxicity (El-Din, 2023). Coenzyme Q10, known for its role in mitochondrial energy production and antioxidative protection, may work synergistically with the phytochemicals found in lemongrass to improve the overall reproductive health and function of treated organisms.

Moreover, studies suggest that polyphenolic compounds derived from lemongrass can inhibit inflammatory mediators, such as cytokines, which contribute to local tissue inflammation and subsequent tissue degeneration (Sanjay *et al*., 2022). By targeting these molecular pathways, *Cymbopogon citratus* demonstrates potential as a Linay

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of the leaves of *Cymbopogon citratus*

Cymbopogon citratus leaves were purchased from New Benin market and authenticated at the Department of Plant biology and Biotechnology, University of Benin, Benin City.

Dr. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, identified and authenticated the *Cymbopogon citratus*. Following that a sample was placed in the departmental herbarium, and a voucher number UBH-C451 was given for referral.

3.2 Extraction of *Cymbopogon citratus* leaves

The extraction was conducted at the Department of Pharmacology, University of benin, Benin City. The leaves were washed thoroughly with distilled water to remove any contaminants. After washing, the leaves were air-dried and ground into a fine powder using a laboratory grinder. Approximately, 50 grams of the lemongrass powder was soaked in 500 ml of ethanol for 24 hr at room temperature. The mixture was continuously agitated on a magnetic stirrer to enhance extraction efficiency and filtered using a fine mesh cloth to separate the plant material from the liquid. The filtrate was dried using a rotary evaporator, yielding a concentrated crude extract. The dried lemongrass extract was dissolved in 1% acetic acid solution to achieve a final concentration of 1% (w/v). The solution was thoroughly mixed and adjusted to pH 4.0 using hydrochloric acid.

3.3 Ethical Considerations

The protocol for this study was approved by the Ministry of Agriculture and Food Security, Animal Ethics Committee (MAFSAEC), Benin City, Edo State and an official approval number was provided, MAFSAEC: 025-07/25/0039. The rats were handled following the International Guidelines for the Care and Use of Laboratory Animals.

3.4.1 Animals

Twenty-four (24) albino rats weighing between 180g and 200g were used for this study at the Vivarium of the Department of Anatomy, University of Benin, Benin City. The animals were kept in cages with proper ventilation and for 7 days to acclimatize. The rats were fed with grower's mash pellets (Standard Feed Nigeria Plc) and water ad libitum under standard conditions of temperature and relative humidity

3.4.2 Acute Toxicity Test

The lethal dose (LD50) of *Cymbopogon citratus* leaves extract was established using Locke's method to know the appropriate dose to be administered. The rats were divided into five (5) groups of two rats in each group. Different concentrations of *Cymbopogon citratus* leaves extract (1000mg/kg, 1600mg/kg and 1900mg/kg per, 2,900mg/kg, and 5,000mg/kg body weight of the albino rats) were administered via oral gavage. They were observed for any signs of adverse effects including lethargy, change in locomotive activity, abnormal behavior, and death for 48hrs.

3.5 Experimental Design

The animals were grouped into four groups where two (n=2) rats were placed in group A, the control whereas groups B-D has 4 rats each.

1. Group A is the control group and the male and female (n=2) rats will receive pellet and distilled water only.
2. Group B had two male and female rats (n=4) and were administered 250mg/kg of *Cymbopogon citratus* leaves extract for 1 month.
3. Group C had two male and female rats (n=4) and were given 500mg/kg of *Cymbopogon citratus* leaves fruit extract for 1 month.
4. Group D had two male and female rats (n=4) and were administered 1000mg/kg of *Cymbopogon citratus* leaves extract for 1 month.

All animals were administered orally via using oral gavage.

3.5.1 Specimen collection

Following the administration period, the animals were euthanized using cervical dislocation, and blood specimen collected using a cardiac puncture and dispensed into lithium heparin containers and plain bottles. The organs were harvested and immediately fixed in 10% formal saline for 24 hours.

3.6 Processing of Specimen

3.6.1 Biochemical Analyses

Blood samples were analyzed for serum levels of reproductive hormones (testosterone, progesterone, LH, and FSH) using enzyme-linked immunesorbent assay (ELISA) kits.

General Considerations for Blood Sample Collection

Blood samples were collected carefully using cardiac puncture to ensure the accuracy of hormone measurement; the blood was collected into serum separator tubes. After collection, the samples were allowed to clot for about 30 minutes at room temperature, followed by centrifugation at 2000–3000 rpm for 10-15 minutes to separate serum from cells (Huang and Sedlak, 2001). This serum was then processed for hormonal analysis.

Procedure for Testosterone Measurement

1. Sample Preparation: Baseline serum testosterone levels were accurately assessed using commercial ELISA kits from Cayman Chemical (Ann Arbor, MI, USA*)*, which are known for their reliability (Panah *et al*., 2024).

2. ELISA Protocol:

a) The serum samples were diluted according to the kit's specifications.

b) The samples were added to the ELISA plate pre-coated with testosterone antibodies using a 500ul automatic pipette.

c) The Elisa plates were incubated at room temperature for about 2 hours, followed by washing the wells to clear unbound substances.

d) The detection reagent was added and incubated again.

e) The substrate solution was then introduced to develop color and measure absorbance at 450 nm using a microplate reader (Thermo Fisher Scientific Multiskan GO) (Huang and Sedlak, 2001).

Procedure for Progesterone Measurement

1. Sample Handling: Progesterone levels were evaluated with kits from DRG International Inc. (Springfield, NJ, USA*)*, which have been validated extensively for hormonal assays (Wu *et al*., 2014).

2. ELISA Implementation:

a) The serum samples were diluted as per the manufacturer's guidelines.

b) The loaded samples were place into the ELISA plate and allowed for incubation.

c) After washing, substrate solution was added and absorbance measured to quantify progesterone levels (Wu *et al*., 2014).

Procedure for Luteinizing Hormone (LH) Measurement

1. Preparation and Enzyme Assay: For LH measurement, the recommended kit from R*and*D Systems was employed (Becerril-García *et al*., 2020).

2. Assay Steps:

a) The serum samples were diluted according to the kit's dilution requirements.

b) The samples were transferred to the designated wells of the ELISA plate using a 500ul automatic pipette.

c) After incubation, the washing solution was used in cycles, and detection reagent was added as provided by the kit's instructions.

d) The absorbance was measured using the ELISA Microplates at 450nm to determine LH levels based on established standards (Becerril-García *et al*., 2020).

Procedure for Follicle-Stimulating Hormone (FSH) Measurement

1. Blood Sample Processing: Following earlier protocols for sample management, FSH levels were analyzed using kits available from Biovendor (Czech Republic) (Becerril-García *et al*., 2020).

2. Implementing the ELISA:

a) The serum samples were diluted as indicated in the product manual.

b) The Samples were introduced into the ELISA wells and allowed to incubate.

c) The samples were washed using washing solutions and substrate added for 15 minutes.

d) The absorbance was measured for the resulting color change to derive FSH concentrations (Becerril-García *et al*., 2020).

3.6.2 Histopathological investigation

The fixed testes, prostate, ovaries and uterus tissues were processed in an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). Tissues were dehydrated in different grades of alcohol, cleared in toluene and impregnated in molten paraffin wax for specified periods in the processor machine. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Paraffin tissue blocks were trimmed at 0µm, sectioned at 3µm, and dried on a hot plate for 15 min. Sections were stained in Cole's haematoxylin for 10 min, rinsed in water, differentiated in 1% acid alcohol briefly, and blued in running water for 10 min. The stained slides were counterstained in 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam (Drury and Wallington, 1980). Sections were microscopically examined using x10 and x40 objective lenses.

3.7 Statistical Analysis

Data collected were organized and analyzed using the Statistical Package for Social Sciences (SPSS) software. Results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted to compare mean values among groups, with Tukey's post-hoc test applied for multiple comparisons. A significance level of $p < 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of *Cymbopogon citratus* fruit extract on FBC analytes

Cymbopogon citratus had no significant effect on the FBC analyte results in the control group (Group A).

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Table 4.1: ANOVA of FBC analytes in the control group

	Mean ± SD	F	p-value
WBC	8 ± 3.4	0.425	0.738
LYM	6.3 ± 2.6	0.443	0.727
LYM (%)	78.4 ± 2.3	1.605	0.24
MID	1 ± 0.5	0.6	0.627
MID (%)	12.9 ± 1.5	2.582	0.102
GRA	0.7 ± 0.3	0.571	0.644
GRA (%)	8.8 ± 0.8	0.853	0.491
HGB	13.7 ± 0.9	2.798	0.085
MCH	19.7 ± 0.3	0.706	0.567
MCHC	32.5 ± 3.1	0.621	0.615
RBC	7 ± 0.5	1.832	0.195
MCV	61.1 ± 5.8	0.257	0.855
HCT	42.7 ± 5.4	0.199	0.895
RDWa	55.9 ± 14.3	0.634	0.607
RDW (%)	19.2 ± 2.7	1.341	0.307
PLT	662.8 ± 227.3	0.268	0.847
MPV	7.8 ± 0.3	0.369	0.777
PDWa	10.1 ± 0.4	0.673	0.585
PDW%	38.7 ± 1.1	0.583	0.637
PCT	0.5 ± 0.2	0.196	0.897
P-LCR	13.4 ± 2.6	0.263	0.851
P-LCC	87.3 ± 29.1	0.091	0.964

Cymbopogon citratus had no significant effect on the FBC analyte results in Group B administered 250mg/kg of *Cymbopogon citratus* leaves extract for 1 month.

Table 4.2: ANOVA of FBC analytes in Group B

	Mean ± SD	F	p-value
WBC	7.5 ± 2.5		
LYM	6.2 ± 2.3		
LYM (%)	80.2 ± 3.7		
MID	0.8 ± 0.2		
MID (%)	11.6 ± 2		
GRA	0.6 ± 0.1		
GRA (%)	8.7 ± 2.1		
HGB	13.7 ± 0.5		
MCH	20.2 ± 0.8		
MCHC	33 ± 0.8		
RBC	6.8 ± 0.3		
MCV	61.2 ± 2.9		
HCT	41.5 ± 1.7		
RDWa	51.7 ± 2.5		
RDW (%)	17.5 ± 1.3		
PLT	563 ± 140.7		
MPV	7.9 ± 0.4		
PDWa	20.8 ± 21.8		
PDW%	33.3 ± 11.4		
PCT	0.4 ± 0.1		
P-LCR	14.3 ± 3.9		
P-LCC	79.3 ± 26		

Cymbopogon citratus had no significant effect on the FBC analyte results in Group C administered 500mg/kg of *Cymbopogon citratus* leaves extract for 1 month.

Table 4.3: **ANOVA of FBC analytes in Group C**

	Mean ± SD	F	p-value
WBC	8.7 ± 5		
LYM	6.9 ± 4.2		
LYM (%)	76.4 ± 4.7		
MID	1.2 ± 0.6		
MID (%)	14.5 ± 2.6		
GRA	0.7 ± 0.2		
GRA (%)	12 ± 5.5		
HGB	14.6 ± 0.4		
MCH	20.2 ± 0.8		
MCHC	34.1 ± 1		
RBC	7.2 ± 0.3		
MCV	59.3 ± 2.4		
HCT	42.7 ± 2.2		
RDWa	48.8 ± 2.7		
RDW (%)	17 ± 0.7		
PLT	585 ± 173.5		
MPV	7.8 ± 0.4		
PDWa	20.6 ± 21.1		
PDW%	32.8 ± 10.6		
PCT	0.5 ± 0.1		
P-LCR	13.4 ± 2.6		
P-LCC	78.5 ± 26.4		

Cymbopogon citratus had no significant effect on the FBC analyte results in Group D administered 1000mg/kg of *Cymbopogon citratus* leaves extract for 1 month.

