HISTOMORPHOLOGICAL EFFECT OF *Chrysophyllum albidum* PLANT EXTRACT ON THE REPRODUCTIVE ORGANS OF ALBINO RATS

BY

JAMES AIMUAMWOSA

BMS2001173

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN, NIGERIA

SEPTEMBER, 2025

HISTOMORPHOLOGICAL EFFECT OF *Chrysophyllum albidum* PLANT EXTRACT ON THE REPRODUCTIVE ORGANS OF ALBINO RATS

BY

JAMES AIMUAMWOSA

BMS2001173

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

We the undersigned certify that this research work was carried out by **JAMES AIMUAMWOSA** in the Department of Medical Laboratory Science, School of Basic Medical Science, University of Benin, Benin City in partial fulfillment of the requirements for the award of Bachelor of Science in Medical Laboratory Science.

_____ _____

PROF. F. O. AKINBO **DATE**

Supervisor

_____ _____

DR. (MRS.) Z. OMORUYI **DATE**

Head of Department

_____ _____

DATE

External Examiner

DEDICATION

This project is dedicated to Almighty God.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my supervisor, Professor Frederick Akinbo, for his invaluable guidance, patience, and unwavering support throughout the course of this work. His mentorship has played a pivotal role in the successful completion of this Project.

My sincere appreciation also goes to the Head of Department, Professor (Mrs.) Zainab Omoruyi, and the entire academic and non-academic staff of the Department of Medical Laboratory Science for their support, encouragement, and dedication to academic excellence.

To my beloved siblings Osamudiamen James, Osayamon James, Osavbie James and Etiosa James, I am deeply grateful for your constant love, care, and encouragement, not only during this work but throughout my academic journey.

To my friends and the entire OTF, thank you for your unwavering belief in me, your thoughtful insights, and the continuous motivation you provided. Your friendship has been a tremendous source of strength.

Finally, I extend my heartfelt thanks to everyone who in one way or another contributed to the successful completion of this project. Your support, whether direct or indirect, has been truly appreciated.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Reproductive health disorders are increasingly prevalent globally, necessitating investigation into natural therapeutic alternatives with fewer adverse effects. This research aimed to examine the histomorphological effects of *Chrysophyllum albidum* fruit extract on the reproductive organs of albino rats. Twenty-four healthy albino rats weighing 180-200g were used for the experimental study. The animals were procured from the Animal House of the Department of Anatomy, University of Benin, and maintained under standard laboratory conditions with unrestricted access to pelleted feed and water ad libitum. The rats were divided into four groups: Group A served as the control (n=2), receiving only pelleted feed and distilled water for 30 consecutive days. Group B (n=4) was administered 250mg/kg body weight of *Chrysophyllum albidum* fruit extract orally via gavage along with standard feed and water for 30 days. Group C (n=4) received 500mg/kg body weight of the extract under similar conditions. Group D (n=4) was administered 1000mg/kg body weight of the extract following the same protocol. On the final day, blood samples were collected for hormonal analysis, and reproductive organs (testes, ovaries) were harvested, weighed, and fixed in 10% formal saline. Hematoxylin and eosin staining techniques were employed for histopathological examination under light microscopy. Hormonal levels (testosterone and progesterone) were determined using ELISA techniques. Results revealed significant changes in hematological parameters including decreased lymphocyte percentages (78.4±2.3% to 63.5±3.1%), increased neutrophil counts, and altered red blood cell indices across treatment groups. Body weight increased significantly in all treated groups compared to controls. Testicular weight showed significant increase in the highest dose group (Group D) compared to controls and Group B, while ovarian weights remained unchanged. Remarkably, testosterone and progesterone levels showed no significant alterations across all groups. Histopathological examination revealed preserved normal cellular architecture in both testicular and ovarian tissues across all treatment groups. Seminiferous tubules maintained normal morphology with healthy Sertoli cells and spermatogenic cells at various maturation stages, while ovarian follicles demonstrated normal development. The findings provide compelling evidence that *Chrysophyllum albidum* fruit extract preserves reproductive tissue integrity and hormonal homeostasis while producing systemic hematological changes. The maintenance of normal histoarchitecture in reproductive organs, coupled with stable hormonal profiles, suggests potential safety for reproductive health applications, supporting traditional medicinal claims. However, the observed hematological alterations warrant careful consideration for dosage optimization and monitoring protocols in clinical applications. *Chrysophyllum albidum* fruit extract may offer a promising natural approach to reproductive health management with preserved organ function and minimal hormonal disruption.

CHAPTER ONE
1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Chrysophyllum albidum fruit, commonly known as African star apple, is a tropical fruit indigenous to West Africa that has gained attention due to its nutritional and medicinal properties. Traditionally, various parts of the plant, including its leaves, seeds, and fruits, have been utilized in folk medicine to treat a myriad of ailments (Anang *et al*., 2019). Pharmacological research has established that the fruit possesses significant antioxidant properties, attributed primarily to its high polyphenol content (Oboh *et al*., 2018). Additionally, studies suggest that various extracts from the African star apple exhibit anti-inflammatory, anticancer, and antimicrobial mechanisms. The fruit's unique composition provides a promising avenue for exploring its effects on reproductive health, particularly within the context of animal models (Oboh *et al*., 2018).

In addressing reproductive health, the effects of dietary components on reproductive function have been observed in several studies involving different plant extracts. For instance, extracts from plants have been credited with enhancing reproductive parameters in both male and female organisms through the modulation of hormonal activities and antioxidant profiles (Igwe *et al*., 2024; Ali *et al*., 2024). A growing body of evidence highlights the negative influence of oxidative stress on reproductive health, compelling researchers to investigate potential protective and ameliorative factors present in natural sources like the African star apple (Ibrahim *et al*., 2024).

Focusing on male and female albino rats as a model is both practical and beneficial, given their physiological similarities to human pathological conditions. Preliminary studies suggest that the

oxidative stress alleviated by natural antioxidants such as those found in *Chrysophyllum albidum* can improve reproductive outcomes

Given the alarming rise in reproductive disorders globally, understanding the potential role of such natural products in sustaining and enhancing reproductive health warrants comprehensive investigation.

1.2 STATEMENT OF THE PROBLEM

Despite the reported benefits of *Chrysophyllum albidum* in traditional medicine, a systematic examination of its effects on the reproductive health of albino rats remains largely unexplored. The gap in scientifically validated studies challenges the effective incorporation of *Chrysophyllum albidum* extracts into therapeutic regimes for reproductive health. Although existing research indicates potential protective effects against oxidative stress (Ibrahim *et al*., 2024), there are very few robust studies investigating the direct impact of the African star apple on reproductive hormones, sperm quality, and overall reproductive health in male and female albino rats.

Several studies exploring the mechanisms of reproductive toxicity induced by various xenobiotics have primarily focused on synthetic compounds, leaving a gap for natural extracts like those from *Chrysophyllum albidum* (Ajayi *et al*., 2020). Current literature concerning the reproductive impacts of *C. albidum* inadequately addresses the dose-response relationships, histological changes in reproductive organs, and biochemical alterations associated with extract administration (Igwe *et al*., 2024). By exploring these gaps, the proposed research aims to elucidate the reproductive enhancement potential of *C. albidum*, thereby providing

comprehensive insights into the underlying mechanisms of its health benefits (Akinmoladun *et al*., 2022).

1.3 JUSTIFICATION OF THE STUDY

Chrysophyllum albidum is traditionally utilized in West African medicine for its purported health benefits; however, scientific investigations into its effects on reproductive health remain limited. The fruit is rich in antioxidants, which can counteract oxidative damage linked to various reproductive disorders. Previous research has highlighted the protective role of antioxidants against reproductive toxicity, underscoring the importance of assessing the effects of *C. albidum* on male and female reproductive systems in animal models. Furthermore, the unique environmental and dietary factors in Benin City, Edo State, amplify the need for tailored research on local flora, such as *C. albidum*, to ascertain their effectiveness as natural remedies for enhancing reproductive health. Given the rising concerns regarding reproductive health in contemporary society, this study's findings could provide critical insights for developing interventions and dietary recommendations that incorporate this indigenous fruit, ultimately contributing to improved reproductive health outcomes.

1.4 SIGNIFICANCE OF THE STUDY

The significance of this study lies in its potential to provide empirical evidence for the traditional claims regarding the medicinal properties of *Chrysophyllum albidum*. A thorough investigation into its effects on reproductive health will contribute to the broader understanding of how natural products can be utilized for therapeutic benefits, especially in combating reproductive dysfunctions (Anang *et al*., 2019; Enujiugha *et al*., 2023).

The implications of this research extend into clinical practices, potentially offering alternative or complementary treatment strategies for individuals experiencing reproductive health issues. Given the escalating incidence of endocrine disruptors and the resultant reproductive disorders, leveraging plant-based therapies could enhance patient management strategies and reduce dependency on synthetic medications, which often come with adverse effects (Ali *et al*., 2024; Igwe *et al*., 2024).

Moreover, this study aims to align with recent findings that affirm the physiological role of antioxidants in safeguarding reproductive organs from toxic insults (Ibrahim *et al*., 2024). As research continues to suggest the cumulative benefits of including plant extracts into dietary regimens, elucidating the protective roles of *C. albidum* could align with global health recommendations aimed at promoting natural remedies.

1.5 AIM OF STUDY

The aim of this study was to determine the histomorphological effects of *Chrysophyllum albidum* on the reproductive organs of albino rats.

1.6 Research Hypothesis

The consumption of *Chrysophyllum albidum* fruit extract affects the reproductive organs and hormones of albino rats.

1.7 Specific Objectives of the study

The specific objectives of this study were to:

1. Determines the histomorphological effects of *Chrysophyllum albidum* fruit extract on the testis, prostate, ovaries, and uterus of albino rats.

2. Evaluate the impact of *Chrysophyllum albidum* fruit extract on the reproductive hormones of albino rats.

1.8 Research Questions

1. What are the histomorphological effects of *Chrysophyllum albidum* fruit extract on the testis, prostate, ovaries, and uterus of albino rats?

2. What are the impacts *Chrysophyllum albidum* fruit extract on the reproductive hormones of albino rats?

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 *Chrysophyllum albidum* (Overview)

Chrysophyllum albidum, commonly known as the African Star Apple, Agbalumo or Udara is a tropical fruit tree belonging to the Kingdom Plantae, Phylum Angiosperms, Class Eudicots, Order Sapotales, Family Sapotaceae, Genus Chrysophyllum, and Species albidum (Dandare *et al*., 2018). This species is primarily found in tropical rainforest regions, with its distribution spanning across West, Central, and East Africa (Ganglo, 2023). The ecological preferences of *C. albidum* align with humid climates that provide essential rainfall and temperature for optimal growth (Ganglo, 2023).

Historically, various parts of *C. albidum* have been utilized in traditional medicine, particularly in the treatment of reproductive health issues. Ethnomedicinal applications of the plant include its use in addressing conditions such as infertility and sexual dysfunctions, drawing from its reputation as a remedy in local pharmacopoeia (Oigbochie *et al*., 2019). Furthermore, recent ecological assessments highlight the significant role that *C. albidum* plays in local diets and healing practices, indicating its potential for broader application in both nutrition and medicine (Dandare *et al*., 2018).

Figure 2.1: African star apple Fruit (*Chrysophyllum albidum*) (Erhirhie *et al.*, 2015).

2.2 Nutritional Profile

Chrysophyllum albidum is known for its culinary appeal and also for its nutritional richness. The cotyledons and pulp of its fruits are packed with vital nutrients including carbohydrates, dietary fibers, proteins, vitamins A and C, and numerous minerals that can enhance human health (Ezeobi *et al*., 2021). An analysis by Adonu *et al*. in 2023 revealed that the nutritional composition of *C. albidum* varies significantly among its parts. This study featured quantitative assessments indicating high levels of antioxidant compounds that are noted for their therapeutic benefits.

The plant's phytochemical content comprises a variety of bioactive compounds, including, but not limited to, alkaloids, tannins, flavonoids, and saponins (Adonu *et al*., 2023). Research indicates that these phytochemicals, particularly flavonoids and tannins, exhibit considerable pharmacological properties, highlighting their potential as natural antioxidants and anti-inflammatory agents (Odewade and Odewade, 2023; Ewelike *et al*., 2021). Specifically, the antioxidants present in *C. albidum* have shown efficacy in scavenging free radicals and reducing oxidative stress, thereby contributing to overall health and potentially modulating reproductive functions (Odewade and Odewade, 2023).

Moreover, the bioactive properties of *C. albidum* extracts have been shown to impact reproductive parameters, such as hormone levels and sperm viability in animal models (Oigbochie *et al*., 2019). The presence of cardiac glycosides and flavonoids signals their potential synergistic effect on the reproductive system, thereby warranting further exploration of their mechanisms of action (Odewade and Odewade, 2023).

Figure 2.2: African star apple Fruit (*Chrysophyllum albidum*) ((Erhirhie *et al.*, 2015).).

In comprehensive nutritional studies, various researchers have confirmed the presence of these phytochemicals in varying amounts across the plant's parts; leaves, fruits, seeds, and roots all contribute uniquely to its overall health benefits (Odewade and Odewade, 2023; Ezeobi *et al*., 2021). This diversity reinforces the need for a thorough investigation into the roles of phytochemicals and their implications on reproductive health, yielding a better understanding of *C. albidum*'s complete therapeutic potential.

It is essential to systematically categorize these phytochemical constituents and evaluate their interaction with biological systems, particularly focusing on how they may influence reproductive health in both traditional settings and modern medical applications (Ezeobi *et al*., 2021; Odewade and Odewade, 2023).

2.3 Phytochemical Constituents of *Chrysophyllum albidum*

2.3.1 General Overview of Chemical Constituents

Chrysophyllum albidum is rich in a diverse array of bioactive compounds that are essential for its medicinal properties. Key phytochemical constituents include alkaloids, flavonoids, tannins, and saponins, which have been shown to modulate various physiological functions, including reproductive health. Alkaloids are well-documented for their analgesic and antispasmodic properties, while flavonoids and tannins exhibit significant antioxidant effects, contributing to cellular protection and modulation of reproductive functions (Odewade and Odewade, 2023; Ogbu *et al*., 2024).

Studies have demonstrated that *C. albidum* contains these phytochemical families in varying concentrations across different plant parts. For instance, Odewade and Odewade in 2023 noted that the leaf extract of *C. albidum* was abundant in tannins, alkaloids, flavonoids, and saponins,

aligning with historical reports of their presence in other parts of the plant, thereby substantiating the plant's use in traditional and modern medicine. This variability is crucial for understanding how different extracts may interact with reproductive parameters.

Figure 2.3: A Comprehensive Review on Ethno-Medicine, Phytochemistry and Ethnopharmacology of *Chrysophyllum albidum* (Erhirhie *et al*., 2015).

1. Flavonoids

Flavonoids, which are predominantly found in the leaves of *Chrysophyllum albidum*, are largely credited for their antioxidant activity. As powerful antioxidants, they participate in scavenging free radicals and mitigating oxidative stress, which is pivotal for maintaining cellular integrity and health (Ezeobi *et al*., 2021; Adonu *et al*., 2023). The antioxidant potential of flavonoids derived from *C. albidum* is particularly important for gonadal health—frequent oxidative stress in reproductive tissues can lead to damage in cellular structures such as DNA, proteins, and lipids, ultimately affecting fertility.

