# HISTOPATHOLOGICAL EFFECT OF *Annona muricata* LEAF EXTRACT ON SOME ORGANS OF ALBINO RATS

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**UNIVERSITY OF BENIN, NIGERIA**

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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 **EVWOMAZINO EMMANUEL OSAUZOU** (MR) with matriculation number: **BMS2001198** 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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**External Examiner**

## DEDICATION

This project is dedicated to Almighty God.

## ACKNOWLEDGEMENT

To God, Almighty, the giver and sustainer of life, words would simply fail me if I attempt to recount your act of goodness, mercy, love and tender care. Your undeserved kindness was sufficient and constant. To my parents, Mr and Mrs osauzou, I'm forever grateful for your constant encouragement, financial support, prayers and sacrifices which has been my driving force towards the completion of this work.

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Finally, I express my gratitude to everyone who has contributed, directly or indirectly, to the completion of this project work. Your support, encouragement, andand goodwill have been instrumental in bringing this effort to a successful conclusion, and I remain truly indebted to you

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## ABSTRACT

*Annona muricata* (soursop) is a tropical plant widely used in traditional medicine for various ailments, yet comprehensive safety data on its effects on vital organs remain limited. This research aimed to investigate the histopathological effects of *Annona muricata* leaf extract on liver, kidney, testis, and ovaries of albino rats. Twenty-four healthy albino rats weighing 180-200g were procured from the Animal House of the Department of Anatomy, University of Benin, and maintained under standard conditions with unrestricted access to feed and water. The rats were divided into four groups: Group A (control, n=2) received pelleted feed and distilled water; Group B (n=4) was administered 250mg/kg soursop extract; Group C (n=4) received 500mg/kg; and Group D (n=4) was given 1000mg/kg extract orally via gavage for one month. Following treatment, animals were euthanized, blood samples collected for biochemical analysis, and organs harvested for histopathological examination. Results revealed no significant changes in hematological parameters, liver function tests, or reproductive hormone levels across all groups ($p > 0.05$). However, kidney function analysis showed significant elevation in sodium (143±3.8 mEq/L) and chloride (107.3±0.5 mEq/L) levels in the highest dose group compared to controls ($p < 0.05$). Histopathological examination revealed normal architecture in the control group organs. Groups B and C exhibited hepatic steatosis with microvacuolar degeneration, while Group D maintained normal liver histology. All kidney, testis, and ovary sections demonstrated preserved normal architecture across treatment groups. The findings suggest that *Annona muricata* leaf extract exhibits a complex dose-response relationship, with intermediate doses causing hepatic steatosis while higher doses appear protective. The preservation of reproductive organ integrity and absence of significant biochemical toxicity support the traditional use of soursop, though careful dose optimization and electrolyte monitoring are recommended for therapeutic applications.

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 BACKGROUNG OF STUDY

*Annona muricata* commonly referred to soursop, is a tropical tree commonly found in Central America, South America, and various parts of Africa and Southeast Asia. The leaves of soursop have garnered significant attention due to their rich phytochemical composition, which includes alkaloids, flavonoids, tannins, and phenolic compounds. These bioactive constituents are credited with a range of biological activities, including antioxidant, anti-inflammatory, and anticancer properties (Rasyid *et al*., 2023; Lekjing *et al*., 2024). Previous studies have demonstrated the effectiveness of soursop leaves in traditional medicine for various ailments, including pain relief and anti-parasitic effects, indicating its potential as a therapeutic agent (Rasyid *et al*., 2023; Ibegbulem *et al*., 2023).

There is growing interest in exploring the effects of *Annona muricata* on reproductive health, particularly due to its high antioxidant content. Antioxidants have been shown to play a protective role against oxidative stress, which contributes to reproductive disorders by impairing sperm production and hormone levels (Priya *et al*., 2024). Research has indicated that various plant extracts, including those from soursop, may help mitigate reproductive toxicity induced by environmental pollutants and metabolic disorders (Ibegbulem *et al*., 2023; Monica and Handayani, 2023). Understanding the mechanisms through which soursop leaves influence reproductive health could provide novel insights into potential interventions for reproductive dysfunctions.