Table 4.4: ANOVA of FBC analytes in Group D

	Mean ± SD	F	p-value
WBC	10 ± 1.3		
LYM	8.2 ± 1.2		
LYM (%)	81.2 ± 1.6		
MID	1.1 ± 0.1		
MID (%)	11.2 ± 0.8		
GRA	0.7 ± 0.1		
GRA (%)	9.5 ± 2.8		
HGB	14.6 ± 0.4		
MCH	20 ± 0.1		
MCHC	33.8 ± 2		
RBC	7.3 ± 0.2		
MCV	59.5 ± 3.6		
HCT	43.4 ± 4		
RDWa	50.3 ± 3.8		
RDW (%)	18 ± 0.7		
PLT	606.5 ± 89		
MPV	7.6 ± 0.8		
PDWa	9.8 ± 1		
PDW%	37.8 ± 1.8		
PCT	0.5 ± 0.1		
P-LCR	12 ± 5.3		
P-LCC	75.5 ± 47.2		

There was no statistically significant change ($p > 0.05$) in the body weight of the rats after administration of *Cymbopogon citratus*

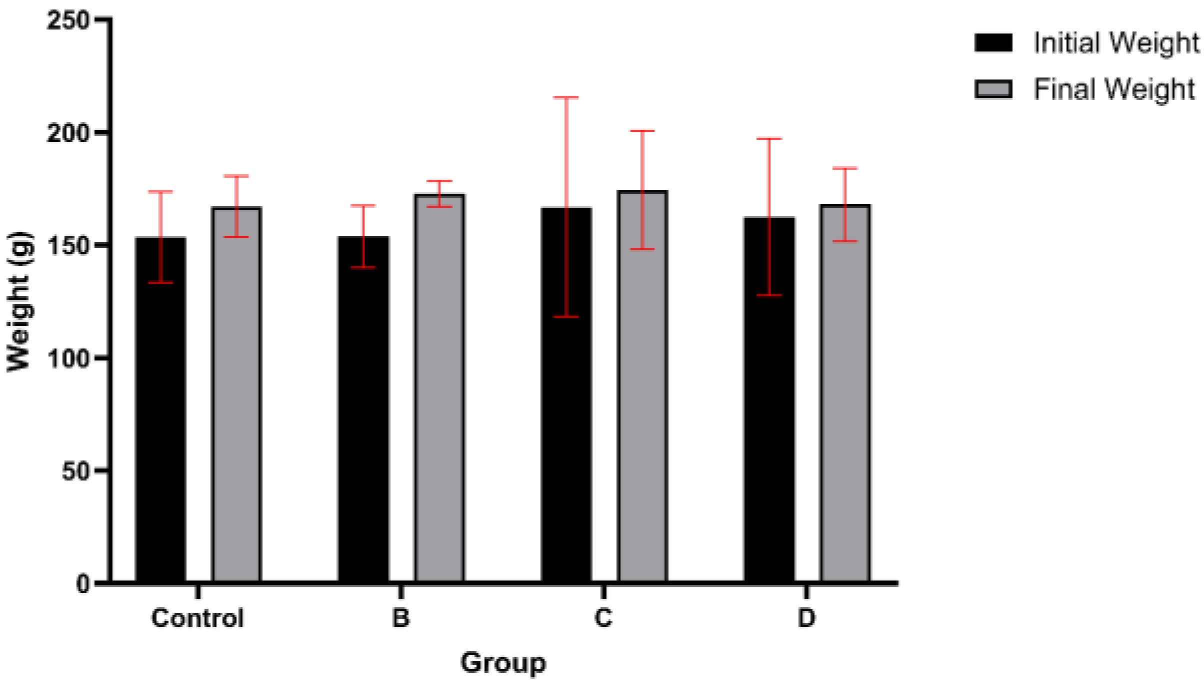

Figure 4.1: Changes in the weight of rats after administration

There was no significant difference ($p > 0.05$) in the weight of the rat testes across the control and 3 treatment groups.

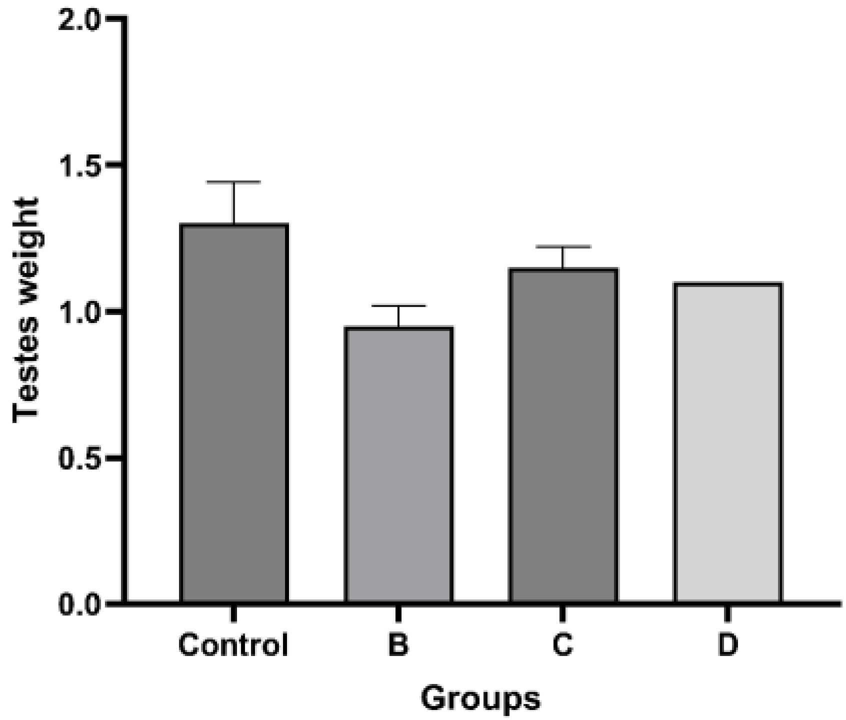

Figure 4.2: Effect of *Cymbopogon citratus* leaves extract on weight of the testes

There was no significant difference ($p > 0.05$) in the weight of the rat ovaries across the control and 3 treatment groups.

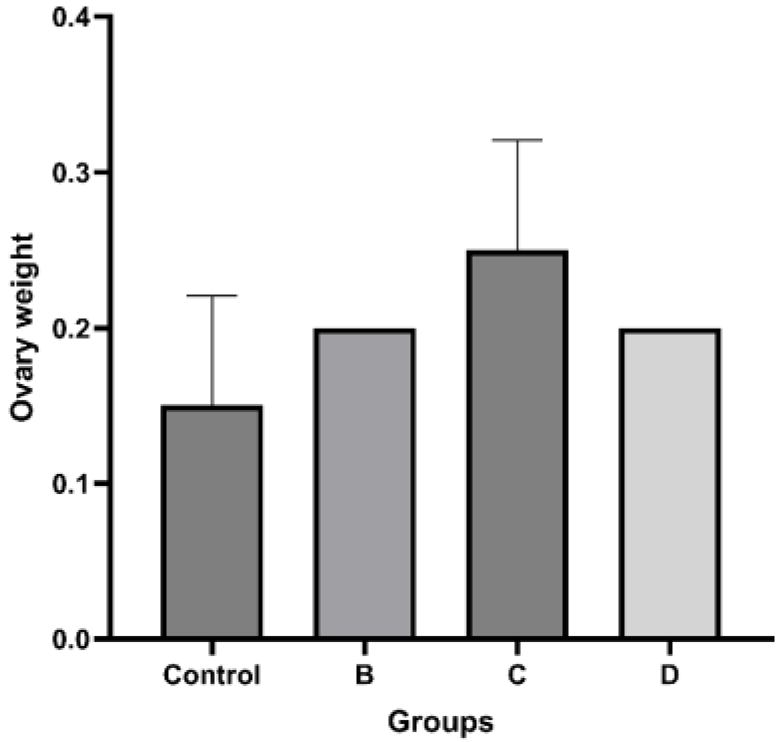

Figure 4.3: Effect of *Cymbopogon citratus* leaves extract on weight of the ovaries

There was a significant difference ($p < 0.05$) in the testosterone levels between the control group and group B.

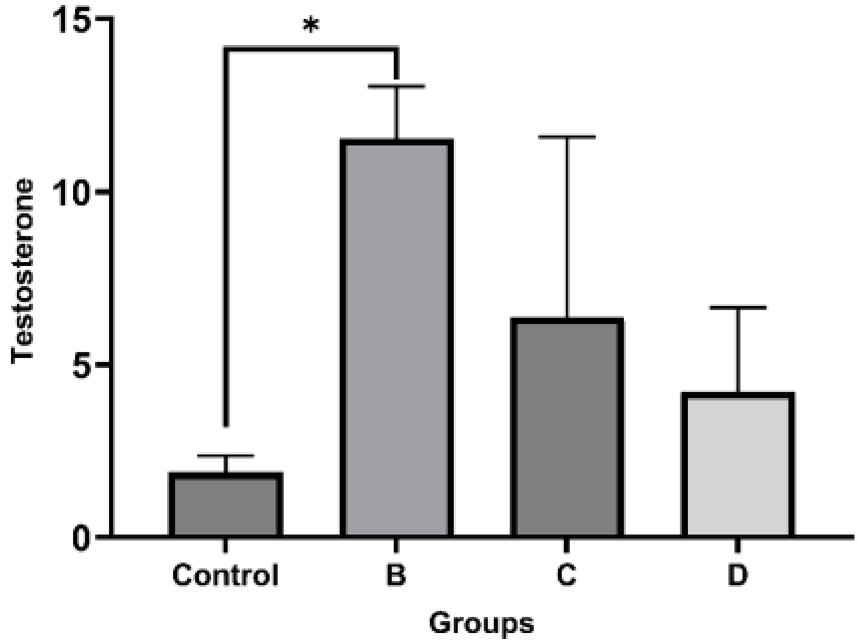

Figure 4.4: Effect of *Cymbopogon citratus* leaves extract on testosterone

There was a significant difference ($p < 0.05$) in the progesterone levels between the control group and group B

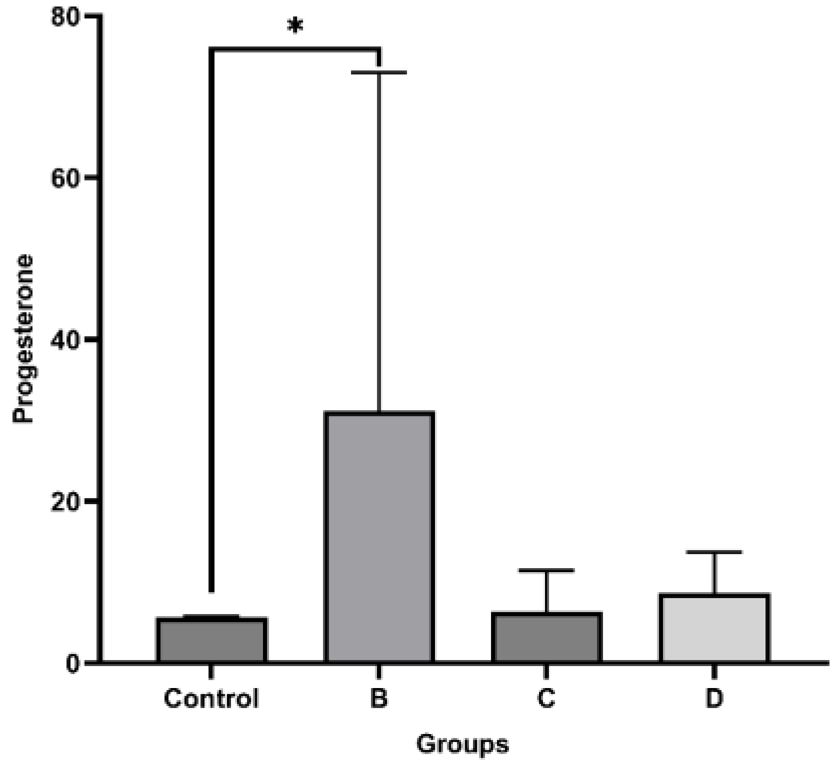

Figure 4.5: Effect of *Cymbopogon citratus* leaves extract on progesterone

A section of the ovary shows the presence of follicles (thin arrow) containing an oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). (H&E stain, x100 Mag)