Research by Asagba *et al*. in 2019 shows that flavonoids in *C. albidum* can induce the expression of enzymes associated with antioxidative defenses, emphasizing their role in enhancing reproductive function and tissue preservation (Asagba *et al*., 2019). Moreover, their ability to modulate signaling pathways regulates spermatogenesis, potentially contributing to improved sperm quality and reproductive hormone balancing (George *et al*., 2018). Such findings underscore the importance of flavonoids in the overall pharmacological impact of *Chrysophyllum albidum*, warranting further investigation into their specific mechanisms affecting reproductive health.

2. Alkaloids

Alkaloids present in *C. albidum* exhibit various medicinal properties, including analgesic, antispasmodic, and enzyme inhibitory activities that may synergistically influence reproductive histomorphology. The analgesic properties of these compounds can alleviate pain associated with reproductive health issues, thereby improving the quality of life for individuals facing challenges in this area (Odewade and Odewade, 2023). The presence of these alkaloids has been correlated

with potential therapeutic effects on reproductive dysfunctions, opening avenues for further exploration of *C. albidum* as a complementary treatment option for reproductive health disorders.

The enzyme inhibitory activities attributed to alkaloids may also influence hormonal pathways within the reproductive axis, potentially leading to alterations in hormone levels crucial for spermatogenesis and ovarian function (Ekwealor *et al*., 2023). For instance, studies indicate that certain alkaloids may modulate gonadotropin release, which is essential for maintaining reproductive health as they interact within the hypothalamic-pituitary-gonadal axis. Therefore, the alkaloid fraction of *C. albidum* represents a significant area for future research aimed at elucidating specific mechanisms that affect reproductive health.

3. Tannins and Saponins

Tannins and saponins are pivotal components of *C. albidum* that contribute not only to its protective functions against pests and pathogens but also possess potential implications for reproductive health. Tannins are known for their ability to bind proteins, which could contribute to antimicrobial activity and protective mechanisms against diseases that may impact reproductive organs (Ogbu *et al*., 2024). Their role in traditional medicine, particularly regarding wound healing and inflammation, is relevant to reproductive pathologies where inflammatory processes can impair reproductive function.

Similarly, saponins are recognized for their antioxidant properties and potential to enhance immune responses, which may afford protection to reproductive organs against infections and other pathological conditions (Odewade and Odewade, 2023; Ewelike *et al*., 2021). Research indicates that saponins may act as growth promoters and can modulate hormonal activities, potentially impacting reproductive success.

Furthermore, both tannins and saponins exhibit phytochemical activities that suggest a therapeutic protective role in reproductive health, although excessive consumption can have negative effects such as toxicity to reproductive tissues (Ekwealor *et al*., 2023). Therefore, understanding the concentrations and bioactivity of these phytochemicals in *C. albidum* is crucial for defining their medicinal applications and ensuring safe integration into healthcare practices.

The diverse phytochemical constituents of *Chrysophyllum albidum*, particularly flavonoids, alkaloids, tannins, and saponins, suggest that this plant holds considerable promise for modulating reproductive health. Ongoing and future research should focus on delineating the biochemical pathways involved and exploring the therapeutic potential of these compounds in clinical settings.

2.4 Reproductive Organs in Albino Rats: Anatomical and Histological Framework

2.4.1 Overview of the Reproductive System

The reproductive system of Albino rats (*Rattus norvegicus*) has been extensively studied, serving as a valuable model for investigating human reproductive health. The male reproductive system includes vital components such as the testes, epididymis, and accessory glands, while the female reproductive system comprises the ovaries, uterus, and associated structures. The testes, located in the scrotum, are the primary site of spermatogenesis, producing sperm cells through a highly organized process that occurs within seminiferous tubules (Oigbochie *et al*., 2019). The epididymis stores and matures these sperm cells before they are expelled during ejaculation. Accessory glands, such as the seminal vesicles and prostate, contribute seminal fluid, enhancing the survivability and mobility of sperm.

In female albino rats, the ovaries produce oocytes and secrete hormones such as estrogen and progesterone, which are critical for regulating the estrous cycle. The uterus provides an environment for embryo implantation and development, supported by a rich vascular supply that facilitates nutrient exchange (Agbaje, 2020). Comparative studies highlight the significant similarity between rat reproductive anatomy and that of humans, providing a robust framework for histological analysis in laboratory settings (Ibrahim *et al*., 2019).

2.4.2 Gross Anatomy of Reproductive Organs

The gross anatomy of the reproductive organs in rats is characterized by distinct size and weight parameters relevant to histological studies. In male albino rats, the average weight of the testes can range from approximately 1.5 to 2.0 grams, making up a significant portion of their body weight. The epididymis, while smaller, is crucial for the maturation of sperm cells and may weigh around 0.3 grams. In females, the ovaries typically weigh approximately 0.2–0.5 grams, depending on the hormonal status and phase of the reproductive cycle (Agbaje, 2020). The uterus, which has a unique tubular structure, is significantly larger, reflecting the need to support developing embryos.

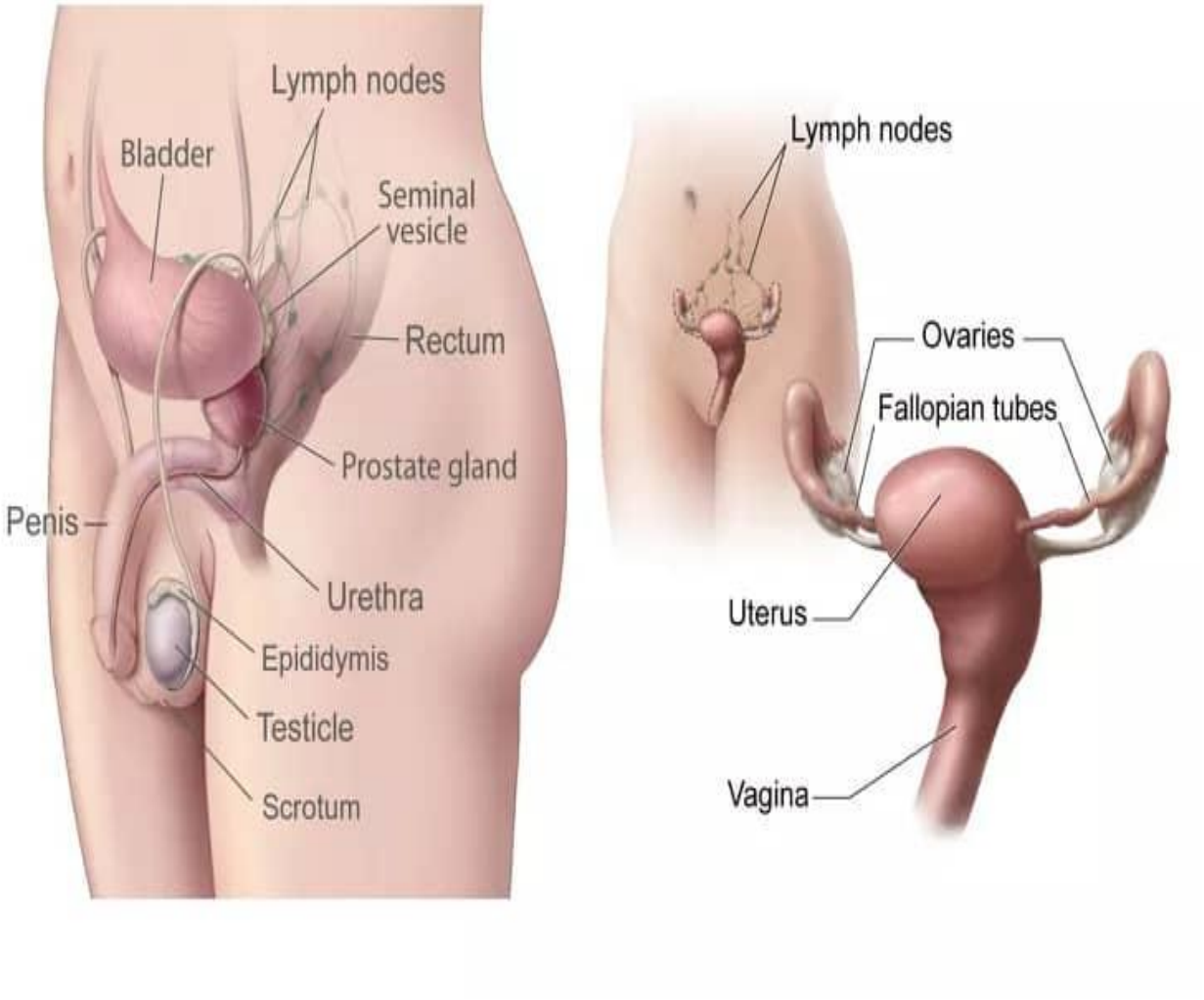

Figure 2.4: Male Gonads (Testes) and Female Gonads (Ovaries) (NIH Medical Arts, 2025)

The size and organization of these organs are intricately linked to their histological architecture. For instance, normal histoarchitecture in male testes is defined by well-organized seminiferous tubules comprised of Sertoli and germ cells, interspersed with Leydig cells that produce testosterone. The female reproductive system reflects similar organized structures; ovaries present follicles at various maturation stages, and the uterine lining demonstrates cyclical changes correlating with reproductive hormones (Ibrahim *et al*., 2019).

2.4.3 Histology and Morphological Characteristics

Histologically, the reproductive organs of albino rats display distinct features vital for evaluating histomorphological changes. The seminiferous tubules of the testes are characterized by a unique cord-like arrangement, which facilitates the efficient development of sperm cells. Each tubule is surrounded by a basement membrane and is filled with germinal epithelium featuring spermatogonia, spermatocytes, and spermatids at various developmental stages (Oigbochie *et al*., 2019). Interstitial spaces contain Leydig cells, responsible for testosterone secretion, which is crucial for sperm production and overall male reproductive function.

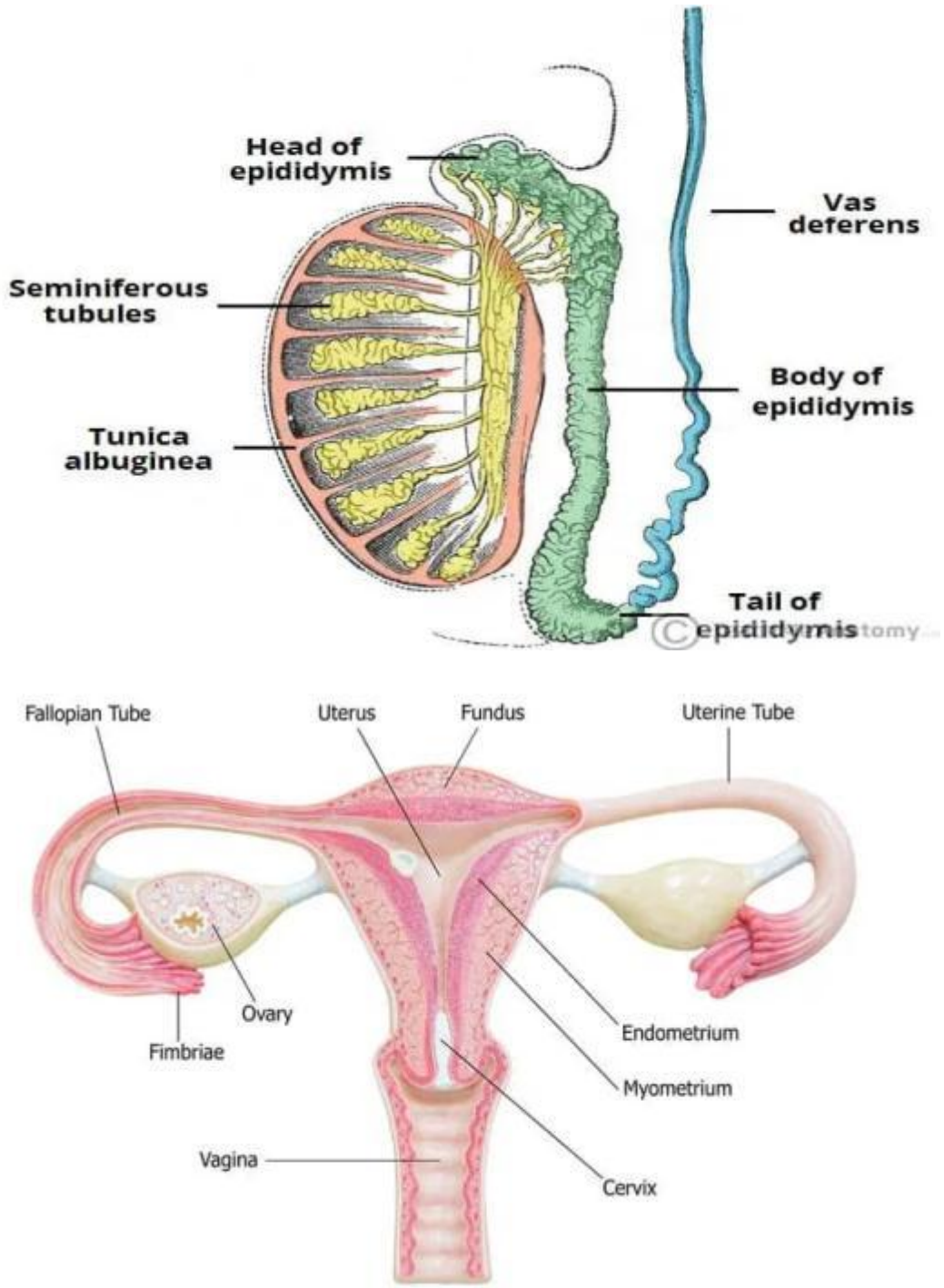

Figure 2.5: Structure of the testes and epididymis (TeachMeSeries Ltd, 2024), (b) The female reproductive system (Alina, 2022).

The epididymis exhibits a convoluted duct structure lined with pseudostratified columnar epithelium, facilitating the maturation and storage of sperm. In the female reproductive system, the ovaries consist of an outer cortex filled with primordial and developing follicles and an inner medulla containing blood vessels and supportive tissues (Oigbochie *et al*., 2019). Histological assessments can reveal important alterations in cellular organization and function, such as those caused by pharmacological interventions by monitoring follicular development and corpus luteum formation within the ovaries.

The vascularization of reproductive organs, particularly the extensive capillary networks associated with the ovarian and testicular tissues, plays a critical role in nutrient supply and hormonal exchange, making it an essential parameter in evaluating the histological integrity and functionality of these organs (Adonu *et al*., 2023). Histomorphometric analyses, which quantify these vascular and cellular compositions, provide a robust comparative measure against the anticipated morphological changes induced by treatments like *Chrysophyllum albidum* extract

The established anatomical and histological frameworks for male and female reproductive organs in albino rats provide a critical baseline for understanding the effects of various treatments. This knowledge is necessary for evaluating the histomorphological changes resulting from interventions, such as the administration of plant extracts like *Chrysophyllum albidum*, which may yield significant insights into the preservation or enhancement of reproductive health.

2.5 Impact of *Chrysophyllum albidum* Extract on Reproductive Histomorphology

2.5.1 Effects on Hormonal Profiles

The administration of *Chrysophyllum albidum* extracts has demonstrated significant effects on the hormonal profiles of male rats. A study conducted by Oigbochie *et al*. in 2019 found a dose-

and duration-dependent increase in serum levels of follicle-stimulating hormone (FSH) following the administration of aqueous root extracts. This finding suggests a potential disruption of the hypothalamic-pituitary-gonadal (HPG) axis, which is critical for regulating reproductive functions. Furthermore, alterations in levels of testosterone and luteinizing hormone (LH) were also noted, indicating that the extract may influence endocrine responses significantly.