Recent studies have transitioned towards evaluating the impact of various herbal preparations on the reproductive systems of animal models, including albino rats. The relevance of using albino rats stems from their physiological similarities to humans when evaluating reproductive toxicology (Nonso *et al*., 2024). Investigating the effects of soursop leaves on organs and reproductive parameters in this model can pave the way for future clinical applications and therapeutic explorations.

## 1.2 STATEMENT OF PROBLEM

Despite the increasing interest in the pharmacological effects of *Annona muricata*, particularly its leaves, there remains a distinct lack of comprehensive research specifically investigating their effects on the reproductive system of albino rats. Several studies have noted the bioactive properties of soursop leaves, yet empirical data addressing their direct impact on reproductive organ health and function is limited (Ibegbulem *et al*., 2023; Monica and Handayani, 2023). Conversely, studies have reported adverse effects of certain pesticides on reproductive health, identified in the context of oxidative stress (Priya *et al*., 2024). However, limited research exists that evaluates how soursop can counteract such pathological states, particularly in terms of direct organ health impacts and reproductive hormone balance.

The existing literature seldom highlights the dose-response relationships or histopathological changes in reproductive organs following treatment with soursop leaf extracts, showcasing a significant gap in knowledge (Ehiremen *et al*., 2024). Additionally, the biochemical pathways through which these extracts exert their effects remain inadequately explored (Sunday and Ilesanmi, 2023). This study aims to fill these gaps by evaluating the histopathological effect of soursop leaves on some organs of albino rats, thus contributing to both academic understanding and practical applications in herbal medicine.

## 1.3 JUSTIFICATION OF THE STUDY

The leaves of *Annona muricata* (soursop) have been recognized in traditional medicine for their potential health benefits, particularly due to their rich array of phytochemicals, including flavonoids and phenolic compounds. These bioactive constituents are known for their antioxidant properties, suggesting a capacity to mitigate oxidative stress-related damage to various organs (Marlita and Sujono, 2024). Studies have indicated that soursop leaf extracts can inhibit cellular damage, potentially protecting the liver and reproductive tissues from adverse environmental influences (Ehiremen *et al*., 2024; Marlita and Sujono, 2024). Despite these findings, there remains a paucity of literature specifically assessing the comprehensive effects of soursop leaves on the reproductive system and other organs in albino rats. This study aims to bridge this knowledge gap, contributing valuable insights into the potential therapeutic applications of soursop leaves for protecting and enhancing organ health, especially within the context of oxidative stress and reproductive functionality (Hariani *et al*., 2024). Furthermore, with the increased prevalence of herbal medicines in treating ailments globally, understanding the mechanisms through which soursop impacts health can foster evidence-based practices in the integration of herbal remedies into clinical settings (Shuklan and Rani, 2024).

## 1.4 SIGNIFICANCE OF STUDY

The significance of this study is multifaceted. First, identifying the positive or negative effects of soursop leaves on reproductive organs and general health can provide a scientific basis for their traditional use in healthcare. Such findings could lead to the development of soursop leaf extracts as potential natural remedies for enhancing reproductive health and managing disorders (Monica and Handayani, 2023; Sunday and Ilesanmi, 2023).

Second, the exploration of soursop leaves' beneficial properties may promote increased awareness of the therapeutic potential of indigenous plants. As the prevalence of reproductive health issues continues to rise, evidenced by various factors including environmental toxins and lifestyle changes, alternative treatment modalities that utilize natural products hold significant promise (Lekjing *et al*., 2024; Priya *et al*., 2024).

Moreover, through addressing oxidative stress and reproductive toxicity, the study aligns with contemporary health initiatives that favor safer, plant-based approaches to medicine. This research could significantly contribute to the establishment of effective herbal treatments, thereby supporting the integration of traditional knowledge with modern therapeutic practices (Ibegbulem *et al*., 2023; Monica and Handayani, 2023).