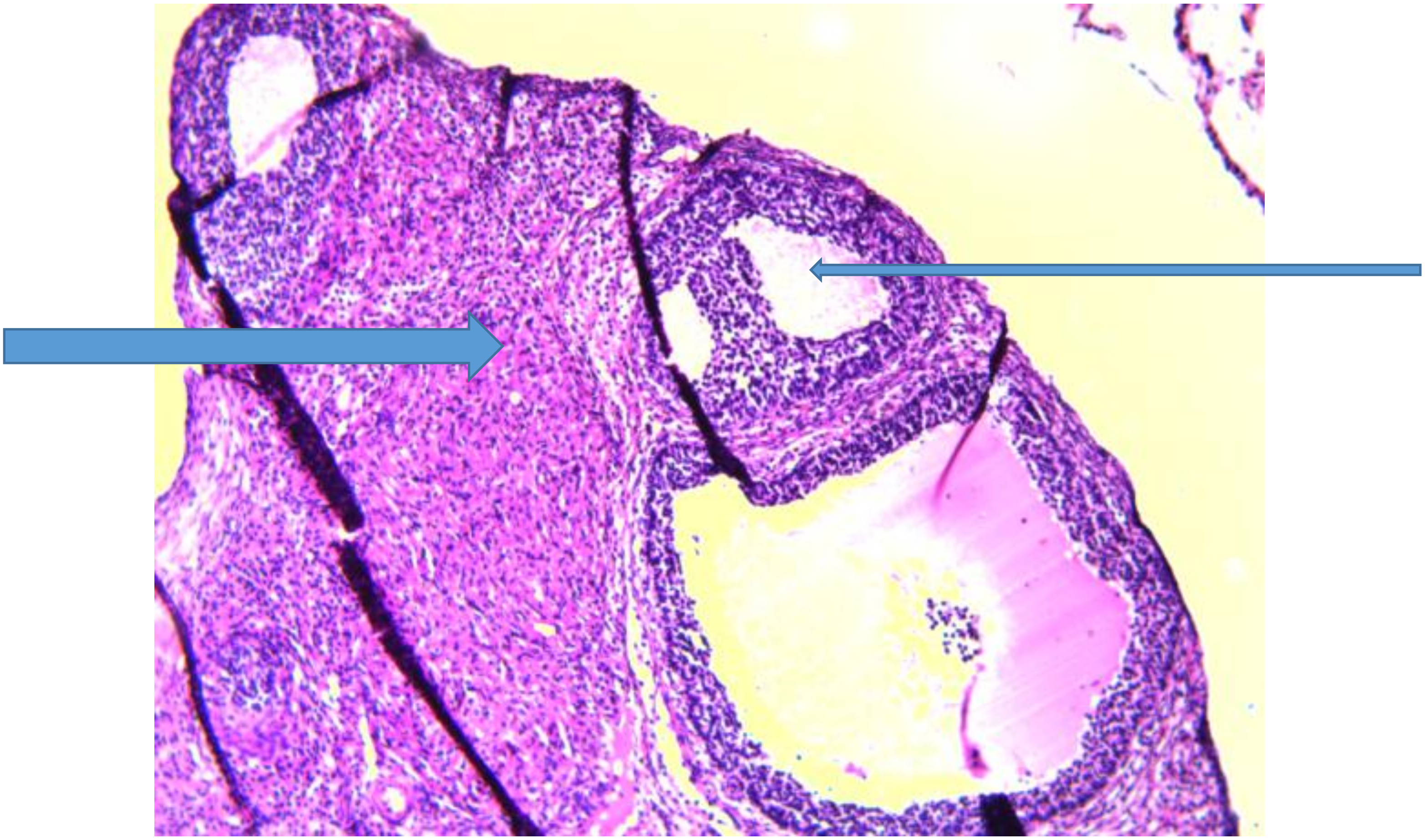

Plate 4.1: Section of ovary of female rat received pellet and distilled water only for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

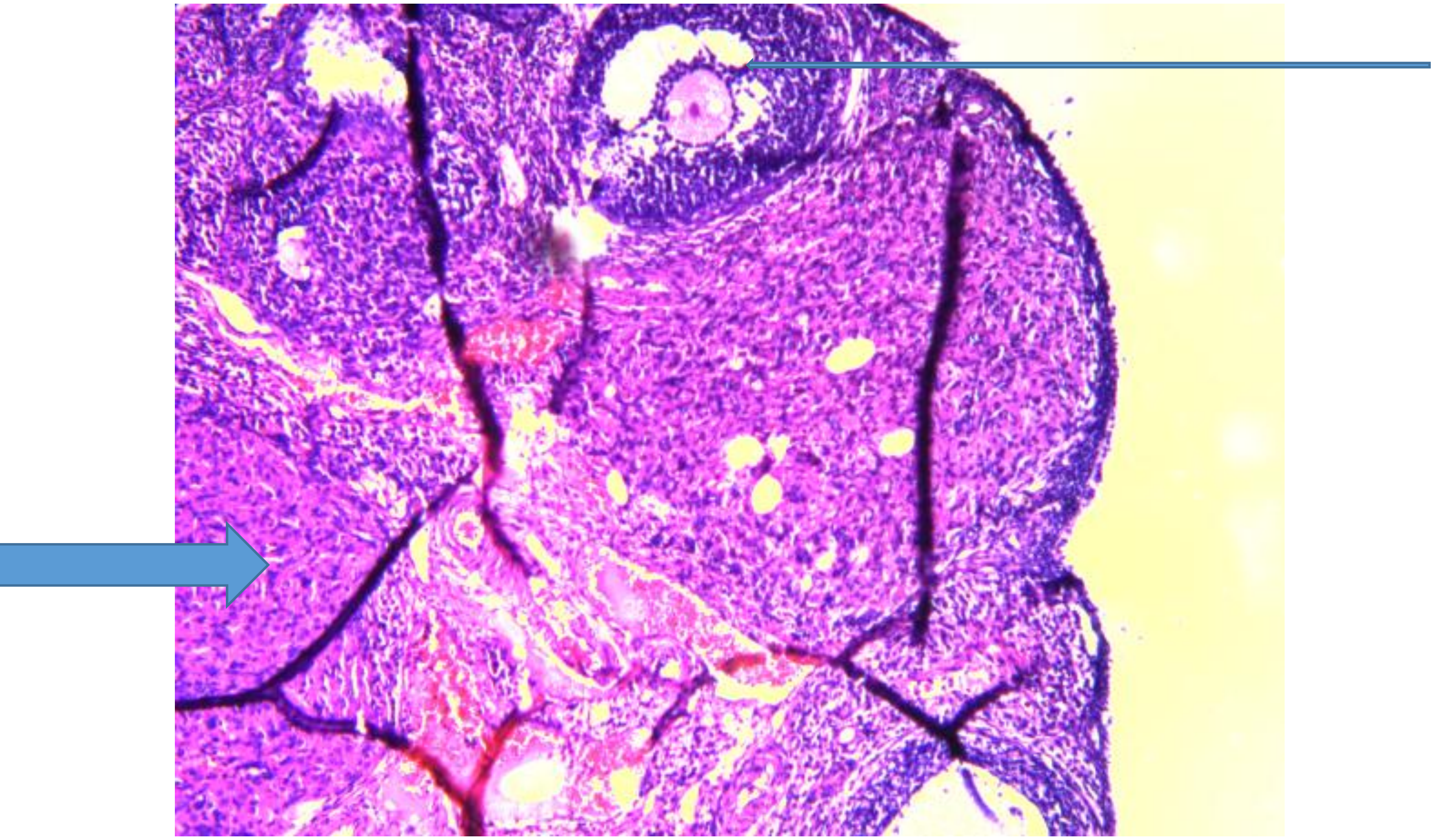

Plate 4.2: Section of ovary of female rat received pellet and distilled water only for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

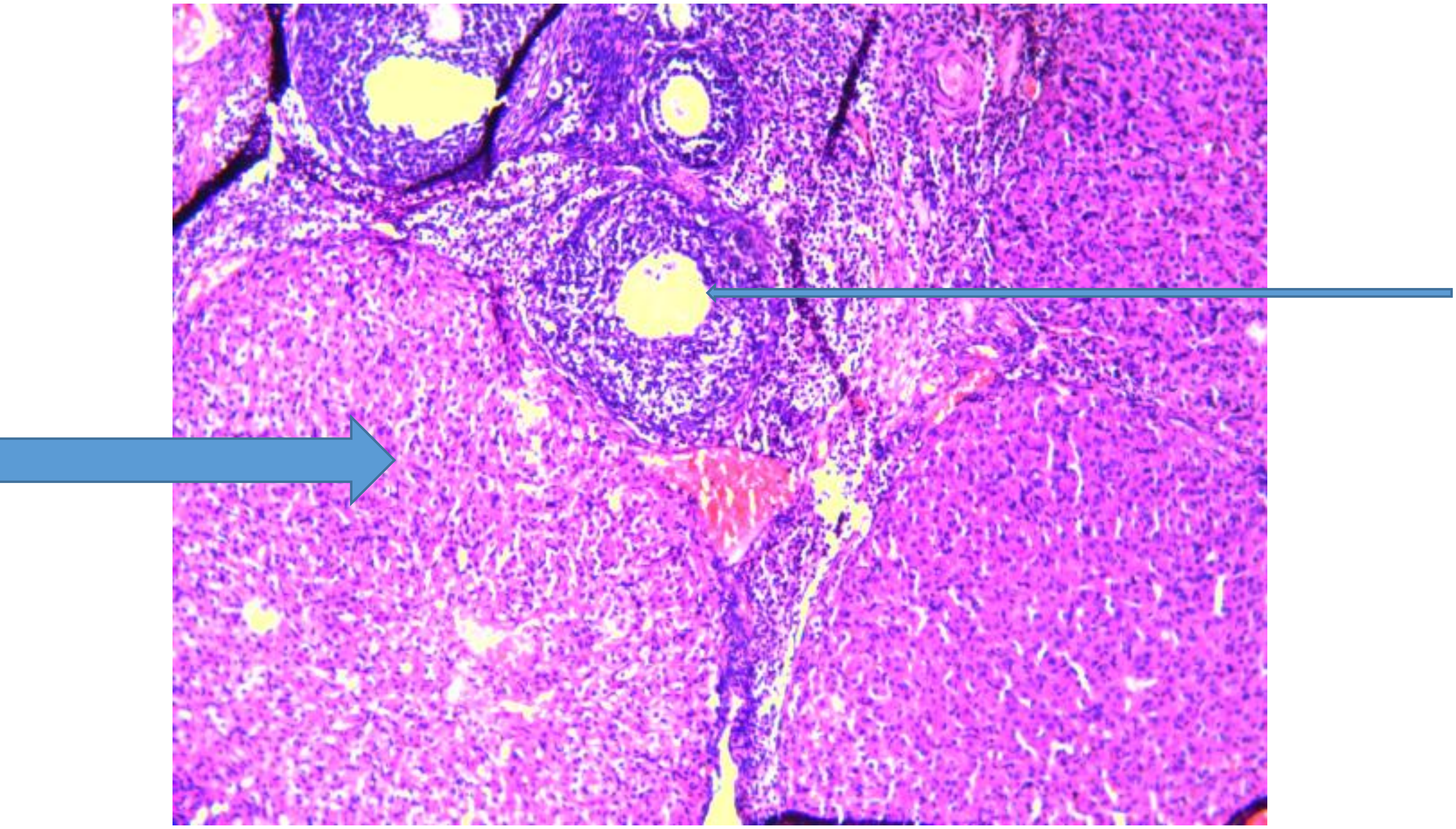

Plate 4.3: Section of ovary of female rat administered 250 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

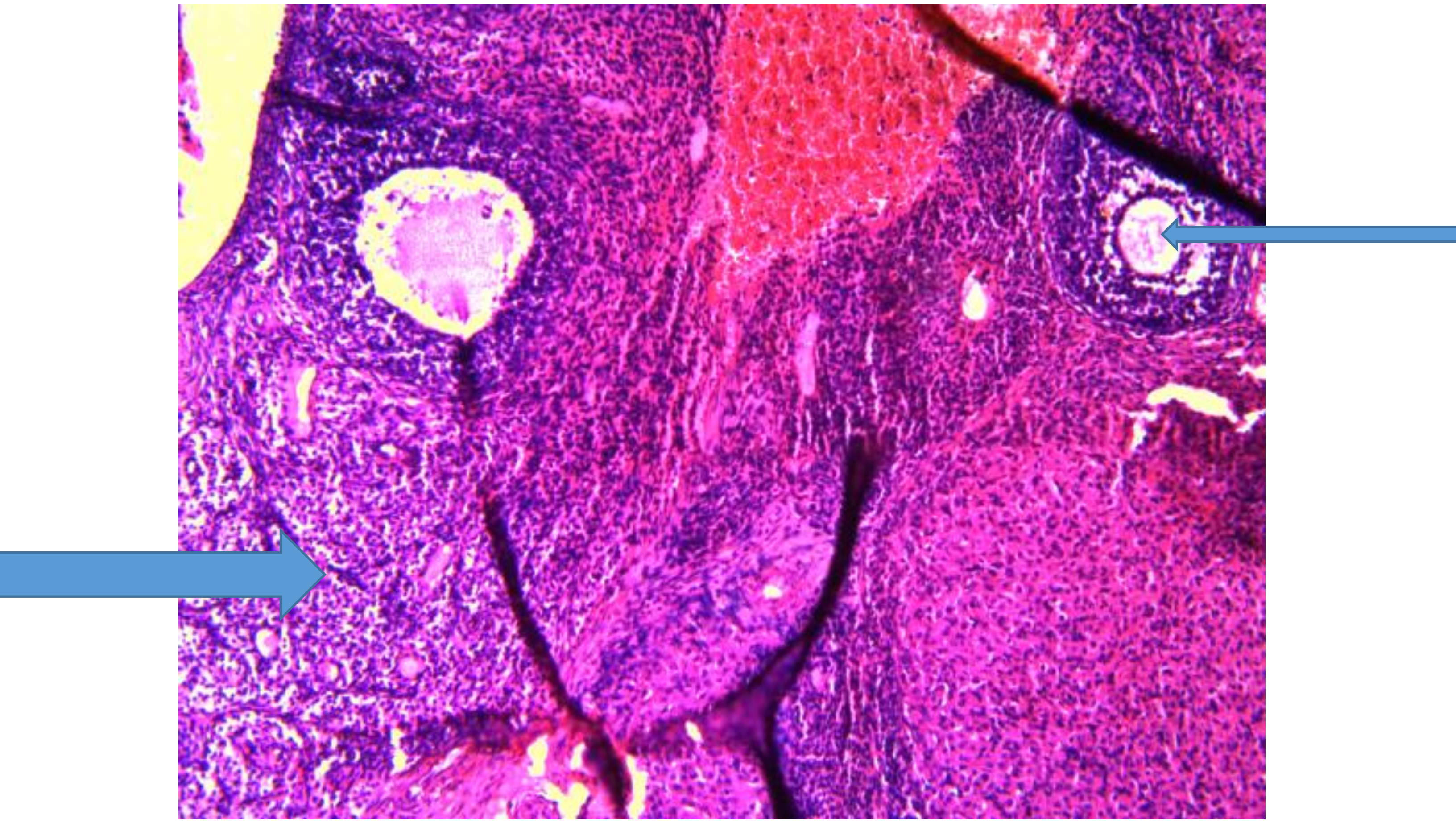

Plate 4.4: Section of ovary of female rat administered 250 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