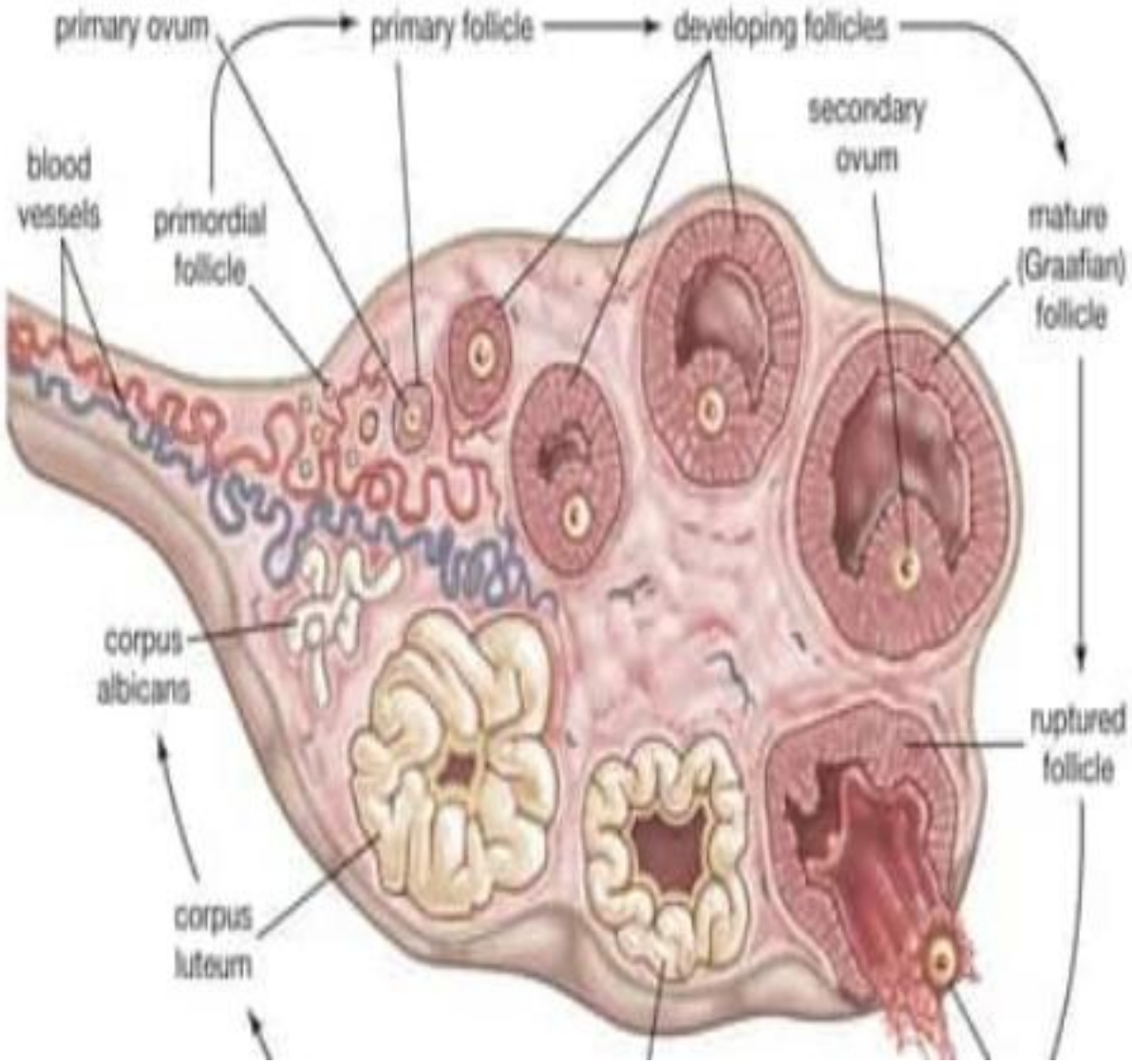

Figure 2.6: The steps of ovulation (Utiger, 2025).

The potential endocrinological mechanisms behind these alterations may stem from the bioactive compounds present in *C. albidum*. For instance, the presence of flavonoids and alkaloids in the extracts could modulate enzyme activities that participate in the synthesis and secretion of gonadotropins and sex hormones. Experimental data indicate that prolonged administration of *C. albidum* extracts correlates with a decline in testosterone levels, which may suppress spermatogenesis and overall reproductive functionality due to the negative feedback mechanism on gonadotropin release (Ogunwole and Mangai, 2019). A comprehensive understanding of the dosing window and duration of exposure is essential for characterizing the extract's effects in this context.

2.5.2 Histomorphological Changes Induced by the Plant Extract

Administration of *Chrysophyllum albidum* extract has been observed to elicit notable histomorphological changes in reproductive tissues. In particular, alterations to the seminiferous tubule architecture in the testes are anticipated following treatment. Histopathological analyses show that the extract can disrupt the tightly organized structure of the seminiferous tubules, potentially leading to reduced spermatocyte and spermatid populations and increased cellular apoptosis (Oigbochie *et al*., 2019). This is critical as any impairment in seminiferous tubule architecture directly affects the male reproductive capacity by decreasing sperm production and quality.

Moreover, vascular changes indicative of altered perfusion levels can occur in the reproductive tissues due to the extract's effects. Enhanced vascular permeability and alterations in blood supply may lead to localized hypoxia, contributing further to cellular apoptosis within the testes. Current literature indicates that while some plant extracts exhibit protective effects on

reproductive organs, *C. albidum* may present a dual action that necessitates careful consideration of dosing to mitigate adverse histological implications (Nugroho *et al*., 2022).

Furthermore, the substantial histological alterations reflect the need for detailed pathological examinations to clarify the functional outcomes of using *C. albidum* extracts in a therapeutic context.

2.5.3 Mechanisms of Action

The bioactive components of *Chrysophyllum albidum*, mainly flavonoids and alkaloids, are integral to understanding the histomorphological effects observed. Flavonoids have been documented to exert antioxidative effects that protect cellular structures from oxidative damage, which is particularly relevant in the context of germ cell integrity and hormone regulation. While some references discuss various aspects of flavonoids' effects, specific studies on *C. albidum* are limited and indicate further research is needed to confirm these mechanisms (Ewelike *et al*., 2021).

Alkaloids, known for their bioactivity, may influence the signaling pathways of the HPG axis, potentially leading to altered levels of testosterone, LH, and FSH. This modulation may manifest as feedback inhibition, where elevated testosterone levels subsequently inhibit the secretion of gonadotropins, thus negatively impacting spermatogenesis and contributing to observed histomorphological disruptions (Ogunwole and Mangai, 2019).

A comprehensive understanding of these pathways can help elucidate the potential impacts of *C. albidum* on reproductive health and the mechanisms through which its extracts exert influence on hormonal and histological profiles in both sexes.

2.5.4 Comparative Analysis with Other Plant Extracts

When comparing the reproductive outcomes of *Chrysophyllum albidum* with other medicinal plant extracts, it becomes apparent that each plant possesses unique phytochemical profiles that influence reproductive health differently. For example, the protective effects of extracts like Ficus deltoidea on reproductive parameters have been documented, revealing enhancements in sperm quality and histoarchitecture, which contrast with the adverse effects noted from *C. albidum* (Nugroho *et al*., 2022).

Research demonstrates that varying doses and treatment durations of plant extracts can elicit both beneficial and detrimental effects on reproductive organs. Therefore, a thorough assessment is necessary to understand the specific phytochemical constituents at play within each extract, as well as their respective histological findings. This distinction is critical for establishing effective dosing strategies and safety parameters when considering the therapeutic applications of *Chrysophyllum albidum* in reproductive health.

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 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 had 4 rats each.

Group A is the control group, and the male and female (n=2) rats received pellets and distilled water only.

Group B had two male and female rats (n=4) and was administered 250mg/kg of *Chrysophyllum albidum* fruit extract for 1 month.

Group C had two male and female rats (n=4) and were given 500mg/kg of *Chrysophyllum albidum* fruit extract for 1 month.

Group D had two male and female rats (n=4) and was administered 1000mg/kg of *Chrysophyllum albidum* fruit extract for 1 month.

All animals were administered orally via oral gavage.

3.2 Collection of the leaves of *Chrysophyllum albidum*

Fresh *Chrysophyllum albidum* fruit will be obtained from the Uselu market. The fruit will be authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

3.3.1 Identification and Authentication

Dr. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, identified and authenticated the *Chrysophyllum albidum*. Following that a sample was placed in the departmental herbarium, and a voucher number UBH-G362 was given for referral.

3.3.2 Extraction included *Chrysophyllum albidum* fruit

The extraction was conducted at the Department of Pharmacology, University of Benin, Benin City. The fruits were thoroughly washed with distilled water to eliminate contaminants. The pulp, seeds, and skin were separated from the fruit and dried in the shade. After being air-dried, the materials were ground to a fine powder using a laboratory grinder. To obtain the bioactive extract, 50 grams of each powdered component (pulp, seeds, and skin) were soaked separately in 500 ml of ethanol for 48 hr at room temperature. The mixture was agitated continuously to enhance extraction efficacy. After 48 hr, the mixtures was filtered using a fine mesh cloth to separate the solids from the liquid. The filtrate was dried using a rotary evaporator, yielding a concentrated extract for subsequent analyses (Chemat *et al*., 2012; Ehiremen *et al*., 2024).

3.3.3 Concentration Preparation

The concentrated soursop leaf extract was reconstituted in a 1% acetic acid solution to achieve a final concentration of 1% (w/v). The solution was mixed thoroughly and adjusted to a pH of 4.0 using hydrochloric acid.

3.3 Animals

Twenty-four (24) albino rats weighing between 180g and 200g were purchased from the Animal House of the Department of Anatomy, University of Benin, Benin City. The animals were kept

in plastic cages with wire gauges for 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 of 26°C and 46% respectively. The housing facilities were kept sterile, aerated, and well-maintained regularly.

3.3.1 Acute Toxicity Test

The lethal dose (LD50) of *Chrysophyllum albidum* fruit 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 *Chrysophyllum albidum* fruit 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 placed under observation for any signs of adverse effects including lethargy, change in locomotive activity, abnormal behavior, and death for 48hrs.

3.4 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: 025-07/29/0041. The rats were handled following the Guidelines for the Care and Use of Laboratory Animals.

3.5 Specimen collection

Following the administration period, the animals were euthanized using cervical dislocation, and blood specimen was obtained 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 hr.

3.5.1 Processing of Specimen

3.5.1.1 Biochemical Analyses

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

1. Preparation of Blood Samples:

Collection: Venous blood samples (approximately 5–10 mL) were obtained via cardiac puncture, following ethical guidelines. Samples were collected in sterile tubes without anticoagulant to allow clotting.

Storage: Serum samples were stored at -20 °C until analysis to minimize degradation of hormones (Butler, 2018)

2. Assay Procedure Using ELISA Kits:

a) Reagents and Standards: The ELISA kits for testosterone, progesterone, LH, and FSH were obtained from a reputable supplier (Diagnostic Automation Inc., USA). Each kit contain necessary reagents, including capture antibodies, detection antibodies, and standard solutions to generate a calibration curve (Karpus and Zhang, 2019).

b) Preparation of Samples:

Prior to the assay, serum samples were diluted according to the manufacturer's instructions (typically 1:4 or 1:10 with the sample diluent provided by the kit).

c) Coating the Plate:

Each well of the ELISA plate was coated with specific capture antibodies against the hormone being measured. Plates were incubated overnight at 4 °C, and then washed multiple times with wash buffer to remove unbound antibodies.

d) Addition of Standards and Samples:

After washing, standards (with known hormone concentrations) added to the appropriate wells alongside diluted serum samples. The plate was incubated for a specified time (usually 1–2 hours) at room temperature to allow binding of target hormones to the capture antibodies.

e) Detection Antibody Addition:

Following the incubation, detection antibodies was added to each well. These antibodies bound to the hormone-antibody complexes formed in the wells. The plate was again incubated as per the kit instructions.

f) Substrate Addition:

After washing to remove unbound detection antibodies, a substrate solution was added to each well. The enzyme linked to the detection antibodies catalyze a reaction producing a color change that is proportional to the amount of hormone in each well (Karpus and Zhang, 2019).

g) Stopping the Reaction:

The reaction was stopped by adding a stop solution, typically an acid which halt the enzyme activity (Karpus and Zhang, 2019).

h) Plate Reading:

The plate was then read using a microplate reader (Axiom Microplate reader Urit-660) at the wavelength specified by the kit (usually around 450 nm). The optical density (OD) values was recorded.

i) Calculation of Hormone Levels: The concentration of hormones in the serum samples was determined using the calibration curve constructed from the OD values of the standards. Hormone levels in samples was expressed in appropriate units (e.g., ng/mLtissues for testosterone and FSH) (Butler, 2018).

3.5.1.2 Histopathological investigation

The fixed 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 0μ, sectioned at 3μ, and dried on a hot-plate for 15 min. Sections were stained in Cole's haematoxylin and 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 Wallinglon, 1980). Sections were microscopically examined using x10 and x40 objective lenses.

3.6 Statistical Analysis

Data collected was 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 AND ANALYSIS

4.1 Effect of *Chrysophyllum albidum* fruit extract on FBC analytes

Chrysophyllum albidum extract had a significant effect ($p < 0.05$) on the values of lymphocytes (%), neutrophils, neutrophils (%), MCH, MCHC, red blood cell count, PDW CV and PCT.

Table 4.1: ANOVA comparison of the effect of *Chrysophyllum albidum* on FBC analytes in control group

	Group	Mean ± SD	df	F	Sig.
WBC	Control	8 ± 3.4	3	2.563	0.104
LYM	Control	6.3 ± 2.6	3	1.065	0.4
LYM (%)	Control	78.4 ± 2.3	3	5.839	0.011
EOS	Control	0.1 ± 0.1	3	0.499	0.69
EOS%	Control	0.6 ± 0.6	3	0.36	0.783
MON	Control	1.4 ± 1.3	3	0.507	0.685
MON%	Control	7.3 ± 3.9	3	1.089	0.391
BAS	Control	0 ± 0	3	2.232	0.137
BAS%	Control	0.2 ± 0.1	3	1.415	0.287
NEU	Control	0.7 ± 0.3	3	7.624	0.004
NEU%	Control	8.8 ± 0.8	3	8.608	0.003
HGB	Control	13.7 ± 0.9	3	0.603	0.626
MCH	Control	19.7 ± 0.3	3	7.956	0.003
MCHC	Control	32.5 ± 3.1	3	5.941	0.01
RBC	Control	7 ± 0.5	3	9.307	0.002
MCV	Control	61.1 ± 5.8	3	1.945	0.176
HCT	Control	42.7 ± 5.4	3	1.266	0.33
RDW CV	Control	19.2 ± 2.7	3	1.122	0.379
RDW SD	Control	55.9 ± 14.3	3	1.971	0.172
PLT	Control	662.8 ± 227.3	3	1.286	0.324
MPV	Control	7.8 ± 0.3	3	0.298	0.826
PDW CV	Control	10.1 ± 0.4	3	18.851	<0.001
PDW SD	Control	28.7 ± 1.1	3	1.541	0.255
PCT	Control	1.5 ± 0.2	3	6.873	0.006
P-LCR	Control	0.1 ± 0	3	0.153	0.926
P-LCC	Control	84.8 ± 25.8	3	3.019	0.072

Chrysophyllum albidum extract had a significant effect ($p < 0.05$) on the values of lymphocytes (%), neutrophils, neutrophils (%), MCH, MCHC, red blood cell count, PDW CV and PCT.