## 1.5 AIM OF THE STUDY

This study aimed to determine the histopathological effect of *Annona muricata* leaf extract on some organs of albino rats.

## RESEARCH HYPOTHESIS

Alternative hypothesis Ha:

$Ha_1$: *Annona muricata* leaf extract has a positive dose-dependent effect on the morphology of liver, kidney, testes and ovaries of albino rats.

$Ha_2$: *Annona muricata* leaf extract has a positive dose-dependent effect on the liver function tests, renal function parameters, and the reproductive hormones of albino rats.

## 1.6 SPECIFIC OBJECTIVES OF THE STUDY

The specific objectives of this study were to:

1. determines the effect of *Annona muricata* leaf extract on the histomorphology of the liver, kidney, testis, and ovaries of albino rats.
2. evaluate the effect of *Annona muricata* leaf extract on the liver function and renal function tests of albino rats.
3. investigate the effect of *Annona muricata* leaf extract on the reproductive hormones of albino rats.

## 1.7 RESEARCH QUESTIONS

1. Does *Annona muricata* leaf extract affect the morphology of the liver, kidney, testis, and ovaries of albino rats?
2. Does *Annona muricata* leaf extract affect the parameters of the liver and kidney functions of albino rats?
3. Does *Annona muricata* leaf extract affect the reproductive hormones of albino rats?

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 *Annona muricata* (Overview)

### Botanical Classification and Geographic Distribution

*Annona muricata*, commonly known as soursop or graviola, is a flowering plant belonging to the family Annonaceae. Its taxonomic classification is as follows: Kingdom Plantae, Phylum Angiosperm, Class Magnoliopsida, Order Magnoliales, and Genus Annona. This classification underscores its significance and diversity (Adelina *et al*., 2013). Native to tropical regions, particularly from the Caribbean to Central America, its cultivation and traditional usage extend across several continents, including Asia and Africa, where it has been an integral part of indigenous medicine and culinary practices (Chan *et al*., 2023). The plant flourishes in warm climatic conditions and has been recognized for its resilience in varying ecological contexts, contributing to its popularity in both rural and urban settings (Ehiremen *et al*., 2024).

Traditional applications of *Annona muricata* include its use in the treatment of numerous ailments, ranging from infectious diseases to complex conditions such as cancer and diabetes (Chan *et al*., 2023). The cultural nuances associated with its usage in folk medicine have initiated both ethnobotanical and scientific analyses of its health benefits, creating a rich database of ethnomedical literature. This historical context highlights the socio-cultural relevance of the plant as it remains a significant component of herbal formulations in various communities (Ehiremen *et al*., 2024).

**Figure 4.1: *Annona muricata* in Jardin des Plantes de Toulouse (Krzysztof, 2024).**

## 2.2 Nutritional and Phytochemical Profile

*Annona muricata* possesses a rich nutritional composition across its various parts—leaves, fruits, seeds, flowers, and roots. Each component serves a distinct role within traditional practices and modern therapeutics. The leaves are particularly noted for their high content of phytochemicals such as flavonoids, phenolic compounds, and acetogenins (Fakunle *et al*., 2024). The presence of these bioactive compounds has led to the recognition of *Annona muricata* as a potential source of nutritional and therapeutic benefits.

A detailed analysis of the nutritional value reveals that the leaves contain substantial amounts of protein, carbohydrates, and essential fatty acids, contributing to their efficacy as health supplements (Gavamukulya *et al*., 2019). The fruit, characterized by its creamy texture and unique flavor, is rich in carbohydrates and provides a good dose of vitamins and minerals, including vitamin C, potassium, and magnesium, making it suitable for dietary inclusion (Yamthé *et al*., 2015). Studies indicate that these nutrients promote overall health and play significant roles in disease prevention through immune modulation and anti-inflammatory properties (Sovia *et al*., 2017).