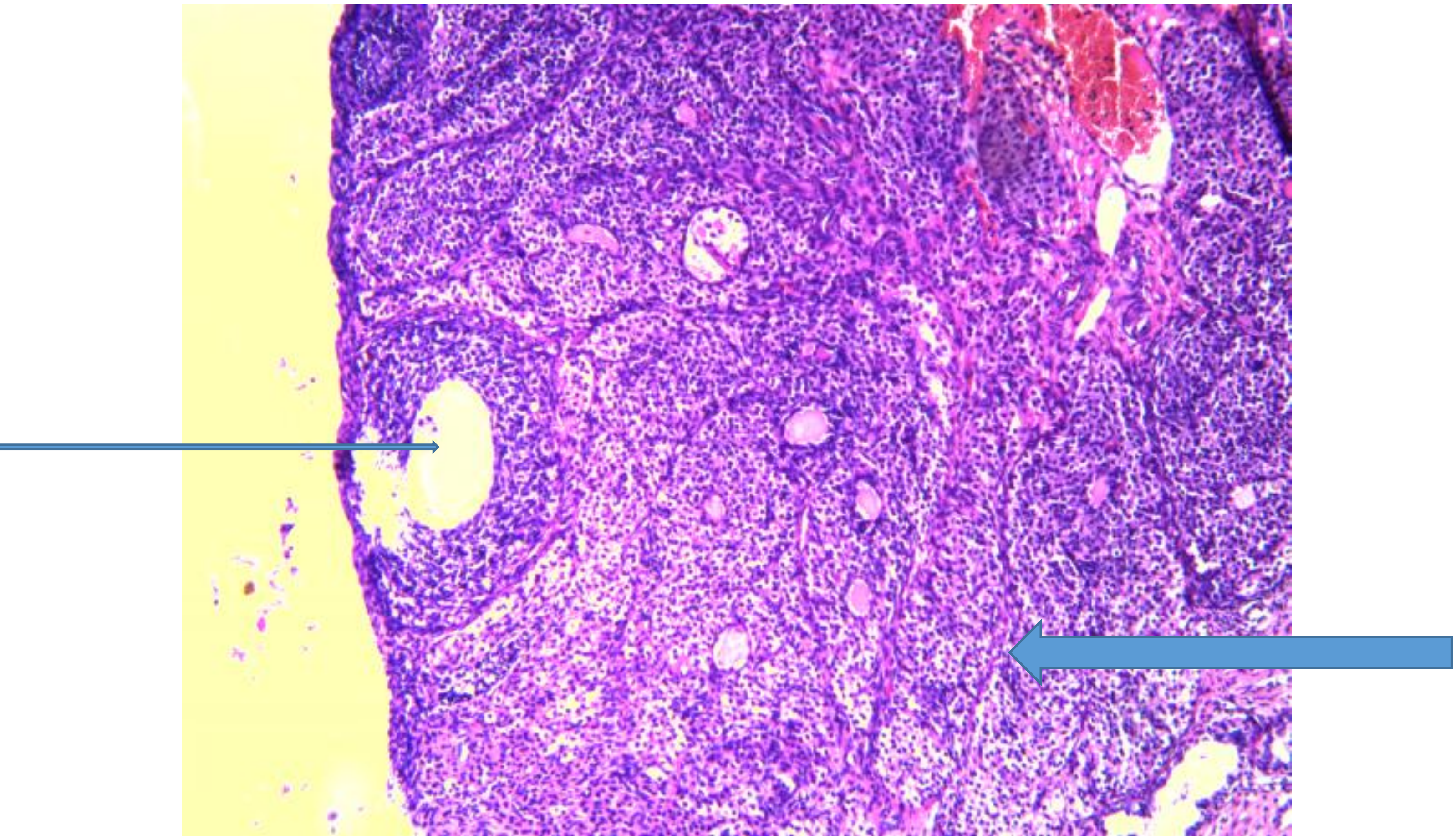

Plate 4.5: Section of ovary of female rat administered 500 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

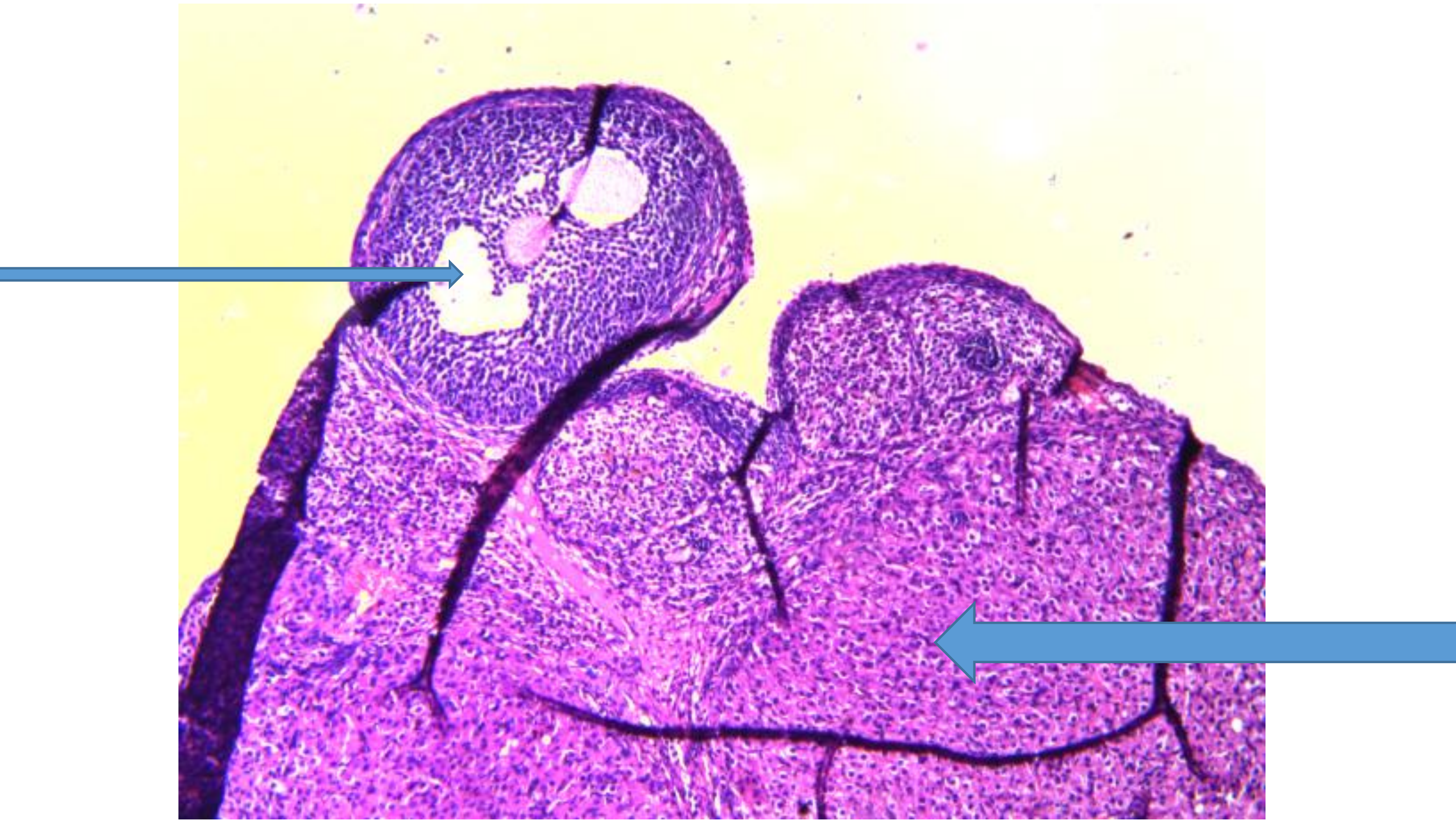

Plate 4.6: Section of ovary of female rat administered 500 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

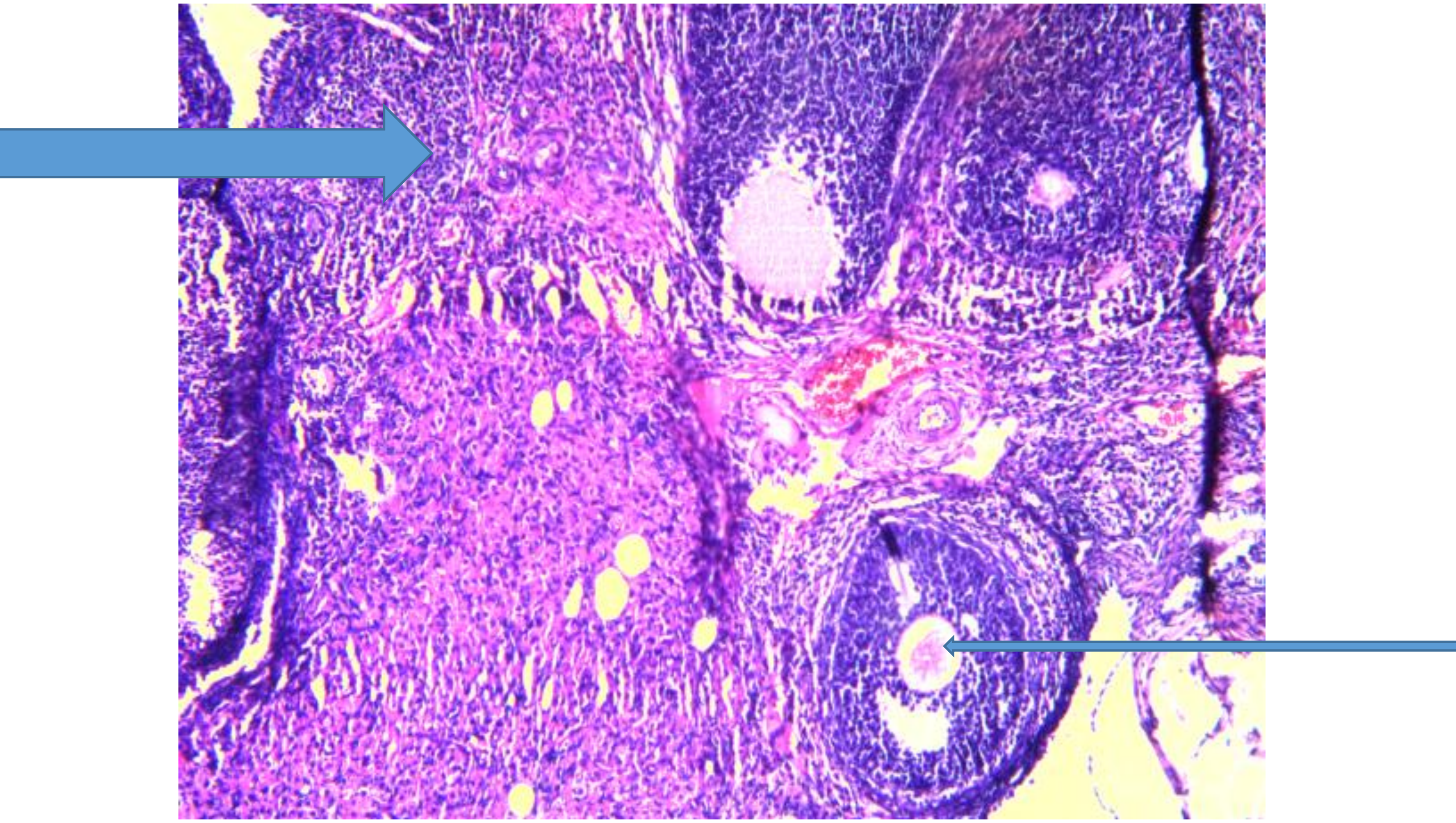

Plate 4.7: Section of ovary of female rat administered 1000 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the ovary shows presence of follicles (thin arrow) containing oocyte surrounded by theca and granulosa cells. Also present are other follicles at different stages of maturation within an ovarian stroma (thick arrow). **FEATURES ARE IN KEEPING WITH NORMAL OVARIAN TISSUE**