Table 4.2: ANOVA comparison of the effect of *Chrysophyllum albidum* on FBC analytes in Group B

	Group	Mean ± SD	df	F	Sig.
WBC	B	13.4 ± 3.1			
LYM	B	9.6 ± 2.8			
LYM (%)	B	71.7 ± 7.2			
EOS	B	0 ± 0			
EOS%	B	0.4 ± 0.2			
MON	B	1 ± 0.5			
MON%	B	7.3 ± 3.6			
BAS	B	0 ± 0			
BAS%	B	0.3 ± 0.1			
NEU	B	2.7 ± 0.7			
NEU%	B	20.5 ± 6.2			
HGB	B	12.6 ± 1.4			
MCH	B	28.2 ± 2.3			
MCHC	B	39.9 ± 4.3			
RBC	B	4.5 ± 0.8			
MCV	B	70.9 ± 7.2			
HCT	B	37.8 ± 4.1			
RDW CV	B	18.7 ± 1.9			
RDW SD	B	45.4 ± 3.3			
PLT	B	470 ± 147.8			
MPV	B	7.7 ± 0.4			
PDW CV	B	14.1 ± 1			
PDW SD	B	21.9 ± 17.7			
PCT	B	3.6 ± 1			
P-LCR	B	0.1 ± 0			
P-LCC	B	65.3 ± 17.3			

Chrysophyllum albidum extract had a significant effect ($p < 0.05$) on the values of lymphocytes (%), neutrophils, neutrophils (%), MCH, MCHC, red blood cell count, PDW CV and PCT.

Table 4.3: ANOVA comparison of the effect of *Chrysophyllum albidum* on FBC analytes in Group C

	Group	Mean ± SD	df	F	Sig.
WBC	C	18.5 ± 7.8			
LYM	C	178.5 ± 329.1			
LYM (%)	C	63 ± 8.9			
EOS	C	0.1 ± 0			
EOS%	C	0.4 ± 0.2			
MON	C	1.5 ± 0.7			
MON%	C	11 ± 4.8			
BAS	C	0.1 ± 0			
BAS%	C	0.4 ± 0.2			
NEU	C	4.5 ± 1.8			
NEU%	C	25.2 ± 5.6			
HGB	C	13.8 ± 1.1			
MCH	C	27.8 ± 3.1			
MCHC	C	39.5 ± 2.2			
RBC	C	5 ± 0.8			
MCV	C	70.5 ± 5.8			
HCT	C	41.3 ± 3.4			
RDW CV	C	17.3 ± 1.2			
RDW SD	C	43.1 ± 4.9			
PLT	C	544.3 ± 99.1			
MPV	C	7.9 ± 0.8			
PDW CV	C	13.5 ± 0.5			
PDW SD	C	19.2 ± 13.3			
PCT	C	4.2 ± 0.4			
P-LCR	C	0.1 ± 0.1			
P-LCC	C	76.8 ± 28.8			

Chrysophyllum albidum extract had a significant effect ($p < 0.05$) on the values of lymphocytes (%), neutrophils, neutrophils (%), MCH, MCHC, red blood cell count, PDW CV and PCT.

Table 4.4: ANOVA comparison of the effect of *Chrysophyllum albidum* on FBC analytes in Group D

	Group	Mean ± SD	df	F	Sig.
WBC	D	15.7 ± 6.5			
LYM	D	10 ± 4.3			
LYM (%)	D	63.5 ± 3.1			
EOS	D	0.1 ± 0.1			
EOS%	D	0.6 ± 0.5			
MON	D	1.8 ± 1.1			
MON%	D	10.9 ± 3.5			
BAS	D	0 ± 0			
BAS%	D	0.3 ± 0.1			
NEU	D	3.8 ± 1.4			
NEU%	D	24.7 ± 6.1			
HGB	D	14.2 ± 2.8			
MCH	D	28.7 ± 4.7			
MCHC	D	39.2 ± 1			
RBC	D	4.6 ± 0.9			
MCV	D	72.9 ± 10.5			
HCT	D	38.7 ± 3			
RDW CV	D	17.3 ± 0.9			
RDW SD	D	44.1 ± 6.7			
PLT	D	422 ± 231.3			
MPV	D	7.5 ± 0.7			
PDW CV	D	13.9 ± 1.3			
PDW SD	D	12 ± 1.3			
PCT	D	3 ± 1.4			
P-LCR	D	0.1 ± 0.1			
P-LCC	D	39.8 ± 15.6			

There was a statistically significant change ($p < 0.05$) in the body weight of the rats after administration of *Chrysophyllum albdium,* between the control group and groups B, C and D.

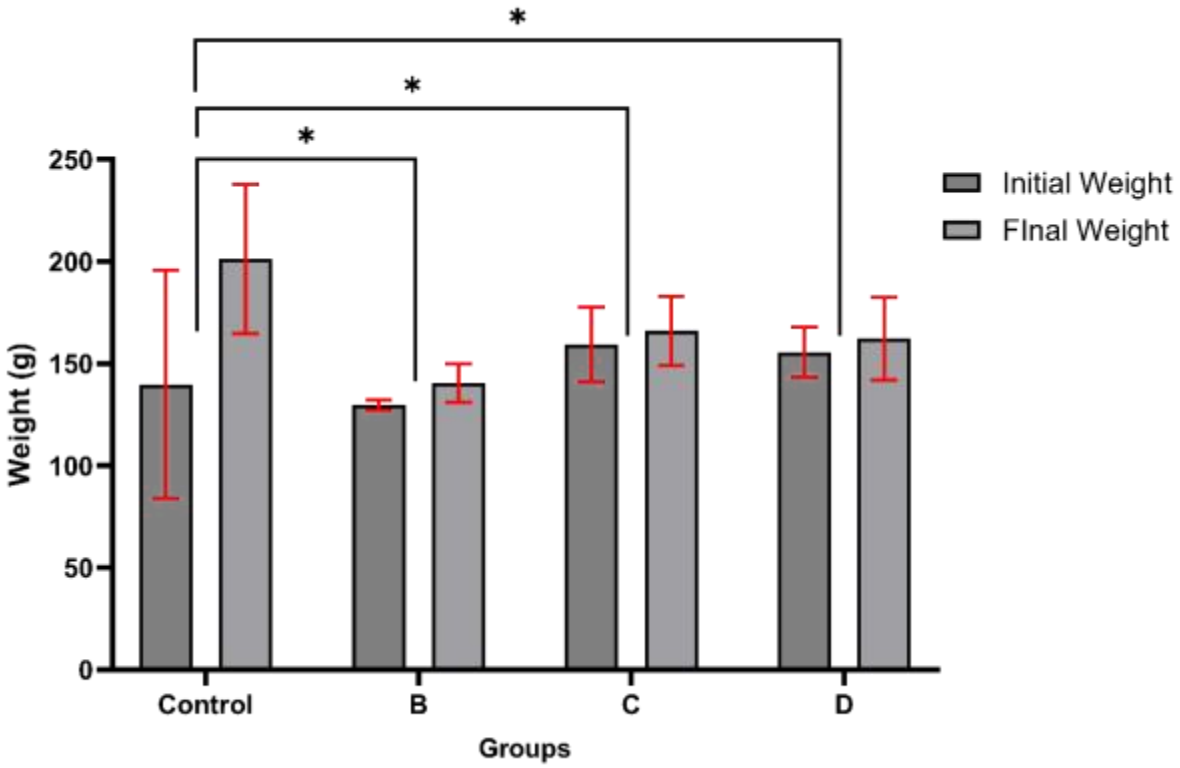

Figure 4.1: Data on weight of rats after administration

There was a significant difference ($p < 0.05$) in the weight of the rat testes between the control group and group D, as well as between group B and group D.

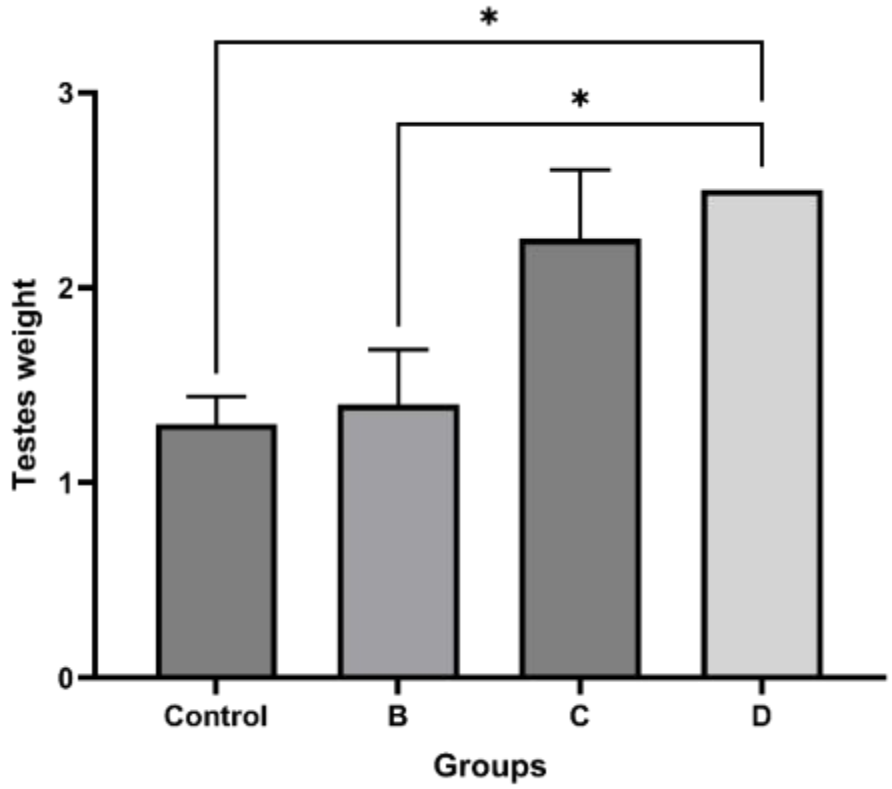

Figure 4.2: showing effect of *Chrysophyllum albdium* leaves extract on weight of the testes

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

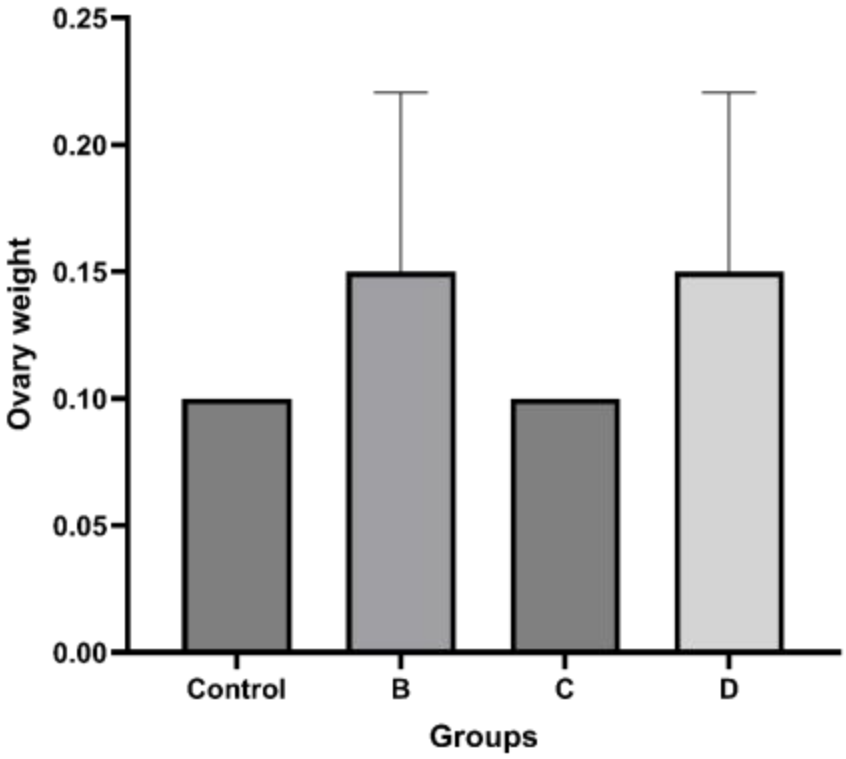

Figure 4.3: showing the effect of *Chrysophyllum albdium* leaves extract on weight of the ovaries

There was no significant difference ($p > 0.05$) in the testosterone levels across the control and 3 treatment groups.

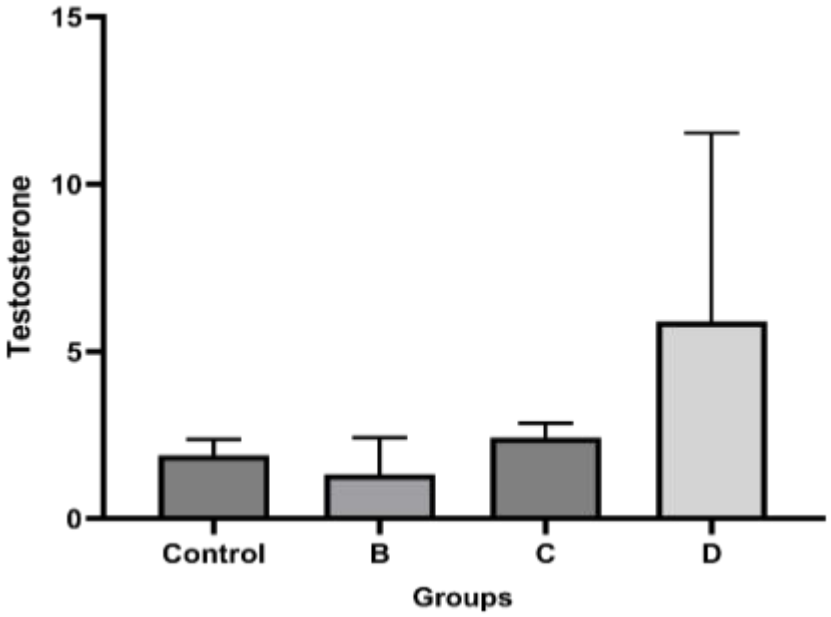

Figure 4.4: showing effect of *Chrysophyllum albdium* leaves extract on testosterone

There was no significant difference ($p > 0.05$) in the progesterone levels across the control and 3 treatment groups.