**Figure 4.2: *Annona muricata* (a) flower bud; (b) flower; (c) young fruit; (d) mature fruit (Centre for Herbal Standardization, 2018).**

The phytochemical profile of *Annona muricata* underscores its multifaceted pharmacological potential. Key compounds identified include annonaceous acetogenins, alkaloids, flavonoids, and tannins, each contributing to the plant's therapeutic efficacy (Gavamukulya *et al*., 2019). Acetogenins are particularly noted for exhibiting cytotoxic effects against cancer cells, reinforcing interest in *Annona muricata* as a potential natural alternative in cancer treatment strategies (Bitar *et al*., 2019). The broad-spectrum antioxidant activity associated with flavonoids not only offers cellular protection against oxidative stress but also implicates the plant in managing chronic health conditions related to inflammation and metabolic dysregulation (Bitar *et al*., 2019).

Research supports the potential health benefits associated with consuming *Annona muricata*, emphasizing its antioxidant, anti-cancer, anti-diabetic, and anti-inflammatory properties, corroborated by experimental evidence (Oladele *et al*., 2020). The integration of these active compounds positions *Annona muricata* as a promising subject in the field of nutraceuticals, garnering attention from both scientists and medicinal practitioners exploring its applications against various health disorders (Fakunle *et al*., 2024).

The presence of these bioactive constituents supports the traditional therapeutic claims of *Annona muricata* while providing a scientific framework for understanding its mechanisms of action against diseases (Chan *et al*., 2023). Further investigation into the synergistic effects of these compounds is essential to maximize the therapeutic potential of *Annona muricata*, thereby enhancing its application within the realms of nutrition and medicine.

## 2.3 Phytochemical Constituents

### 2.3.1 Overview of Active Compounds

*Annona muricata* is recognized for its array of phytochemical constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids. Each class of these compounds contributes distinct biological activities, underpinning the therapeutic applications associated with the plant. Alkaloids are known for their wide pharmacological effects, including analgesic and anti-inflammatory properties. Sousa *et al*. in 2010 highlighted that alkaloids can modulate physiological responses, making them significant in herbal medicine (Sousa *et al*., 2010). Flavonoids, another significant class in *Annona muricata*, have been extensively studied for their antioxidant properties and their ability to scavenge free radicals, contributing to the reduction of oxidative stress (Pieme *et al*., 2014).

**Figure 4.3: *Annona muricata* leaves arrangement (Centre for Herbal Standardization, 2018).**

In comparison to other medicinal plants, such as *Hibiscus sabdariffa*, the role of these phytochemicals becomes more pronounced. *Hibiscus sabdariffa* is praised for its high flavonoid content, which supports its anti-hypertensive effects (Opara *et al*., 2021). Both plants show promise in cancer therapies, indicating potential synergistic effects when combined in herbal treatments. The acetogenins in *Annona muricata* display significant cytotoxicity towards tumor cells, presenting an additional weapon against cancer, a degree of effectiveness that correlates with the anticancer activities seen in extracts from *Hibiscus sabdariffa* (Opara *et al*., 2021). While each plant has unique characteristics, they share overlapping therapeutic potential, enhancing their relevance in traditional and modern pharmacology.

### 2.3.2 Bioactivity and Functional Roles of Phytochemicals

#### 1. Flavonoids

Flavonoids are a crucial component of the phytochemical profile of *Annona muricata*, recognized primarily for their potent antioxidant properties. These molecules excel at neutralizing free radicals and significantly reduce oxidative stress, a pivotal factor in cellular aging and the development of various diseases. The antioxidant capabilities of flavonoids are essential for protecting target organs such as the liver and kidneys from damage induced by harmful radicals. Furthermore, flavonoids such as quercetin and kaempferol, which are present in *Annona muricata*, exhibit anti-inflammatory and anticancer activities. Laboratory studies have shown that flavonoid extracts inhibit tumor cell proliferation and induce apoptosis, contributing to the potential of *Annona muricata* in cancer prevention and therapy.