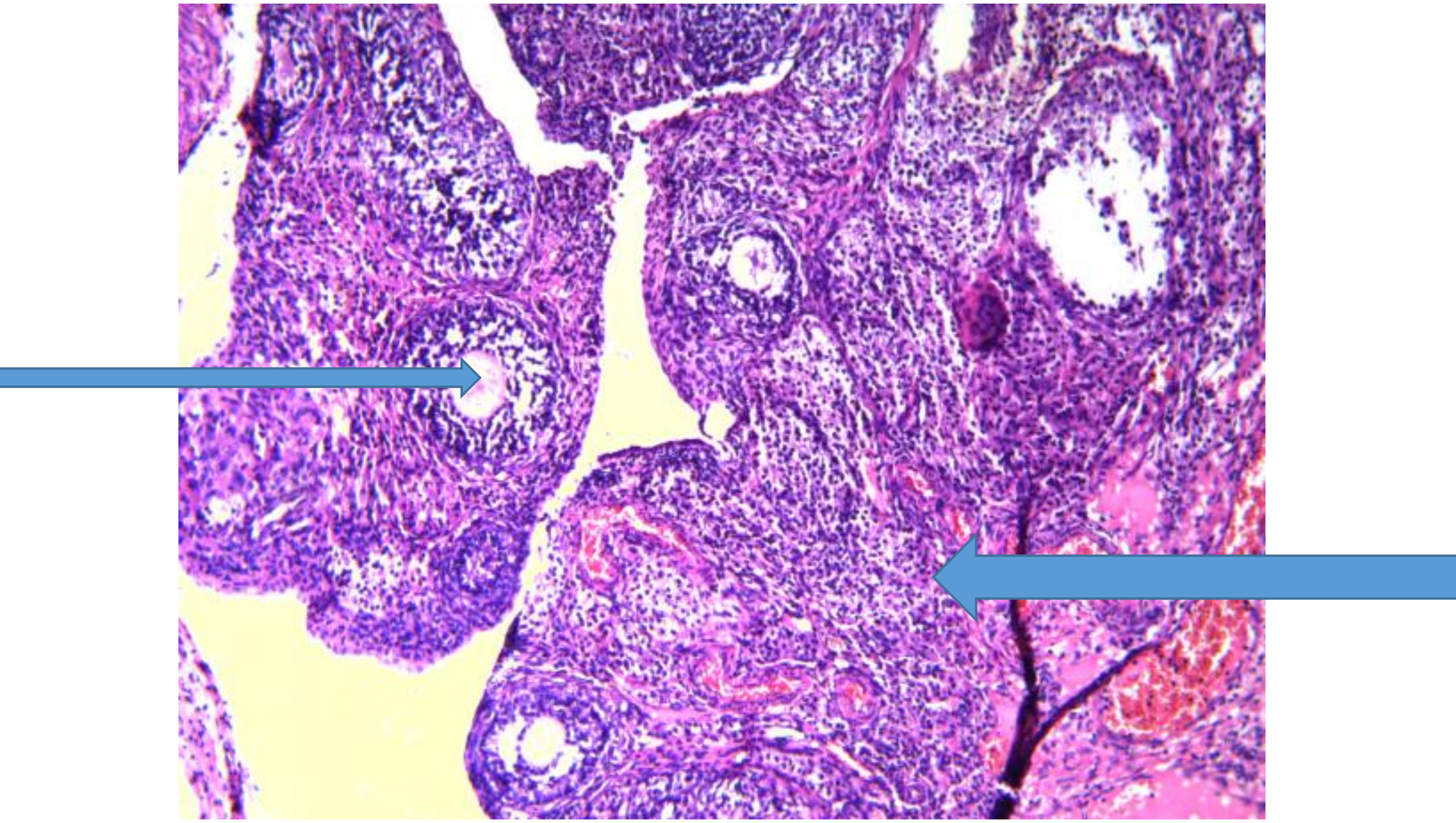

Plate 4.8: Section of ovary of female rat administered 1000 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

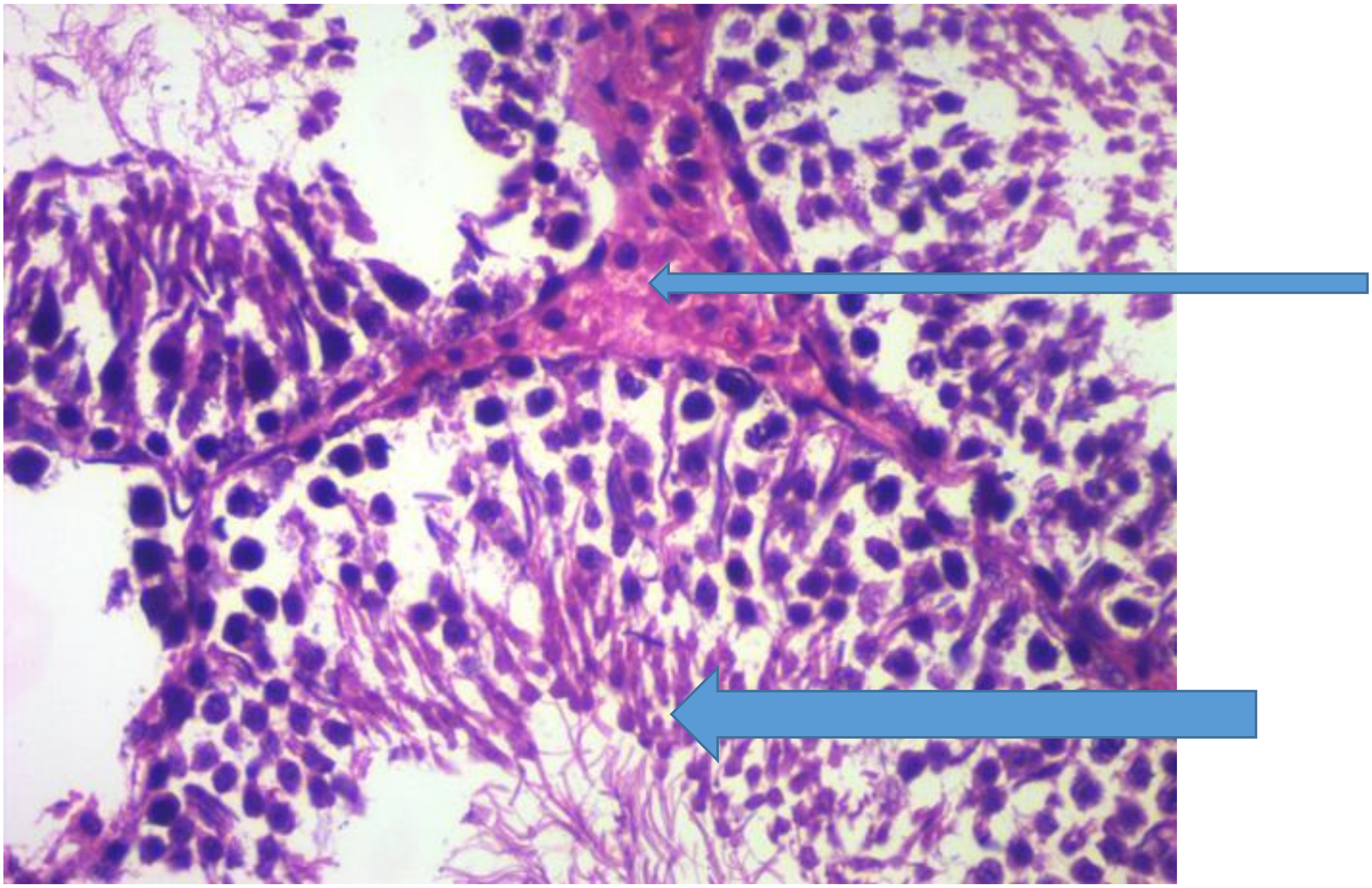

Plate 4.9: Section of testes of male rat received pellet and distilled water only for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

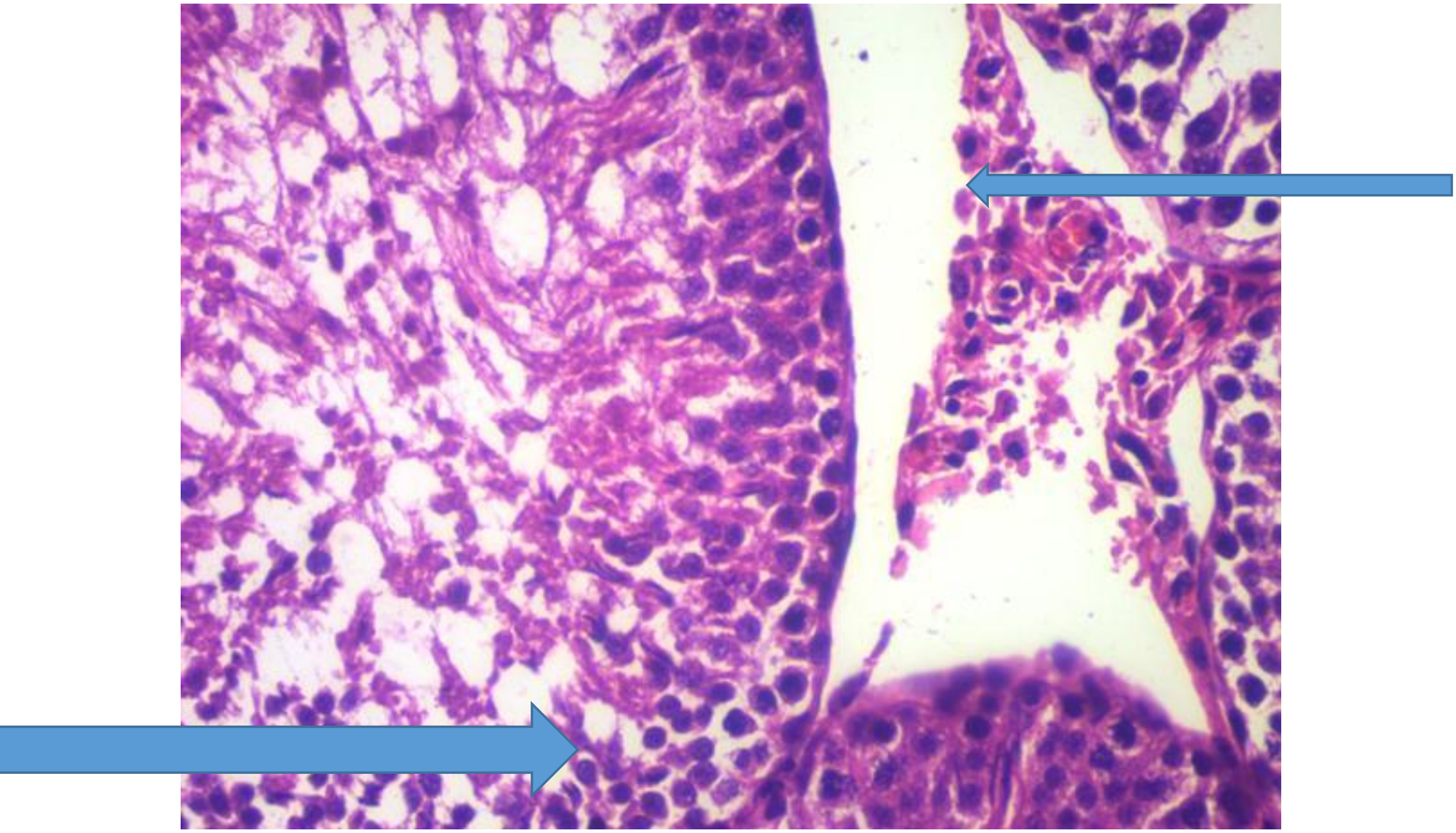

Plate 4.10: Section of testes of male rat received pellet and distilled water only for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