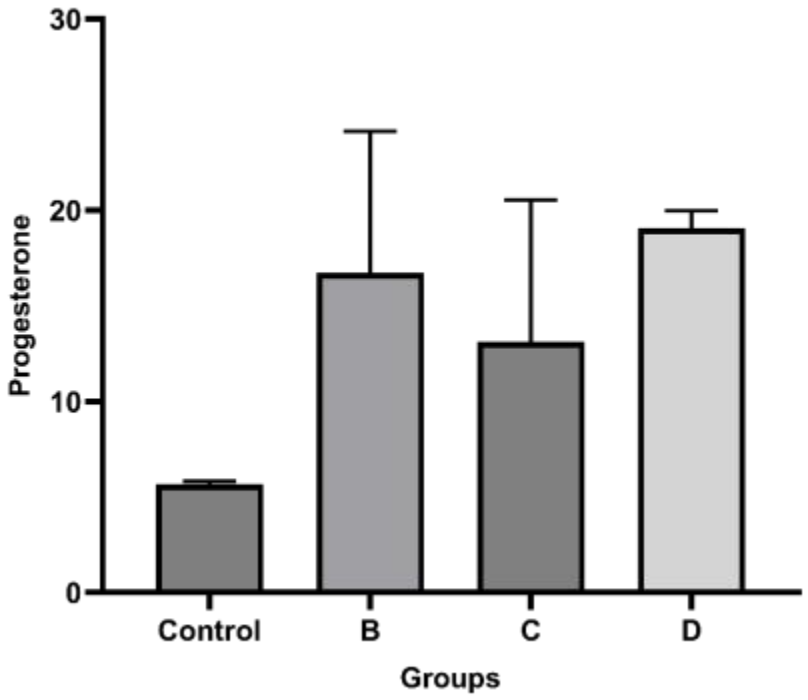

Figure 4.5: showing effect of *Chrysophyllum albdium* leaves extract on progesterone

Section of testes of male rat received pellet and distilled water only for 1 month showed 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 the presence of Leydig cells (thin arrow) in the interstitium. (H&E Stain, x 100 Mag)

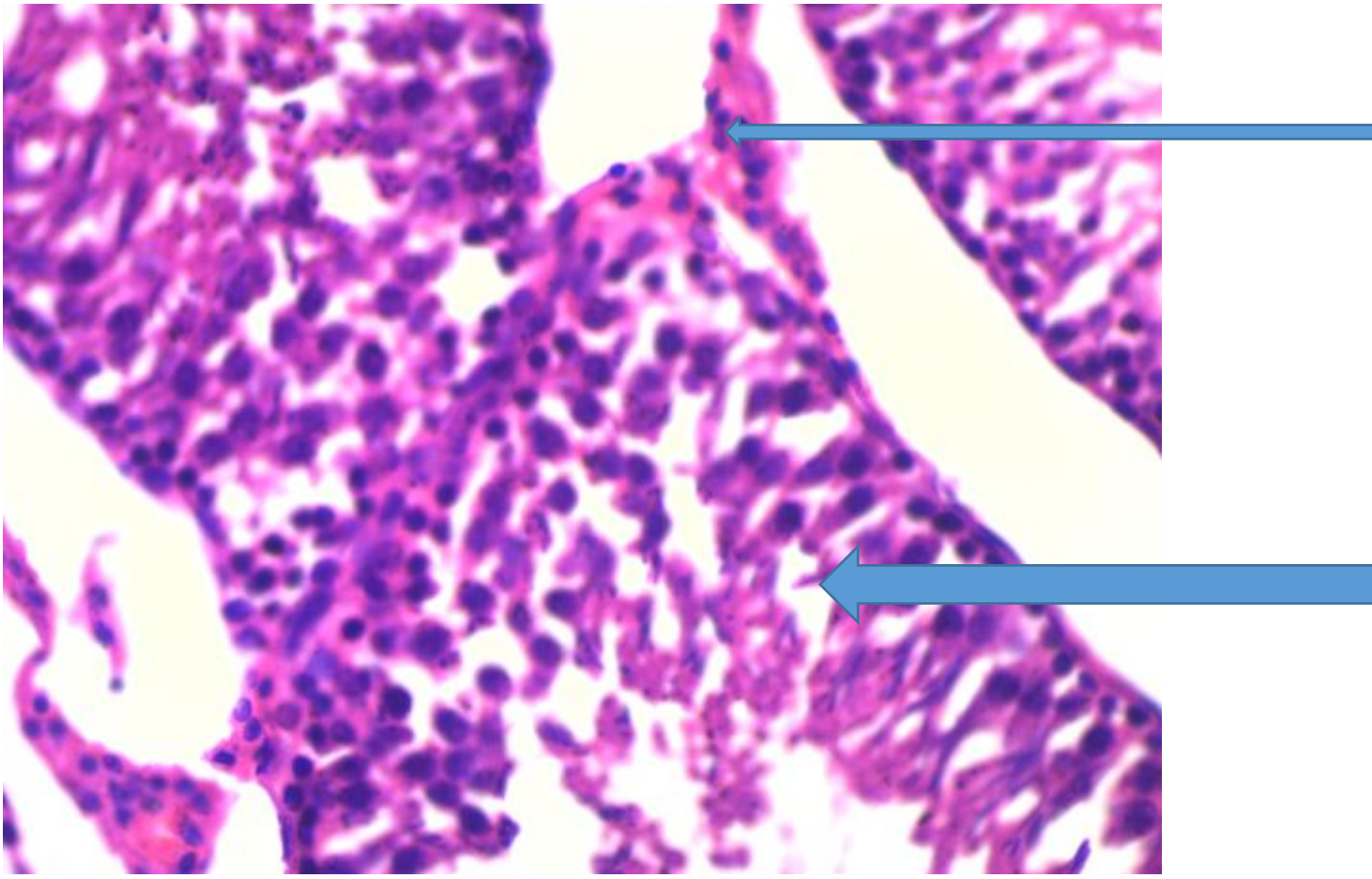

Plate 4.1: Section of testes of male rat received pellet and distilled water only for 1 month

Section of testes of male rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

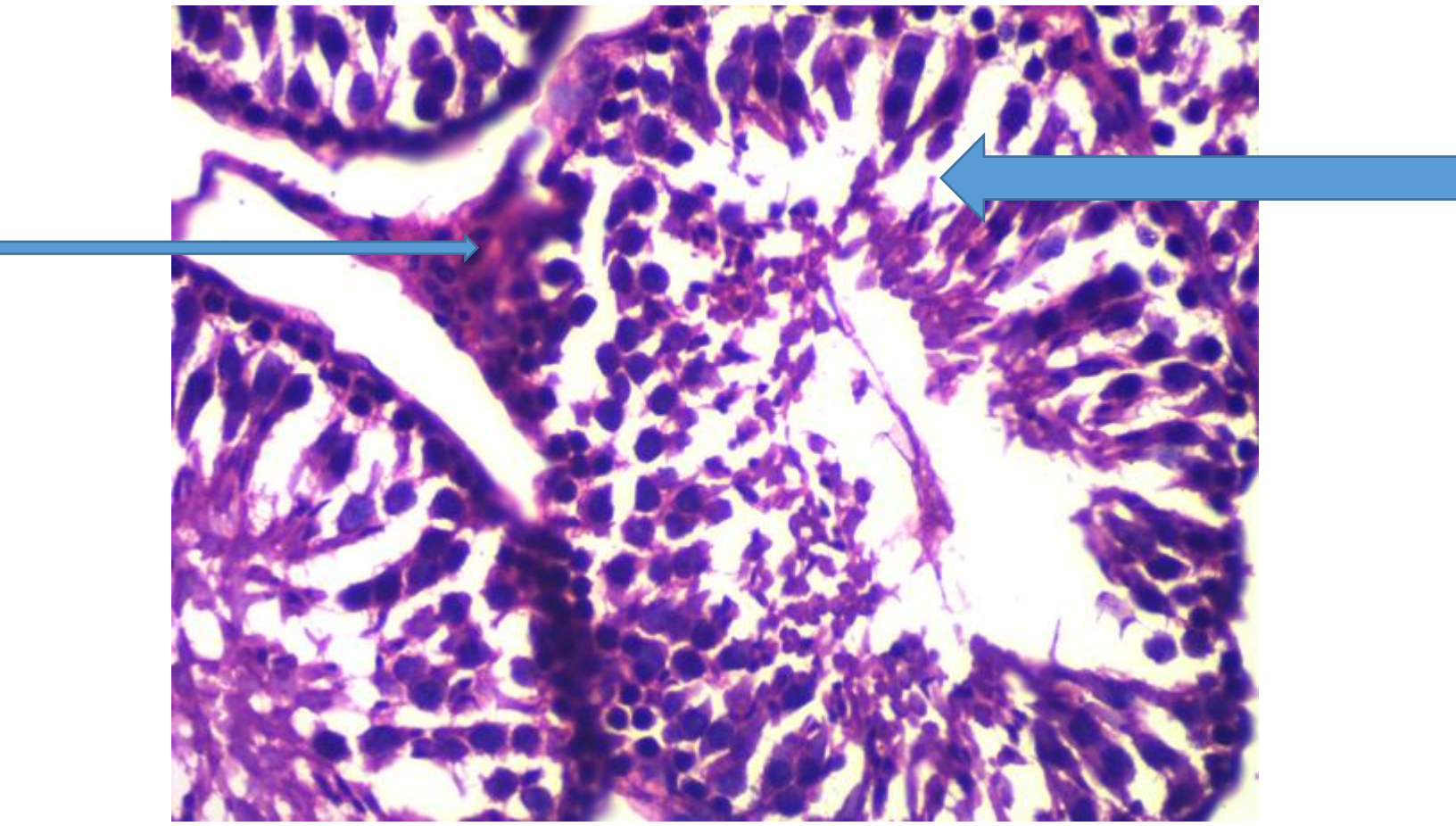

Plate 4.2: Section of testes of male rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of testes of male rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

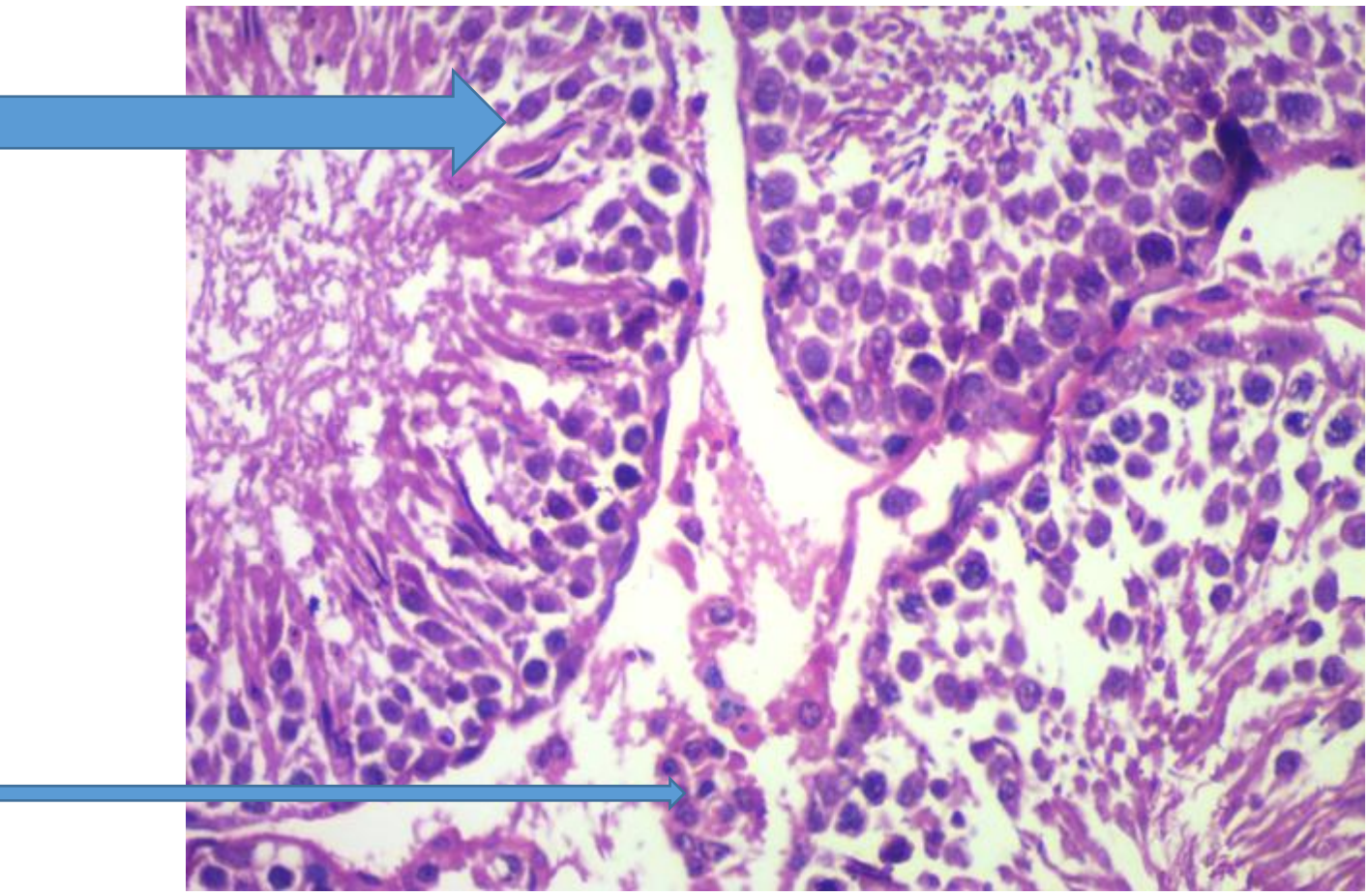

Plate 4.3: Section of testes of male rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of testes of male rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

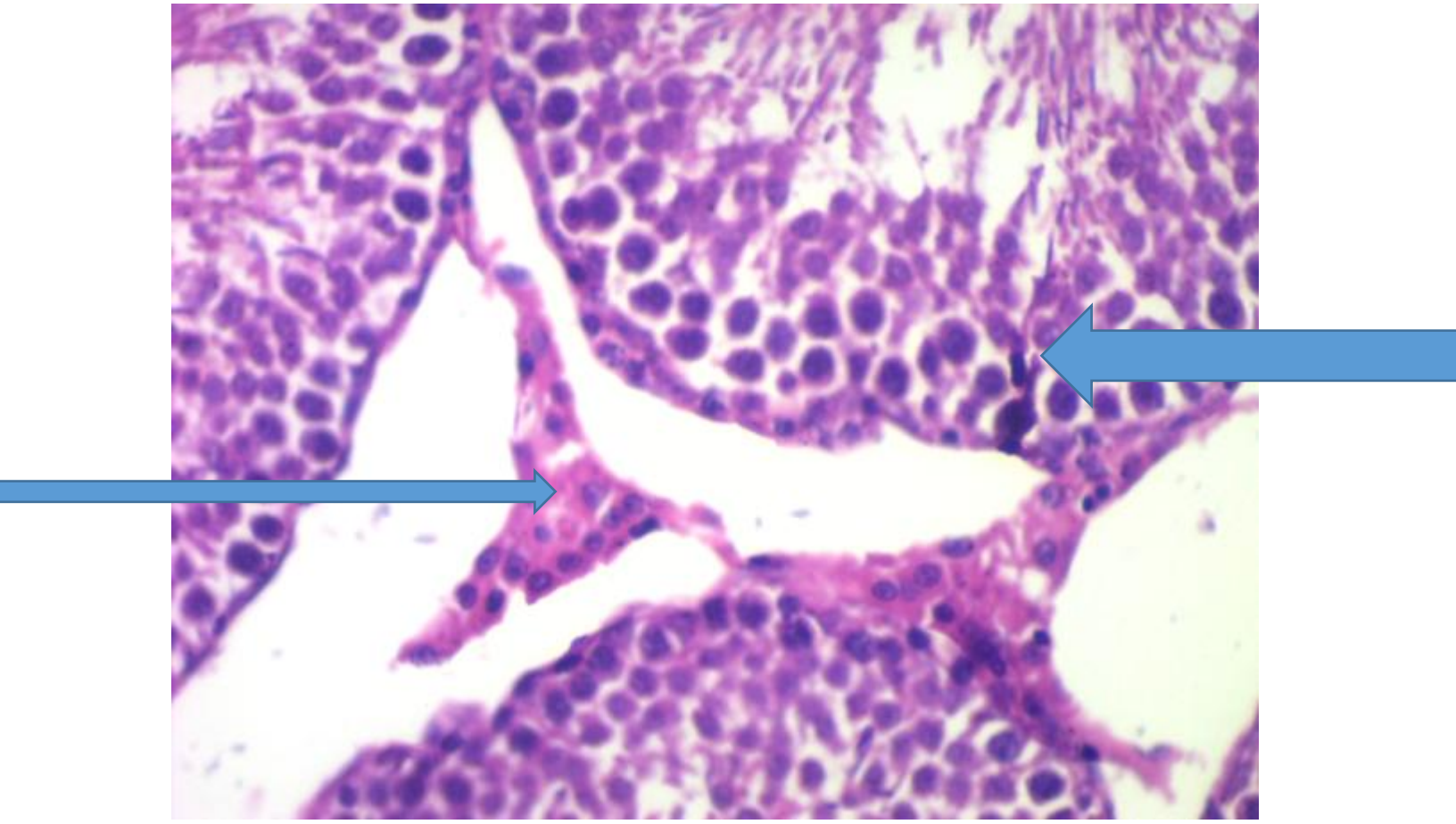

Plate 4.4: Section of testes of male rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat received pellet and distilled water only for 1 month 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**