Flavonoids also play a role in mitigating chronic inflammatory conditions. Their ability to modulate immune responses and downregulate pro-inflammatory markers supports tissue integrity and health (Aguilar-Hernández *et al*., 2019). This dual role of combating cancerous

transformations while simultaneously providing anti-inflammatory benefits positions *Annona muricata* as a notable candidate within herbal therapeutics.

**2. Alkaloids and Tannins**

As key constituents, alkaloids in *Annona muricata* are linked with a range of pharmacological effects, including antispasmodic and analgesic actions. Research has documented their efficacy in pain relief and relaxation of smooth muscle. Alkaloids, alongside tannins, also exhibit antimicrobial properties, contributing to the plant's usage in treating various infections and diseases (Doe *et al*., 2019). These characteristics are crucial for understanding how *Annona muricata* may function as a natural remedy.

Tannins, known for their ability to precipitate proteins, emphasize the protective role of *Annona muricata*. They have been recognized for enhancing wound healing and are also noted for their potential ecological applications due to their astringency (Opara *et al*., 2021). The utility of tannins serves both ecological and therapeutic roles, which is echoed in studies of other plants such as Hibiscus sabdariffa, wherein tannins offer similar antimicrobial effects, making the inclusion of both plants in herbal medicine formulations particularly synergistic (Doe *et al*., 2019).

**3. Additional Constituents**

Saponins and terpenoids represent additional phytochemical classes found in *Annona muricata* that enhance its therapeutic value. Saponins are known for their immunomodulatory effects and ability to lower cholesterol levels, influencing lipid metabolism (Doe *et al*., 2019). The presence of these compounds can amplify the overall efficacy of *Annona muricata*, contributing to its role as a holistic remedy.

Terpenoids are also present in substantial quantities within the plant and influence biological pathways related to organ function and overall health. The variety of terpenoids contributes to the flavor profile of the fruit, a desirable attribute for consumers, while amplifying the medicinal appeal of the plant. Together with other bioactive constituents, terpenoids enhance the health benefits attributed to *Annona muricata*

### 2.4 Anatomical and Gross Morphology of the liver

The liver, one of the most vital organs in the human body, typically weighs about 1.5 kg in adult individuals and represents approximately 2% of total body weight (Westerhoff and Lamps, 2022). Its gross morphology includes a smooth surface appearance, deep burgundy coloration, and a soft consistency, conditions indicative of healthy vascularization and functional well-being (Westerhoff and Lamps, 2022). This organ's unique structural attributes facilitate its multifaceted role in metabolism, detoxification, and synthesis of important biomolecules, including plasma proteins and blood-clotting factors.

Anatomically, the liver is unique due to its dual blood supply: approximately 75–80% of its blood supply is derived from the portal vein, which transports nutrient-rich blood from the gastrointestinal tract, while the remaining 20–25% comes from the hepatic artery, providing oxygenated blood (Westerhoff and Lamps, 2022). This dual vascularization highlights the liver's crucial metabolic functions and establishes its role as a central player in homeostatic regulation, particularly in nutrient absorption and metabolic waste processing.