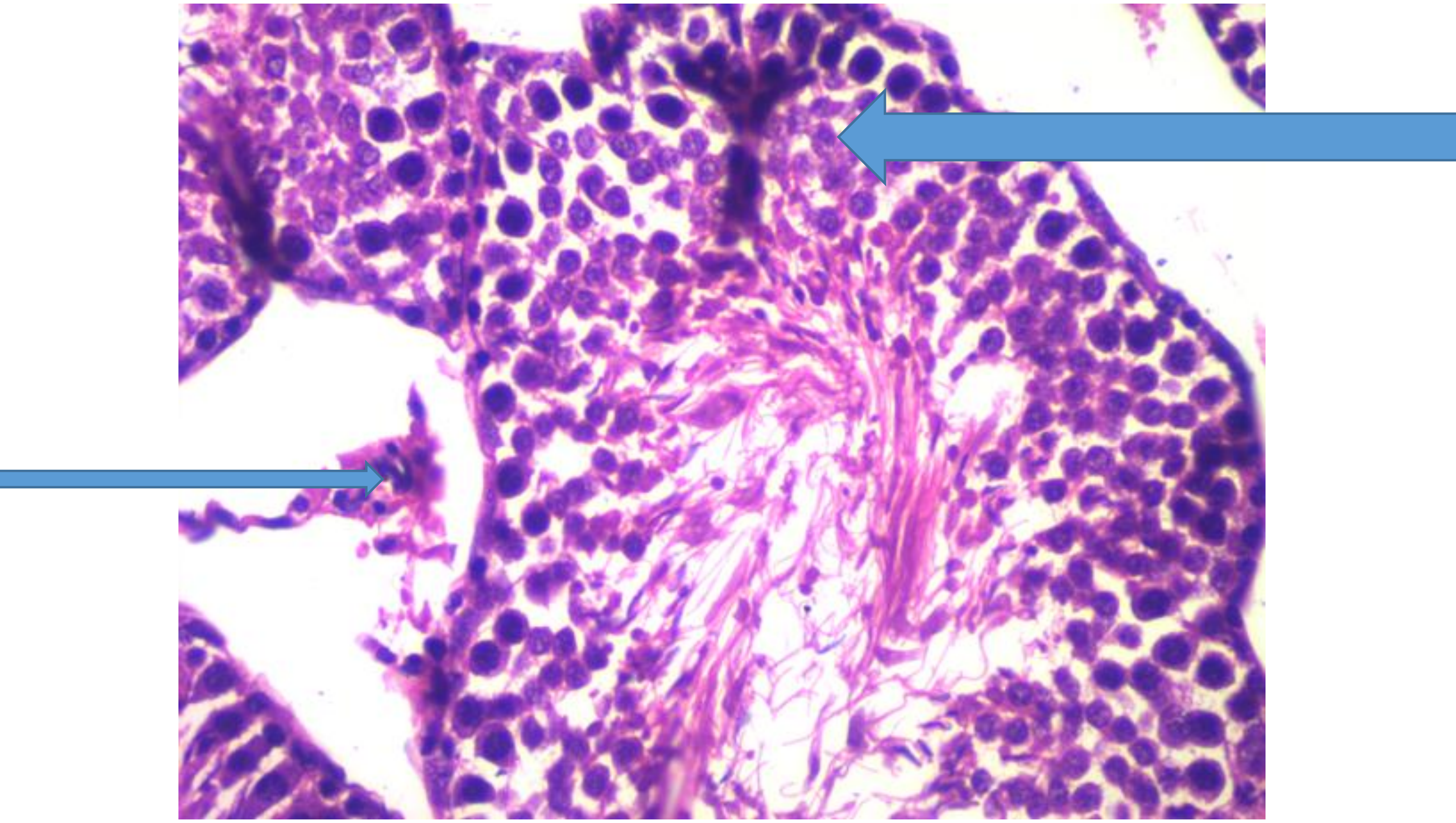

Plate 4.11: Section of testes of male rat administered 250 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

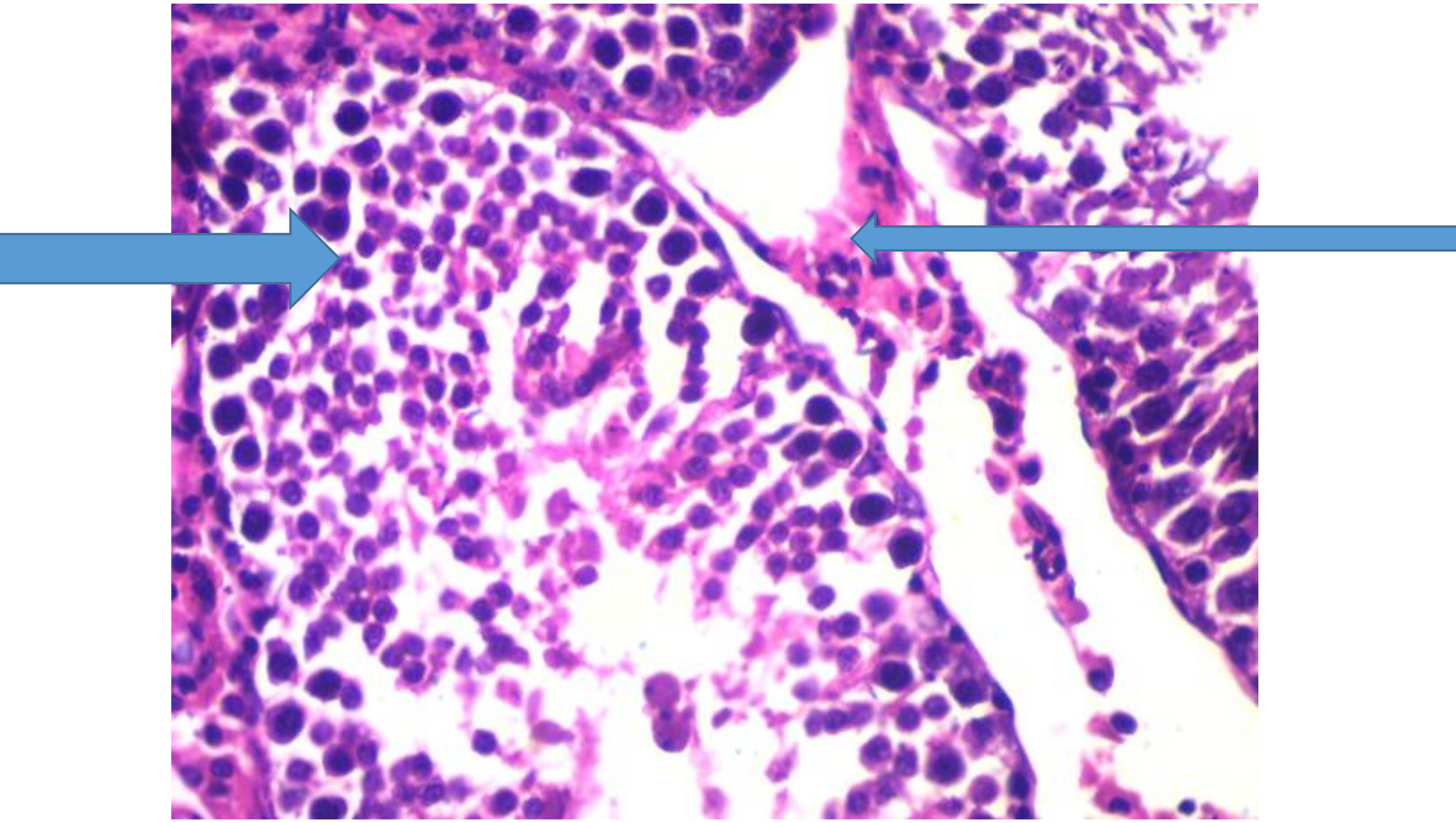

Plate 4.12: Section of testes of male rat administered 250 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the testis shows seminiferous tubules (thick arrow) that appears shrunken with thickened basement membrane. The tubules contain reduced number of germ cells. The Leydig cells (thin arrow) appears normal. **FEATURES ARE SUGGESTIVE OF TESTICULAR ATROPHY**

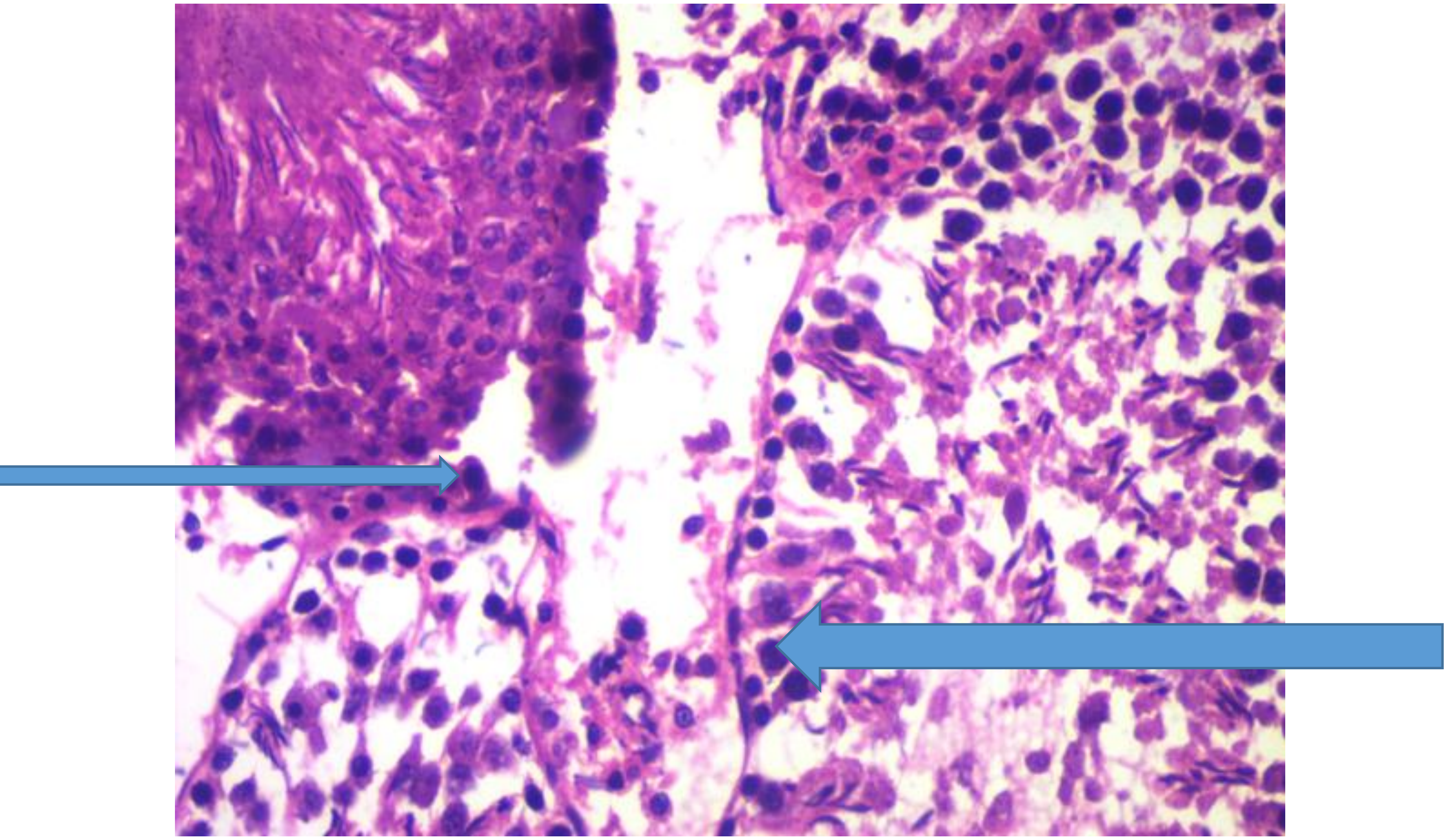

Plate 4.13: Section of testes of male rat administered 500 mg/kg body weight of Cymbopogon citratus for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