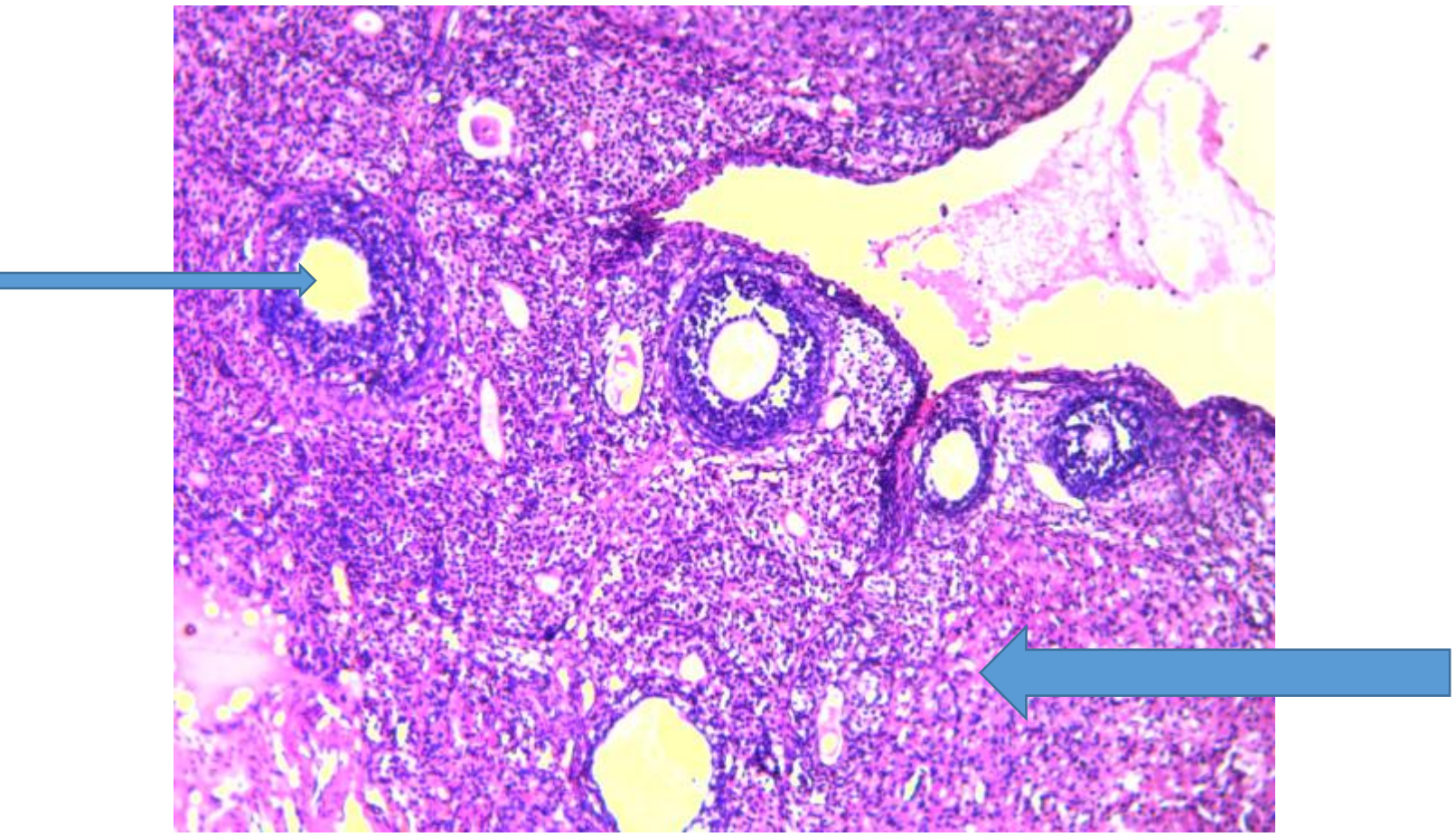

Plate 4.5: Section of ovary of female rat received pellet and distilled water only for 1 month

Section of ovary of female rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

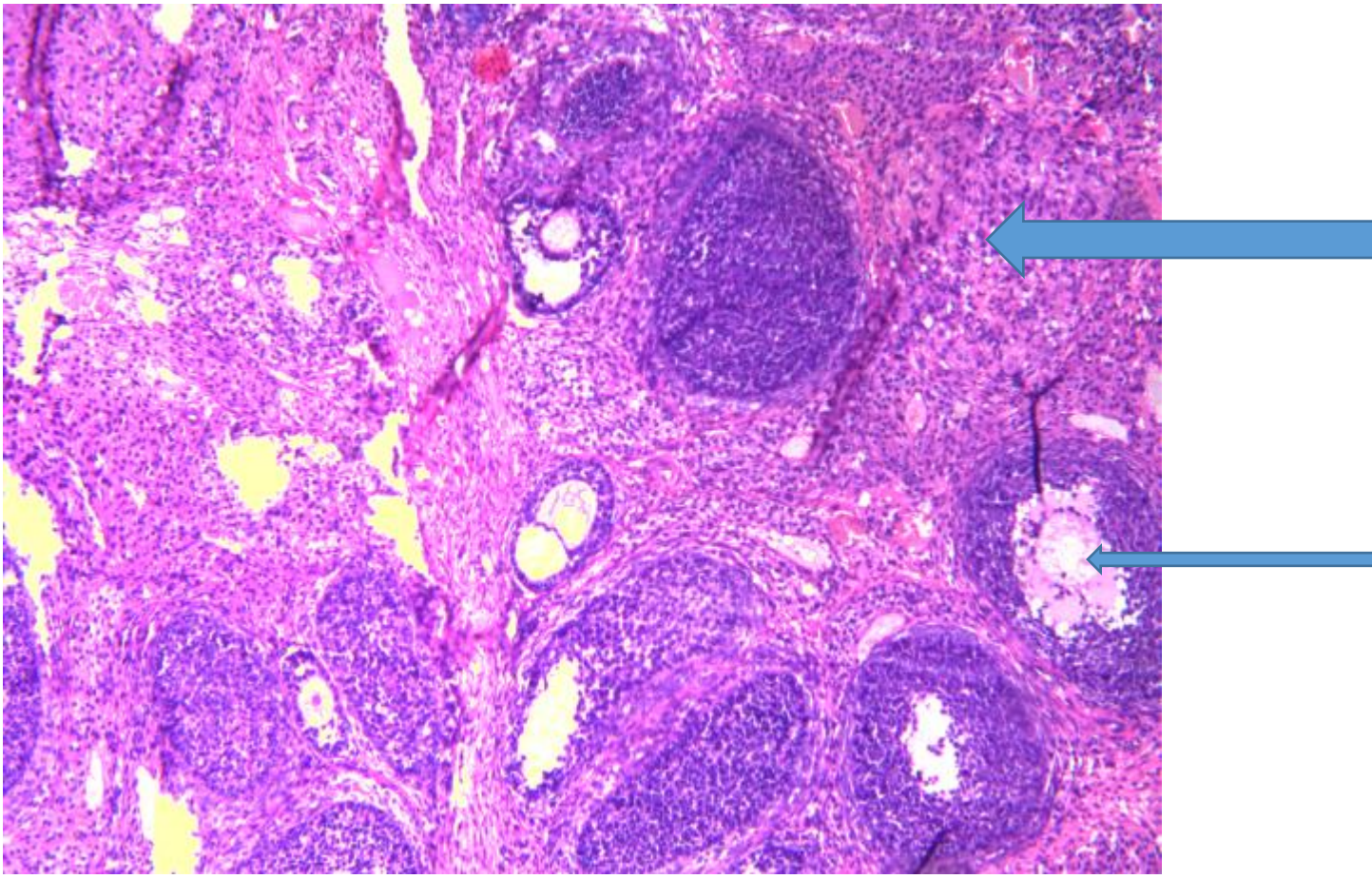

Plate 4.6: Section of ovary of female rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

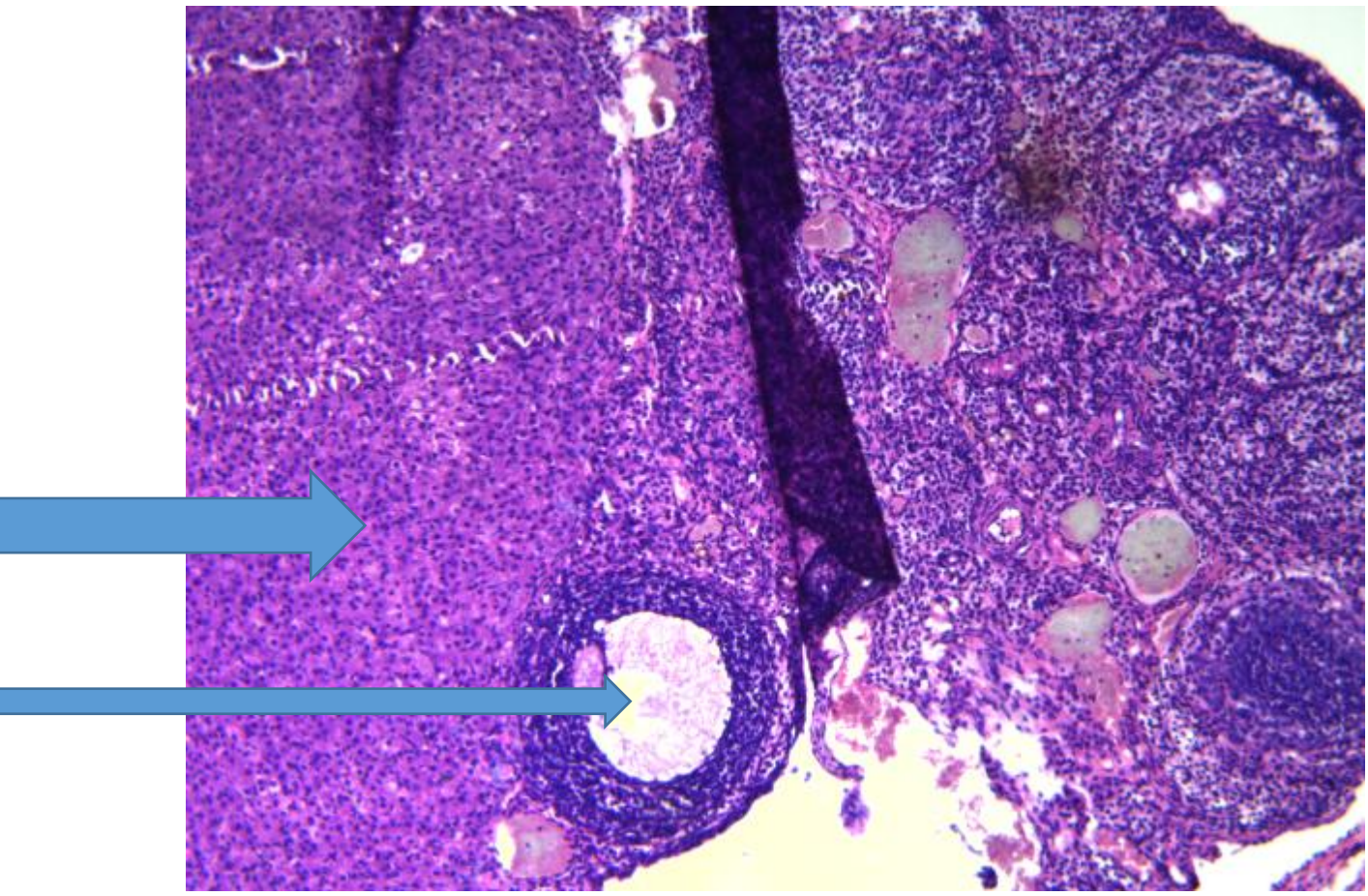

Plate 4.7: Section of ovary of female rat administered 250 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

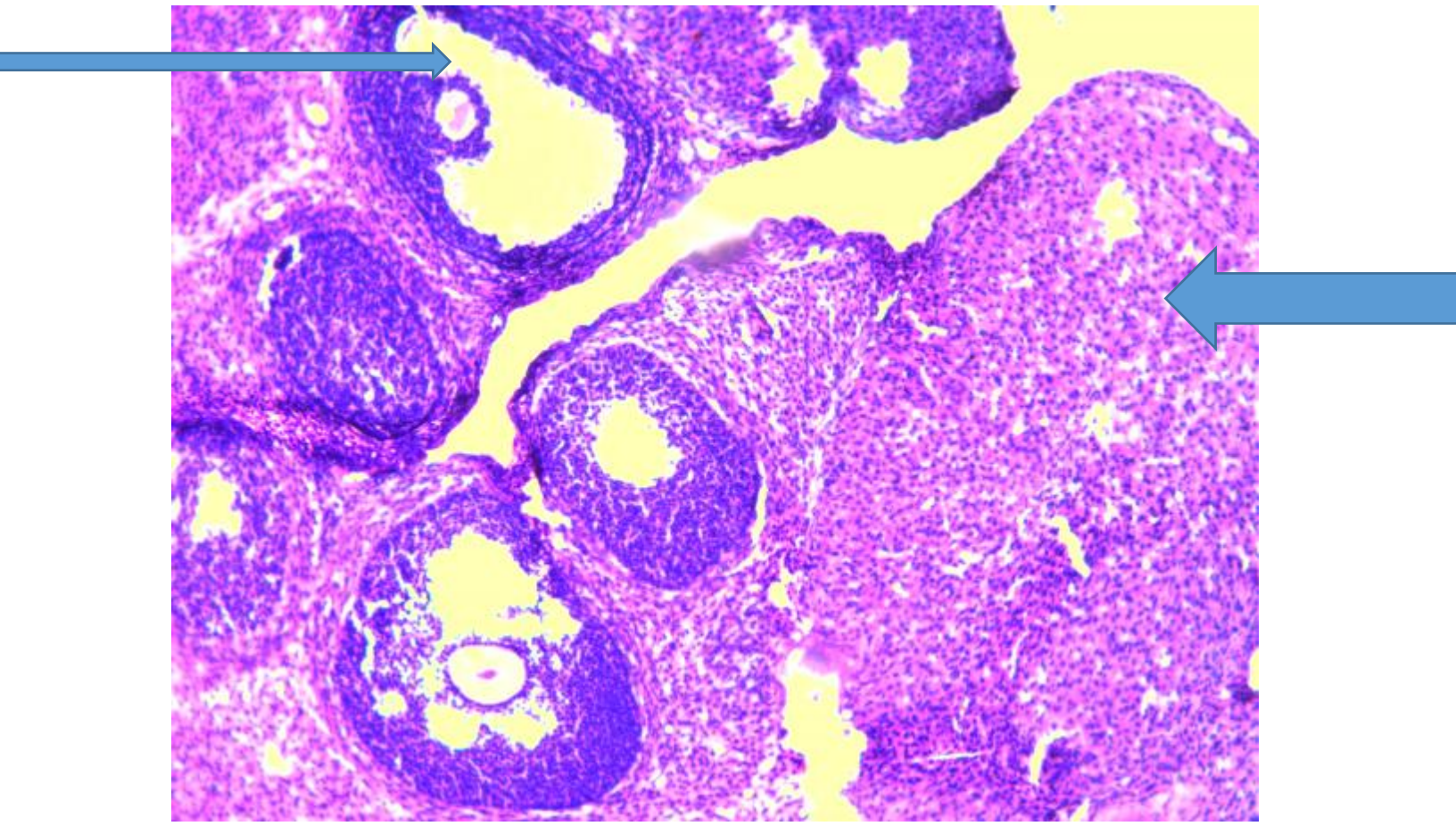

Plate 4.8: Section of ovary of female rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