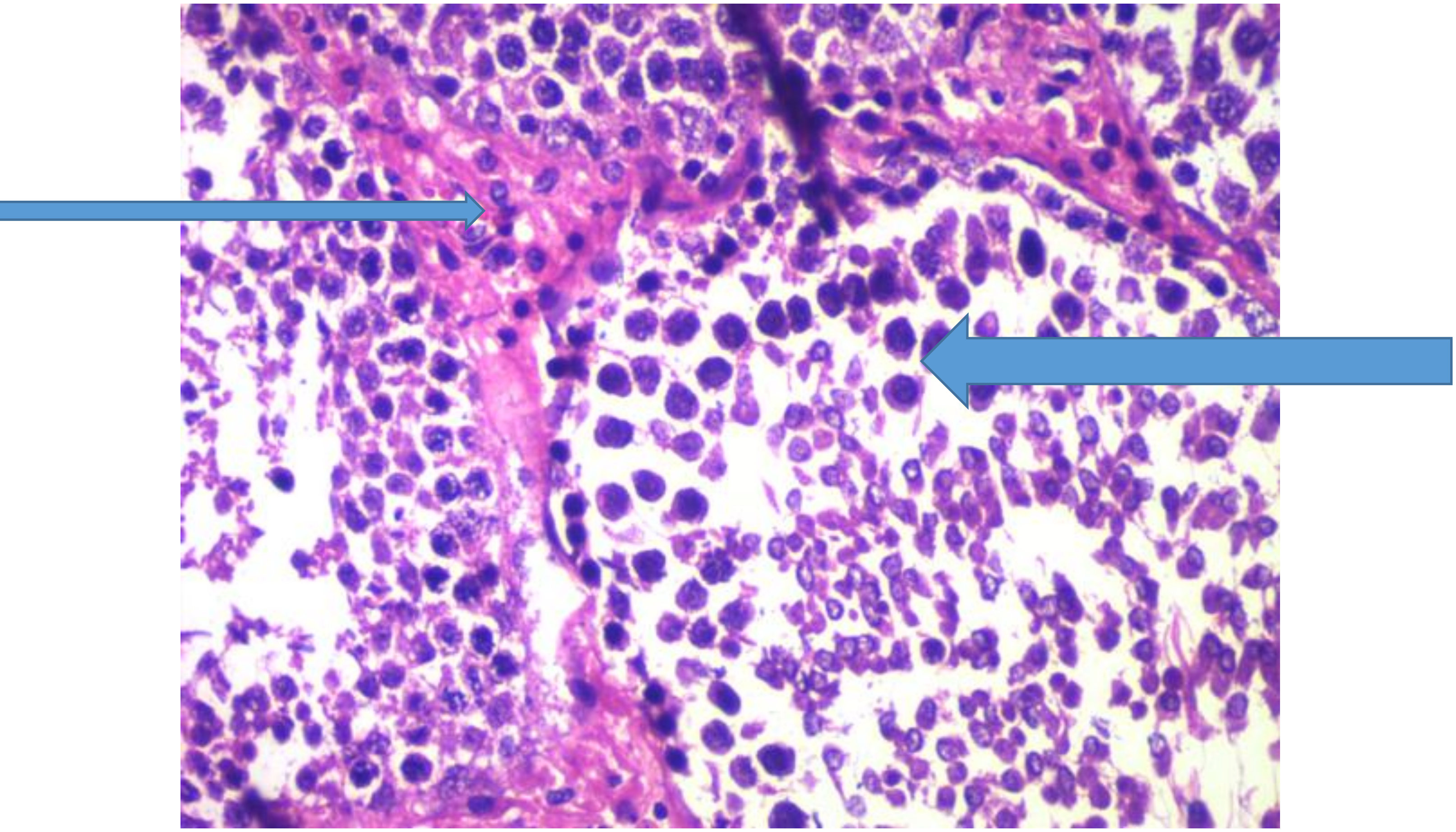

Plate 4.14: Section of testes of male rat administered 500 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

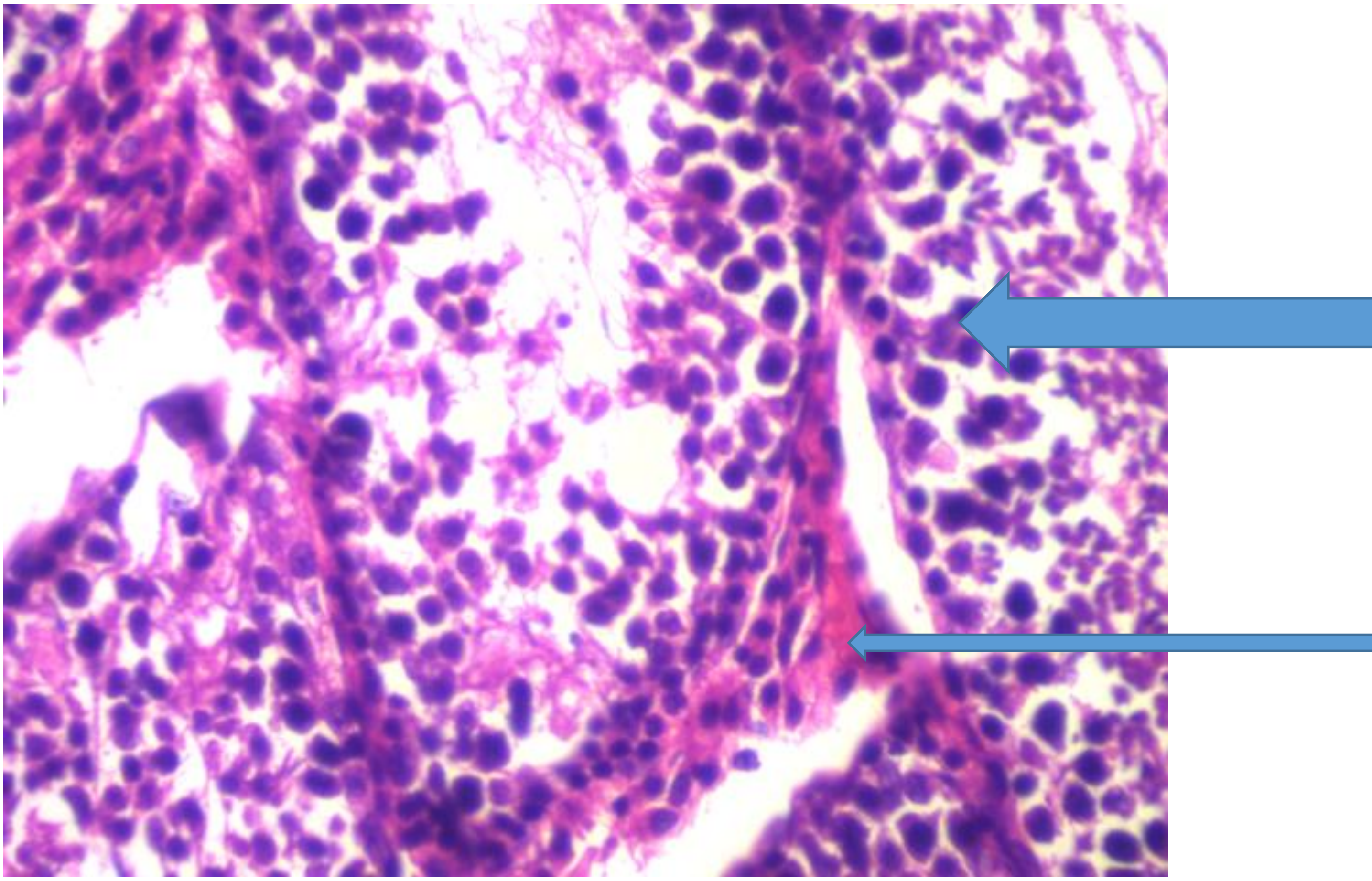

Plate 4.15: Section of testes of male rat administered 1000 mg/kg body weight of *Cymbopogon citratus* for 1 month

Section of the testis shows oval shaped seminiferous tubules (thick arrow) containing sertoli cells and sperm cells at different stages of maturation. The tubules are surrounded by a thin membrane with presence of Leydig cells (thin arrow) in the interstitium. **FEATURES ARE IN KEEPING WITH NORMAL TESTIS**

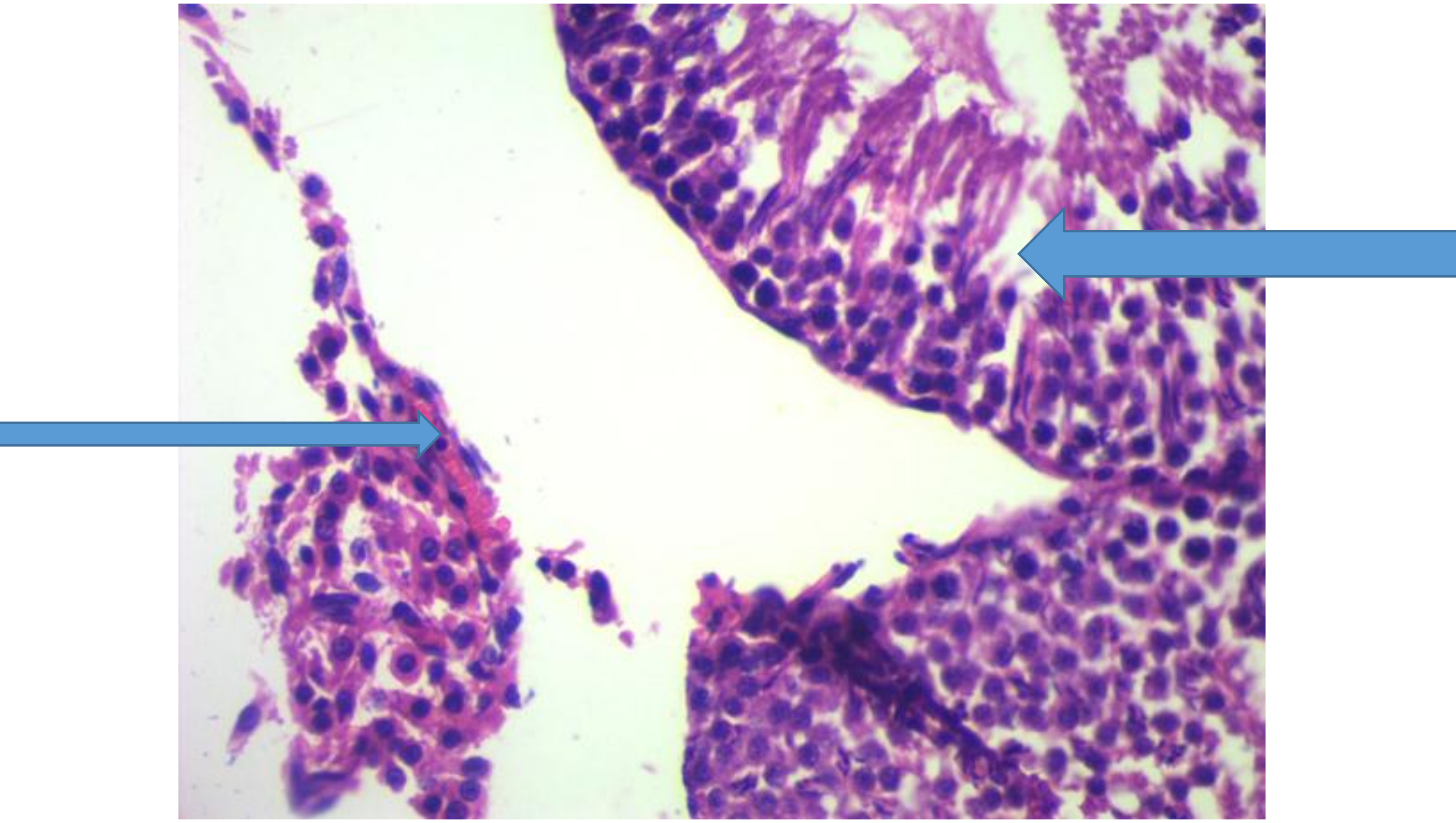

Plate 4.16: Section of testes of male rat administered 1000 mg/kg body weight of *Cymbopogon citratus* for 1 month

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigated the histopathological effects of *Cymbopogon citratus* leaf extract on the reproductive organs of albino rats, examining both morphological changes and hormonal impacts across different dosage groups. The findings provide important insights into the dose-dependent effects of lemongrass extract on reproductive health, revealing both protective and potentially adverse outcomes depending on the concentration administered (El-Din, 2023). The research demonstrated that while the extract had no significant effect on hematological parameters or organ weights across all treatment groups, it exhibited significant hormonal modulation at the 250 mg/kg dose level, with testosterone and progesterone levels showing notable changes compared to controls (Wadhwa *et al*., 2023). Histopathological examination revealed predominantly normal tissue architecture across most treatment groups, with the critical exception of testicular atrophy observed in one specimen from the 500 mg/kg group, suggesting the existence of a therapeutic window beyond which beneficial effects may be compromised by potential toxicity (Anna and Tony, 2020).

Effect on Full Blood Count (FBC) Analytes

The present study demonstrated that *Cymbopogon citratus* leaf extract had no significant effect on full blood count (FBC) analytes across all treatment groups when compared to controls ($p > 0.05$). This finding is consistent with previous research by Ayembilla *et al*. (2023), who reported that conventional Soxhlet *Cymbopogon citratus* leaf extracts showed minimal hematological toxicity in acute and subchronic toxicity assessments. The absence of significant changes in white blood cell counts, red blood cell parameters, and platelet indices suggests that the extract does not induce systemic hematological toxicity at the doses tested, which is encouraging for potential therapeutic applications. The preservation of normal hematological parameters across doses ranging from 250 mg/kg to 1000 mg/kg indicates a favorable safety margin for blood-forming tissues and immune function.

Effect on Body Weight

Similarly, the lack of significant changes in body weight across treatment groups aligns with findings from Umukoro *et al*. (2017), who observed that *Cymbopogon citratus* administration did not significantly alter body weight in mice models. This suggests that the extract does not adversely affect general metabolic processes or appetite regulation, which is important for long-term safety considerations. The maintenance of normal growth patterns across all dosage groups indicates that the extract does not interfere with normal physiological development or energy homeostasis mechanisms.