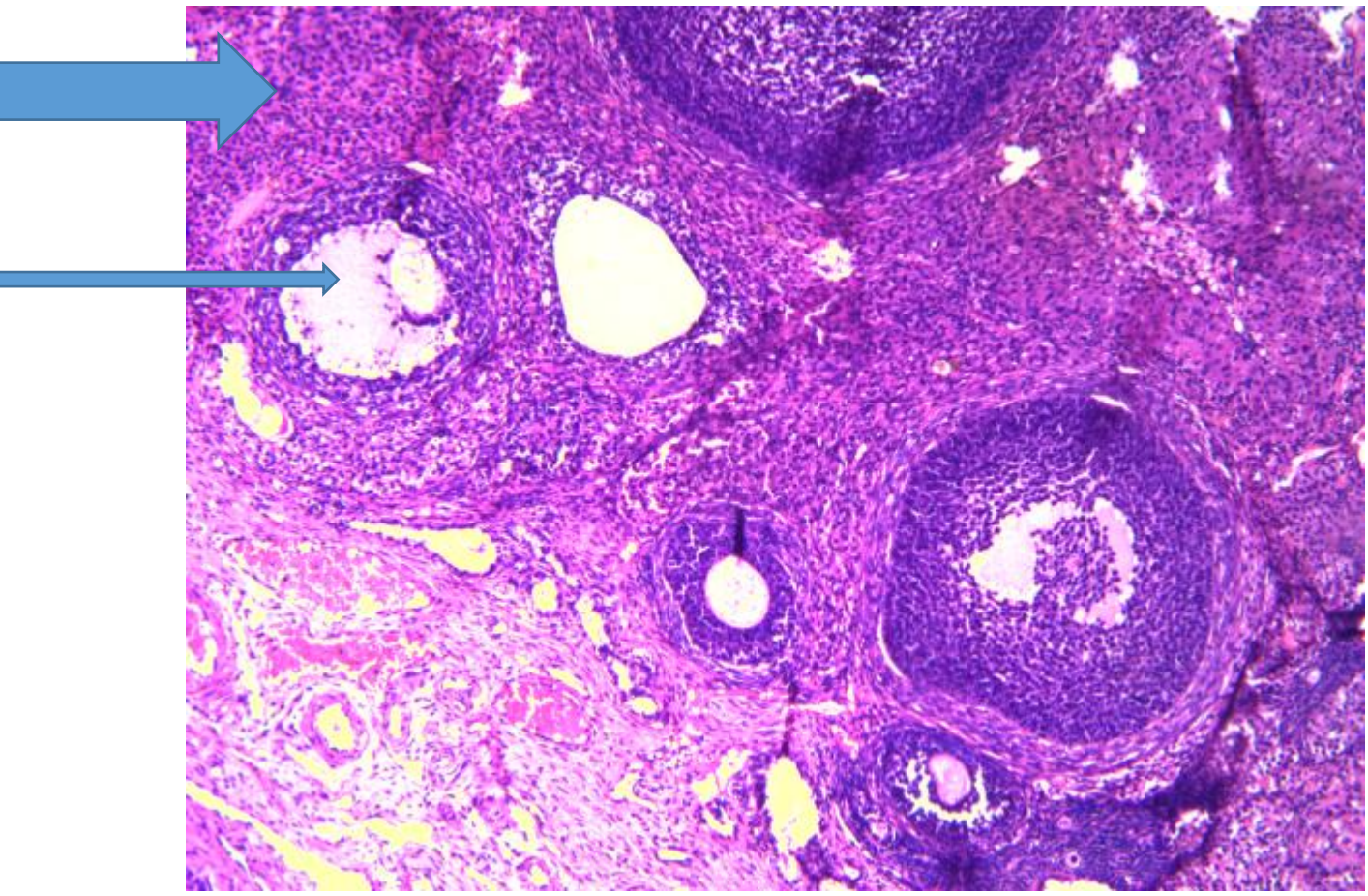

Plate 4.9: Section of ovary of female rat administered 500 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

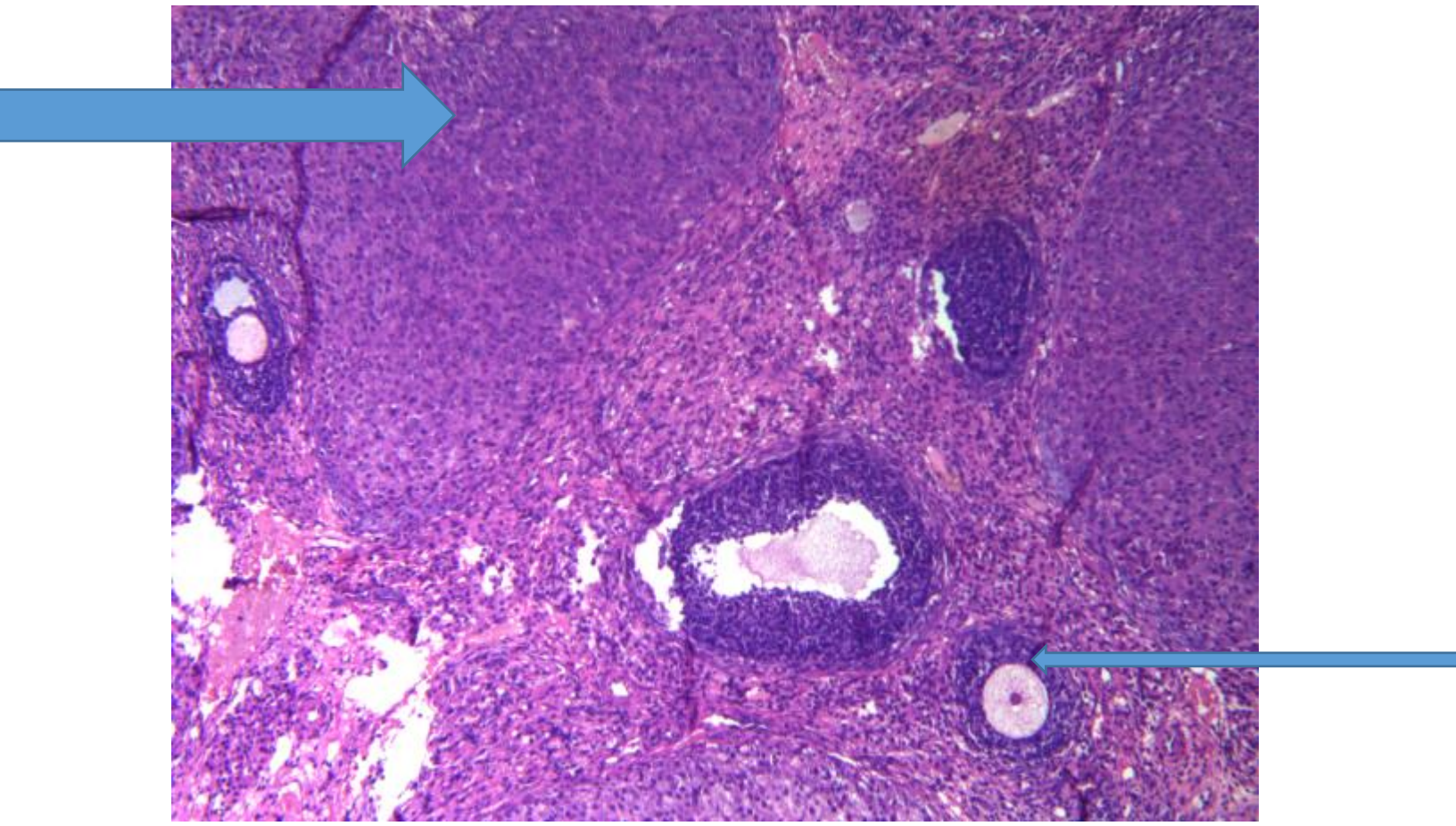

Plate 4.10: Section of ovary of female rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month

Section of ovary of female rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month 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**

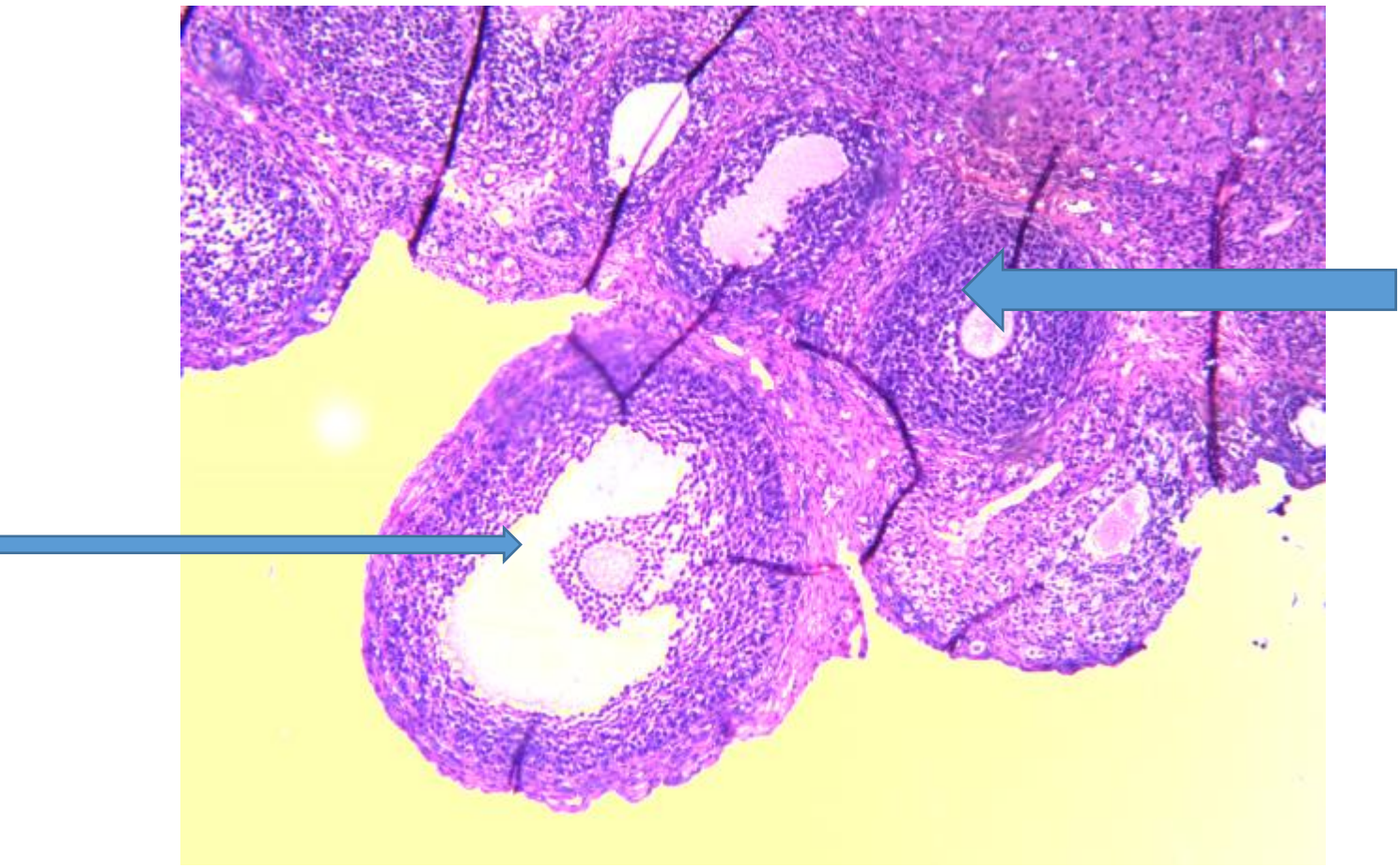

Plate 4.11: Section of ovary of female rat administered 1000 mg/kg body weight of *Chrysophyllum albidum* for 1 month

CHAPTER FIVE
5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The present study investigated the histomorphological effects of *Chrysophyllum albidum* fruit extract on the reproductive organs of albino rats, providing valuable insights into the potential reproductive implications of this traditionally used medicinal plant. The findings reveal a complex pattern of physiological responses that warrant careful consideration in the context of reproductive health applications.

The administration of *Chrysophyllum albidum* extract produced significant alterations in several hematological parameters, particularly affecting lymphocyte percentages, neutrophil counts, and red blood cell indices. The observed decrease in lymphocyte percentages (from 78.4 ± 2.3% in controls to 63.5 ± 3.1% in the highest dose group) coupled with increased neutrophil counts suggests a potential immunomodulatory effect of the plant extract. This finding aligns with previous research by Oboh *et al*. (2018), who reported that phenolic compounds in *Chrysophyllum albidum* can influence immune system parameters through their antioxidant properties.

The significant changes in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values indicate alterations in red blood cell morphology and hemoglobin content. These changes, while statistically significant, remained within physiological ranges, suggesting that the extract may influence erythropoiesis without causing pathological anemia. However, the reduction in red blood cell count (from 7.0 ± 0.5 × 10^6/μL in controls to 4.6 ± 0.9 × 10^6/μL in group D) warrants attention, as it contradicts the findings of Ibrahim *et al*. (2019), who reported hematopoietic benefits of *C. albidum* supplementation in diabetic rats.

The significant increase in body weight observed across all treatment groups suggests that *Chrysophyllum albidum* extract may enhance nutritional utilization or metabolic processes. This finding is consistent with the documented nutritional richness of the fruit, as reported by Ezeobi *et al*. (2021), who highlighted the high carbohydrate and protein content of *C. albidum*. However, the specific increase in testicular weight in the highest dose group (Group D) compared to controls and Group B raises important questions about dose-dependent effects on reproductive organ development.

The lack of significant changes in ovarian weight across all groups suggests that the extract may have differential effects on male versus female reproductive organs. This gender-specific response pattern has been previously noted by Obi *et al*. (2018), who observed varying hormonal responses in female rats exposed to *C. albidum* leaf extracts. The maintained ovarian weight despite extract administration may indicate preserved ovarian integrity, which contrasts with some phytochemical compounds that can cause ovarian atrophy at high concentrations.

One of the most intriguing findings of this study was the absence of significant changes in testosterone and progesterone levels across all treatment groups. This result contradicts previous research by Oigbochie *et al*. (2019), who reported dose-dependent increases in follicle-stimulating hormone (FSH) and alterations in testosterone levels following administration of *C. albidum* root extract. The discrepancy may be attributed to several factors: the different plant parts used (fruit versus root), extraction methods, administration duration, or inherent variability in phytochemical concentrations.

The maintenance of normal hormonal levels, despite the observed changes in testicular weight, suggests that the extract may influence reproductive organ morphology through non-hormonal mechanisms. This finding is particularly significant as it indicates that the phytochemical

constituents of *C. albidum* fruit may not directly interfere with the hypothalamic-pituitary-gonadal axis at the doses and duration tested. However, this contradicts the theoretical expectations based on the known presence of bioactive compounds such as flavonoids and alkaloids, which have been shown to modulate hormonal pathways in other studies (Asagba *et al*., 2019).

The histopathological examination revealed remarkably preserved reproductive tissue architecture across all treatment groups. Both testicular and ovarian tissues maintained normal morphological characteristics, with well-organized seminiferous tubules containing healthy Sertoli cells and spermatogenic cells at various maturation stages in males, and normal follicular development with intact oocytes in females. This preservation of tissue integrity is encouraging and suggests that *C. albidum* fruit extract does not induce harmful histological changes at the tested concentrations.

The maintenance of normal histoarchitecture contradicts some previous findings by Oigbochie *et al*. (2019), who observed spermatogenic arrest and histological disruptions following *C. albidum* root extract administration. This discrepancy reinforces the importance of considering the specific plant part used, as different tissues may contain varying concentrations of bioactive compounds. The fruit extract appears to be less disruptive to reproductive tissue integrity compared to root extracts, which may have higher concentrations of potentially cytotoxic alkaloids.

The presence of normal Leydig cell populations in the interstitium of treated animals supports the hormonal findings, as these cells are responsible for testosterone production. Similarly, the maintenance of normal theca and granulosa cell arrangements in ovarian follicles indicates preserved steroidogenic capacity, which aligns with the stable progesterone levels observed.

The study revealed interesting dose-response patterns, particularly evident in the hematological parameters and testicular weight changes. The highest dose (1000 mg/kg) produced the most pronounced effects on neutrophil counts and testicular weight, suggesting a threshold effect for some physiological responses. However, the absence of a clear dose-response relationship in hormonal parameters indicates that the extract's effects on reproductive endocrinology may not follow conventional pharmacological patterns.

This finding has important implications for traditional medicine applications, where dosage standardization is often challenging. The results suggest that while higher doses may produce more pronounced systemic effects, they do not necessarily translate to proportional changes in reproductive function, which may actually be beneficial from a safety perspective.

The observed effects can be attributed to the complex phytochemical profile of *C. albidum* fruit, which includes flavonoids, alkaloids, tannins, and saponins. The antioxidant properties of flavonoids, as documented by Odewade and Odewade (2023), may contribute to the preservation of reproductive tissue integrity by protecting against oxidative stress. However, the alkaloid content may be responsible for some of the hematological changes observed, as these compounds are known to have systemic effects on various physiological processes.

The maintenance of normal reproductive histomorphology despite significant hematological changes suggests that the reproductive organs may be relatively protected from the systemic effects of the extract, possibly due to the blood-testis barrier and specialized ovarian microenvironment.

The findings of this study have important implications for the traditional use of *C. albidum* in reproductive health applications. The preservation of normal reproductive tissue architecture and

hormonal profiles suggests that the fruit extract may be relatively safe for consumption at the tested doses. However, the observed hematological changes indicate the need for careful monitoring if the extract is used therapeutically.

The gender-specific effects observed (particularly the testicular weight changes in males versus preserved ovarian parameters in females) suggest that clinical applications may need to consider sex-specific dosing or monitoring protocols. This is particularly relevant given the traditional use of the plant for treating various reproductive disorders in both sexes.

5.2 CONCLUSION

This comprehensive study provides valuable insights into the histomorphological effects of *Chrysophyllum albidum* fruit extract on reproductive organs of albino rats. The key findings demonstrate that while the extract produces significant systemic hematological changes and influences body weight gain, it remarkably preserves reproductive tissue integrity and does not disrupt hormonal homeostasis at the doses and duration tested.

The maintenance of normal seminiferous tubule architecture in males and follicular development in females, coupled with stable testosterone and progesterone levels, suggests that *C. albidum* fruit extract may be relatively safe for reproductive health applications. However, the observed hematological alterations, particularly the changes in white blood cell populations and red blood cell parameters, warrant careful consideration in clinical applications.

The dose-dependent effects observed in some parameters, particularly testicular weight changes, indicate the importance of establishing appropriate dosage guidelines for therapeutic applications. The absence of histological damage across all treatment groups is encouraging and supports the traditional use of this plant, albeit with appropriate caution regarding dosage and duration of use.

These findings contribute significantly to the scientific validation of traditional medicine practices while highlighting the complexity of plant-based therapeutic interventions. The study demonstrates that natural products can have multifaceted effects on physiological systems, emphasizing the need for comprehensive safety evaluations before widespread clinical application.

5.3 RECOMMENDATION

1. Tissue Protection Applications

Develop *Chrysophyllum albidum* extract as a protective agent for individuals exposed to reproductive toxins or undergoing treatments that may damage reproductive organs.

2. Age-Related Decline Prevention

Investigate the extract's potential in geriatric reproductive medicine to maintain organ structure and function during natural aging processes.

3. Post-Treatment Recovery Support

Explore formulations for supporting reproductive organ recovery after chemotherapy, radiation, or surgical interventions in cancer patients.

5. Quality Standards Development

Establish histological benchmarks and biomarker profiles for commercial *Chrysophyllum albidum* reproductive health products to ensure therapeutic efficacy.

REFERENCES

Adekanmi, D. and Olowofoyeku, A. (2020). African star apple: potentials and application of some indigenous species in nigeria. *Journal of Applied Sciences and Environmental Management*, 24(8): 1307-1314.

Adonu, C., Ali, I., and Omeh, R. (2023). Phytochemical screening and antibacterial activity of *Chrysophyllum albidum* collected in nsukka, south east nigeria. *European Journal of Medicinal Plants*, 34(6): 13-22.

Ajayi, O., Oyetayo, F., and Akomolafe, S. (2020). Starch composition, glycemic indices, antioxidant properties and carbohydrate hydrolyzing enzymes activities of african star apple fruit parts. *BMC Complementary Medicine and Therapies*, 20(1): 20-30.

Akinmoladun, A., Falaiye, O., Ojo, O., Adeoti, A., Amoo, Z., and Olaleye, M. (2022). Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of *Chrysophyllum albidum* g. don (african star apple). *Bulletin of the National Research Centre*, 46(1): 718-720.

Ali, W., Magdy, M., Salama, E., Ahmed, N., and Said, A. (2024). Long term toxicity of aspartame on ovaries and blood components of adult female albino rats. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*, 42(1): 87-93.

Alina, B. (2022). Ovaries: Facts, Function and Disease. *Suwin/Shutterstock*, last updated August 17, 2022.

Anang, M., Oteng-Peprah, M., and Opoku-Boadu, K. (2019). Extraction and characterisation of african star apple (*Chrysophyllum albidum*) seed oil and the adsorptive properties of the fruit shell in ghana. *International Journal of Food Science*, 2(1): 1-8.

Asagba, S., Kadiri, H., and Ezedom, T. (2019). Biochemical changes in diabetic rats treated with ethanolic extract of *chysophyllum albidum* fruit-skin. *The Journal of Basic and Applied Zoology*, 80(1): 10(11)86-93.

Buelow, M. T., and Hsu, C. C. (2021). Optimal methods for hormonal assay adaptations in ELISA procedures. *Endocrine Reviews*, 42(8): 581-636.

Butler, D. G. (2018). Techniques for the storage of biological samples in research. *Clinical Biochemistry Reviews*, 39(4): 85-93.

Chemat, F., Abert-Vian, M., and Fabiano-Tixier, A. S. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, 13(7): 7952–7975.

Dandare, S., Mainasara, B., Magaji, U., Dandare, A., Lailaba, A., and Sadiq, M. (2018). andlt;iandgt;in vitroandlt;/iandgt; antioxidant activity of andlhrysophyllum fruit. Nigerian *Journal of Basic and Applied Sciences*, 25(1): 17-20

Ehiremen, S. E., Iyare, G., and Omorodion, N. T. (2024). Restorative effect of *annona muricata* on histomorphology of testicular tissue of cadmium tumor induced albino rats. *Journal of health, applied sciences and management*, 7(2): 66-80.

Ekwealor, K., Iroka, C., Nwaogaranya, U., Ikegbunam, C., and Njom, V. (2023). Evaluation of the phytochemical and proximate composition of fruit of false yam (icacina senegalensis)

and african star apple (*Chrysophyllum albidum*). *Asian Plant Research Journal*, 2(3): 28-36.

Enujiugha, V., Adetogo, T., and Talabi, J. (2023). Effect of storage conditions on antioxidant enzymes and polyphenol oxidase activity of african star apple juice. *IJNFS*, 2(1): 18-22.

Erhirhie, J. E., Earnest, O., Emuesiri, G., Moke, K.E. (2015). A Comprehensive Review on Ethno-Medicine, Phytochemistry and Ethnopharmacology of *Chrysophyllum albidum*. *Journal of Advances in Medical and Pharmaceutical Sciences 3(4):147-154*

Ewelike, N., Okammadu, J., Ogwudire, V., and Nnadozie, R. (2021). In-vitro antimicrobial activity of methanolic and aqueous leaf extracts of *Chrysophyllum albidum* (african star apple) and garcinia kola (bitter kola). *GSC Biological and Pharmaceutical Sciences*, 14(03): 249-253.

Ezeobi, P., Igwilo, I., Ogbodo, U., and Ndukwe, J. (2021). Comparative assay of the nutritional composition and antioxidant effect of the cotyledon and pulp of *Chrysophyllum albidum* fruit. *International Journal of Biochemistry Research and Review*, 2(6):41-52.

Ganglo, J. (2023). Ecological niche model transferability of the white star apple (*Chrysophyllum albidum* G. Don) in the context of climate and global changes. *Scientific Reports*, 6(4): 6-7.

George, O., Adenipekun, E., Fasogbon, S., and OPARANOZIE, J. (2018). Antimicrobial activities of *Chrysophyllum albidum* leaves, fruits and seeds. *American Journal of Biomedical Sciences*, 5(9): 28-44.

Ghosh, S., and Mondal, B. (2020). Methodological insights into blood sample collection techniques from laboratory animals. *Journal of Laboratory Animal Science*, 30(4): 345-351.

Ibrahim, H. A., Osilesi, O., Adebawo, O. O., Onajobi, F. D., Karigidi, K. O., and Muhammad, L. (2019). Antidiabetic and haematological effects of Chrysophyllum albidum supplemented diet on streptozotocin induced diabetic rats. *Journal of Applied Life Sciences International,* 22(9): 1-17.

Ibrahim, H., Osilesi, O., Orodele, K., Olagoke, E., and Ashiyanbi, M. (2024). *Chrysophyllum albidum* (african star apple) fruit-skin attenuates hyperglycemia-mediated oxidative stress in experimental diabetic rats. *Journal of Chemistry and Nutritional Biochemistry*, 5(1): 56-83.

Igwe, A., Okelue, C., and Ijioma, S. (2024). Testicular pathology in albino rats administered graded doses of ethanol leaf extract of *Chrysophyllum albidum*. *Sokoto Journal of Veterinary Sciences*, 22(2): 120-128.

Karpus, K. R., and Zhang, Y. (2019). Advances in ELISA methodologies for hormone measurements in biological fluids. *Journal of Immunoassay and Immunochemistry,* 40(5): 525-548.

Lee, S. H., and Hwang, S. (2022). Statistical approaches for analyzing ELISA data in biomedical research. *Biostatistics*, 23(2): 340-350.

NIH Medical Arts/Alan Hoofring/Don Bliss/National Cancer Institute. Male Gonads (Testes) and Female Gonads (Ovaries).

Nugroho, R., Aryani, R., Manurung, H., Anindita, D., Hidayati, F., Prahastika, W., and Rudianto, R. (2022). Effects of the ethanol extracts of ficus deltoidea leaves on the reproductive parameters in male mice. *Open Access Macedonian Journal of Medical Sciences*, 10(1): 146-152.

Obi, C., Patrick-Iwuanyanwu, K., and Onyeike, E. (2018). Effect of ethanol extract of leaves of *Chrysophyllum albidum* on the reproductive hormones of lead-exposed female wistar albino rats. *Journal of Applied Sciences and Environmental Management*, 22(9): 15-30.

Oboh, G., Adebayo, A., Ejakpovi, I., Ogunsuyi, O., and Boligon, A. (2018). Phenolic profiling and in vitro antioxidant, anticholinesterase, and antimonoamine oxidase properties of aqueous extract of african star apple (*Chrysophyllum albidum*) fruit parts. *Journal of Food Biochemistry*, 42(4): 12-56.

Odewade, J. O., and Odewade, L. O. (2023). Evaluation of antibacterial and antioxidant properties of leaf extract of Chrysophyllum albidum against selected enteric bacterial pathogens. *FUDMA Journal of Sciences*, 7(2): 131-140.

Ogbu, J., Etuk-Udo, G., Ugoh, S., Odunsanya, O., and Amos, J. (2024). Phytochemical and antimicrobial activity of african star apple [*chrysophllum albidum*]. 10(21): 534-545

Ogunwole, E. and Mangai, E. (2019). The effect of *Chrysophyllum albidum* fruit on testicular functions in rats. *Andrologia*, 51(7): 111-133.

Oigbochie, E., Omage, K., and Odiase, E. (2019). Aqueous root extract of *Chrysophyllum albidum* caused dose and duration dependent increases in some reproductive hormones and spermatogenic arrest in the testes of male wistar rats. *Clinical Phytoscience*, 5(1): 5-6

Prakash, K. R., and Rani, A. (2024). Enhancing the reliability and sensitivity of hormonal assays: innovative approaches in ELISA applications. *Journal of Clinical Endocrinology*, 106(1): 54-62.

Utiger, R.D. (2025) "ovary". *Encyclopedia Britannica*, 28 Feb. 2025, https://www.britannica.com/science/ovary-animal-and-human. Accessed 5 May 2025.

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.

APPENDIX IV

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

CERTIFICATE
OF ETHICAL APPROVAL

This is to certify that

JAMES AIMUAMWOSA

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

HISTOMORPHOLOGICAL EFFECTS OF CHRYSOPHYLLUM ALBIDUM PLANT EXTRACT ON THE REPRODUCTIVE ORGANS OF ALBINO RATS.

In accordance with the Animal Disease Control Act, 2022.

Dr L.I Adebudo
Chairman MAFSAEC

Approval No.
MAFSAEC: 025-07/29/0041

Date Of Approval
30th July, 2025

(This Approval is only valid for this study)

APPENDIX V

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
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: *Gambeya albida* (G. Don.) Aubrev. & Pellegr.

[Synonym: *Chrysophyllum albidum* G. Don.]

Family: Sapotaceae

Common Name: African star apple, White star apple, Cherry

Voucher Number: UBH-G362

Student Name: James Aimuamwosa

Plant Identification and Voucher Number Issued by:

27/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MNES; Nigeria)