Effect on Reproductive Organ Weights

The study revealed no significant differences in testicular and ovarian weights across treatment groups compared to controls. This finding contrasts with some previous studies that reported organ weight changes following herbal extract administration. For instance, Igwe *et al*. (2024) observed variations in testicular weights in rats administered graded doses of *Cymbopogon citratus* ethanol leaf extract, although their methodology and extraction process differed from the current study. The preservation of normal organ weights in the present study suggests that the extract, at the doses administered, does not cause significant organ atrophy or hypertrophy, which is a positive indicator for reproductive health. The consistency of organ weights across both testicular and ovarian tissues indicates that the extract's effects are likely mediated through functional rather than structural mechanisms.

Effect on Reproductive Hormone Levels

One of the most significant findings of this study was the impact on reproductive hormones, particularly testosterone and progesterone levels. The study demonstrated significant differences in testosterone levels between the control group and Group B (250 mg/kg dose), with similar patterns observed for progesterone levels. This hormonal modulation aligns with previous research by Rahim *et al*. (2013), who reported that *Cymbopogon citratus* could influence reproductive hormone levels, potentially through its antioxidant properties and interaction with the hypothalamic-pituitary-gonadal axis.

The hormonal changes observed in Group B, but not in higher dose groups (Groups C and D), suggest a complex dose-response relationship. This pattern is consistent with the concept of hormesis, where low doses of bioactive compounds may have beneficial effects while higher doses may be neutral or potentially harmful. El-Din (2023) reported similar findings, noting that moderate doses of lemongrass extract provided optimal benefits for reproductive function, while higher doses showed diminishing returns or adverse effects.

The increase in hormone levels at lower doses could be attributed to the antioxidant properties of flavonoids and other phytochemicals present in *Cymbopogon citratus* extract. As discussed in the literature review, these compounds can protect reproductive tissues from oxidative stress, potentially enhancing hormonal production and regulation (Wadhwa *et al*., 2023). The antioxidant mechanism may help preserve Leydig cell function in males and support ovarian follicle development in females, leading to improved hormonal output.

Histopathological Findings of Reproductive Organs

The histopathological examination revealed predominantly normal tissue architecture across most treatment groups, with one notable exception in Group C testis, which showed features suggestive of testicular atrophy. The affected testis displayed shrunken seminiferous tubules with thickened basement membranes and reduced numbers of germ cells, while Leydig cells appeared normal. This finding is particularly significant as it suggests that the 500 mg/kg dose may represent a threshold where adverse effects begin to manifest.

The observation of testicular atrophy in one specimen from the 500 mg/kg group is consistent with previous research by Anna and Tony (2020), who reported that high doses of plant extracts could lead to degenerative changes in testicular tissues. However, the fact that this change was observed in only one specimen suggests that individual susceptibility may play a role, or that this dose represents a borderline toxic level for some animals.

The preservation of normal ovarian histology across all treatment groups is encouraging and suggests that female reproductive tissues may be more resilient to the effects of *Cymbopogon citratus* extract, or that the doses used were below the threshold for ovarian toxicity. This finding is supported by Igwe and Okelue (2024), who reported that lemongrass extract had protective effects on reproductive tissues, particularly in females. The consistent observation of normal follicular development, intact theca and granulosa cells, and appropriate ovarian stroma across all treatment groups indicates that the extract does not disrupt normal ovarian physiology or folliculogenesis at any of the tested doses.

Dose-Response Relationships and Therapeutic Implications

The results of this study highlight the importance of dose optimization in herbal medicine applications. The pattern observed suggests that 250 mg/kg (Group B) may represent an optimal therapeutic dose, providing hormonal benefits without adverse histological changes. The 500 mg/kg dose (Group C) appears to be at or near a toxicity threshold, as evidenced by the testicular atrophy observed in one specimen. The 1000 mg/kg dose (Group D) showed neither significant hormonal changes nor consistent histological abnormalities, which may indicate that at very high doses, the extract's effects become unpredictable or that compensatory mechanisms are activated.

This dose-response pattern is consistent with findings from Haggag (2015), who demonstrated that *Cymbopogon citratus* exhibited protective effects at moderate doses but showed potential for toxicity at higher concentrations. The concept of therapeutic windows is well-established in pharmacology, and these results suggest that *Cymbopogon citratus* extract follows similar principles.

The observed effects can be attributed to the rich phytochemical profile of *Cymbopogon citratus*, particularly its flavonoid content. Flavonoids are known to possess antioxidant properties that can protect reproductive tissues from oxidative damage (Sousa *et al*., 2021). The mechanism likely involves the scavenging of reactive oxygen species (ROS), which are known to damage reproductive cells and disrupt hormonal balance.

The selective effects observed at different doses may be explained by the complex interactions between various phytochemicals present in the extract. As noted by Zhang *et al*. (2019), synergistic interactions among flavonoids, alkaloids, terpenoids, and tannins can produce more potent biological activities than individual compounds. At optimal doses, these interactions may enhance beneficial effects, while at higher doses, they may lead to pro-oxidant effects or direct cellular toxicity.

The findings of this study have several important clinical implications. The demonstration that *Cymbopogon citratus* extract can modulate reproductive hormones at specific doses suggests potential therapeutic applications for reproductive health disorders. However, the observation of testicular atrophy at higher doses underscores the importance of careful dose selection and monitoring in any potential therapeutic applications.

The preservation of normal hematological parameters and general health indicators at all tested doses is encouraging for the safety profile of the extract. However, the reproductive effects observed emphasize the need for further research to establish safe and effective dosing guidelines.

5.2 Conclusion

This study evaluated the histopathological effects of *Cymbopogon citratus* leaf extract on reproductive organs in albino rats across different dosage levels. The key findings demonstrate that the extract exhibits dose-dependent effects on reproductive physiology while maintaining an acceptable safety profile for most parameters examined.

The extract showed no significant impact on hematological parameters, body weight, or reproductive organ weights across all treatment groups, indicating minimal systemic toxicity at the doses tested. However, significant hormonal effects were observed, with testosterone and progesterone levels being notably enhanced in Group B (250 mg/kg dose) compared to controls, while higher doses showed no such benefits.

Histopathological examination revealed predominantly normal tissue architecture across treatment groups, with the important exception of testicular atrophy observed in one specimen from the 500 mg/kg group. This finding suggests that 500 mg/kg may represent a threshold dose where adverse effects begin to manifest, while the 250 mg/kg dose appears to provide optimal therapeutic benefits without structural damage.

The results indicate that *Cymbopogon citratus* leaf extract possesses a narrow therapeutic window, with the 250 mg/kg dose demonstrating the most favorable risk-benefit profile for reproductive health applications. The study confirms that while natural products can offer therapeutic benefits, careful dose optimization is essential to maximize benefits while avoiding potential toxicity.

These findings provide scientific validation for the traditional use of *Cymbopogon citratus* in reproductive health management, while emphasizing the critical importance of appropriate dosing in herbal medicine applications.

5.3 Recommendations

1. Clinical Application for Reproductive Health Enhancement

Given the significant increase in testosterone and progesterone levels observed at the 250 mg/kg dose, *Cymbopogon citratus* extract should be considered for development as a natural therapeutic intervention for individuals with low reproductive hormone levels. Healthcare practitioners could explore its potential use as an adjunct therapy for conditions such as hypogonadism, low libido, and age-related hormonal decline, particularly in patients seeking natural alternatives to synthetic hormone therapies.

2. Integration into Sexual Wellness Programs

The hormonal enhancement effects demonstrated in this study support the incorporation of standardized *Cymbopogon citratus* extract into comprehensive sexual wellness programs. Fertility clinics and reproductive health centers could consider including this extract as part of holistic treatment approaches for couples experiencing fertility challenges or individuals seeking to optimize their reproductive health naturally.

3. Development of Standardized Herbal Formulations

Based on the optimal therapeutic effects observed at 250 mg/kg, pharmaceutical and nutraceutical companies should develop standardized *Cymbopogon citratus* extract formulations for commercial use in sexual health products. These formulations should maintain the bioactive compound concentrations that produced the beneficial hormonal effects while ensuring safety through adherence to the established therapeutic dose range.

4. Educational Implementation for Traditional Medicine Practitioners

The scientific validation of *Cymbopogon citratus*'s reproductive health benefits should be integrated into continuing education programs for traditional medicine practitioners, herbalists, and naturopaths. This will ensure evidence-based application of lemongrass extracts in clinical practice, emphasizing the importance of proper dosing (250 mg/kg equivalent) and avoiding potentially toxic higher doses that may cause adverse effects such as testicular atrophy.

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.

a. Feeding flat plate

b. Feeding water bottles

c. Feed (pellets)

d. ISOL disinfectant

e. Digital thermometer

f. Plastic cage

g. Weighing balance

h. Indian ink and plate

2. For Sacrificing

a. Hand gloves

b. Sterile Lancet

c. Cotton wool

d. Chloroform

e. Plastic container sterile with a cover

f. Dissenting set

g. Sterile containers

h. Formalin

3. Histology Laboratory

a. Scrape blade

b. Spatula

c. Block holder

d. Automatic tissue processor

e. Molten basket

f. Tissue basket

g. L-shaped mould

h. Rotary type microtome

i. Water bath

j. Hot plate

k. Metal pencil

l. Slides and cover slip

m. Stain (Haematoxylin and eosin)

n. Binocular microscope

o. Dibutylphthalate polysterene xylene (DPX),

p. Xylene, alcohol and water

APPENDIX II

I. The mould was filled with molten paraffin wax

II. With a pair of warm blunt-nosed forceps, tissues were transformed from the paraffin bath to the mould

III. Forceps were warmed and tissues oriented until lying in the desired plane.

IV. Corresponding labels from the paraffin bath were removed and placed against the side of the mould adjacent to the tissues.

V. Air was blown on the surface until a thin film of wax has solidified.

VI. The mould was transferred to a container of cold water and submerged until wax hardens.

After embedding, the block is to left harden up while placed on the ice for some hours before sectioning.

The Hertz microtome (Cambridge model) was used for trimming and sectioning at varying microns and the block clamp adjusted so that sections at 3-5microns were obtained in a ribbon-like manner, which was floated in a water bath to flatten by gentle heat.

The section or short ribbon was picked using a clean grease-free slide to ensure that the sections were thoroughly dried before staining by placing on a hot plate. After which, slides were stained according to Hematoxylin and Eosin method.

APPENDIX III

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

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

Reagents

Distilled water, Ethanol, Acetic acid, Sodium hydroxide, Hydrochloric acid, Eosin dye, 1% acid-alcohol, xylene, Haematoxylin dye, distrene plasticizer, normal saline and 10% neutral buffered formalin including other ingredients. NOTE: All the reagents were standardized before use.

Equipment and Apparatus

Glass-wares (Pyrex), Measuring Cylinder, Cover slip, Slides, Conical Flask, 5ml Syringes, Universal Container. Dissecting materials: Dissecting Board, Dissecting Set, Gauze, Cotton wool, Husks. Tissue Processing Materials: Binocular Soxhlet extractor, Analytical weighing balance, plastic cages, Automatic tissue processor machine (Shandon 2000,Leica, Frankfurt, Germany*)*, Microscope (Olympus England*)*, Embedding Machine (Hesition- E500 Germany*)*, digital electronic balance (Gilbertini, Italy; sensitivity =0.001g*)*, Leuckhart molds, Water bath (Gallenkamp*)*, Digital rotary microtome (Hestion ERM 4000 Germany*)*, Hot plate, Muslin cloth, Staining rack, Forceps and Swifts.

APPENDIX IV

MINISTRY OF AGRICULTURE AND FOOD SECURITY,
ANIMAL ETHICS COMMITTEE (MAFSAEC)

CERTIFICATE
OF ETHICAL APPROVAL

This is to certify that

ONUMBA ADAORA JOAN

Has been given MAFSAEC Approval for the Animal Component of the research titled:

EFFECT OF LEMON GRASS (CYMBOPOGON CITRATUS) ON THE REPRODUCTIVE ORGANS OF MALE AND FEMALE ALBINO RATS.

In accordance with the Animal Disease Control Act, 2022.

Dr L.I Adebudo
Chairman MAFSAEC

Approval No.
MAFSAEC: 025-07/25/0039

Date Of Approval
28th July, 2025

(This Approval is only valid for this study)

APPENDIX V

APPENDIX VI

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
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Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Cymbopogon citratus* (DC.) Stapf.

Family: Poaceae

Common Name: Lemon grass, West Indian lemon grass

Voucher Number: UBH-C451

Student Name: Onumba Adaora Joan

Plant Identification and Voucher Number Issued by:

27/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MBOSON, MAEIAN, MFBAN; Nigeria)