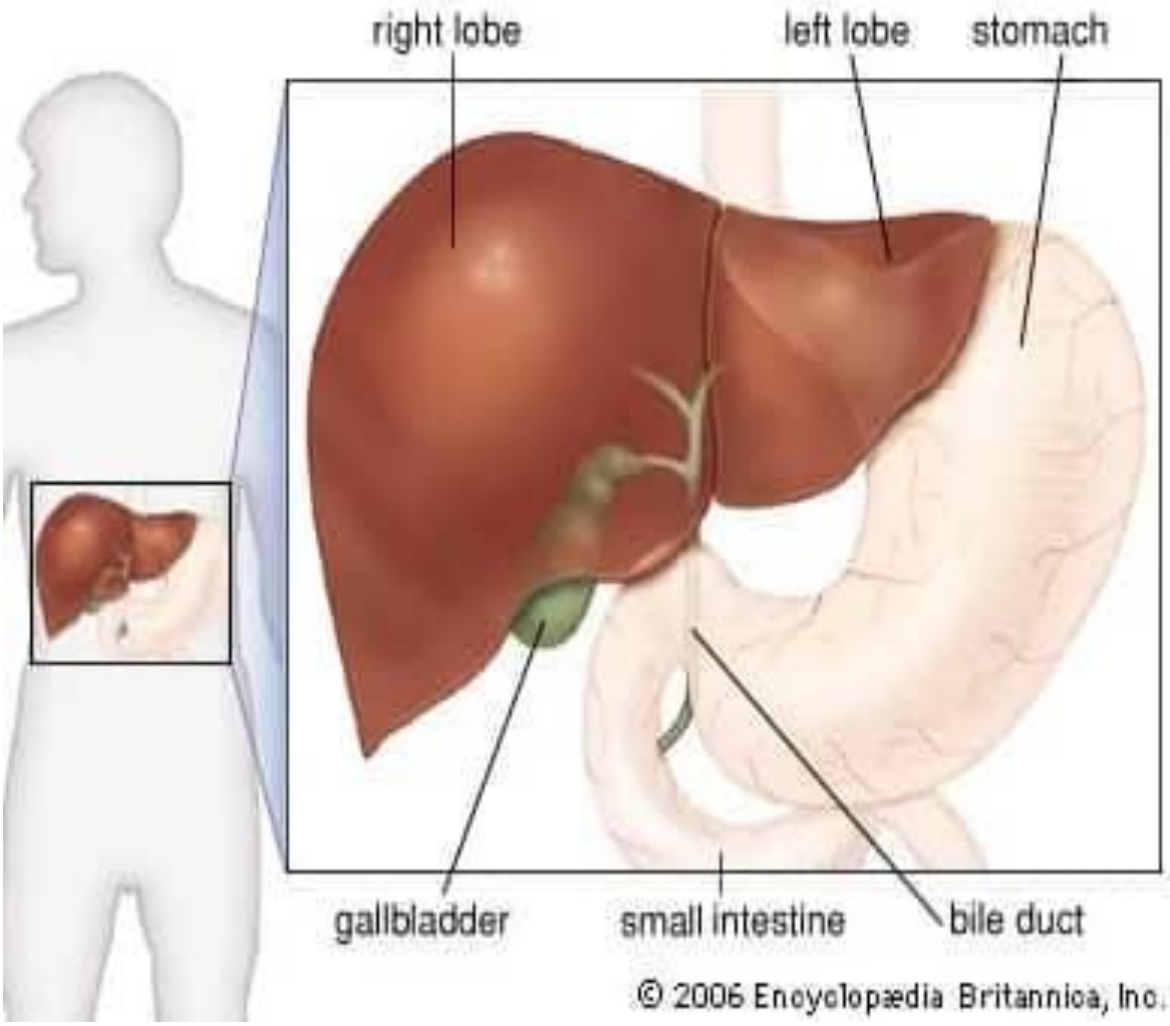


**Figure 4.4: Human liver in relation to other organs (Britannica, 2025).**

### 2.4.1 Histology and Cellular Composition of the Liver

#### 1. Histological Organization

Histologically, the liver is organized into functional units known as lobules, enveloped by a connective tissue capsule. Each lobule contains plates of hepatocytes radiating from a central vein, with blood perfusing through sinusoidal spaces, allowing for the efficient exchange of nutrients and waste. The arrangement of hepatocytes into these structures supports centripetal blood flow, where blood moves from the periphery towards the center, facilitating metabolic exchange and detoxification processes.

Kupffer cells, resident macrophages located within the sinusoidal spaces, are integral to the liver's immune surveillance capabilities. They play a critical role in recognizing and phagocytizing pathogens, clearing apoptotic cells, and modulating inflammatory responses by releasing various cytokines. The presence of these immune cells marks the liver as an immune-organizing center, further emphasizing its pivotal role in both metabolic and immune functions.

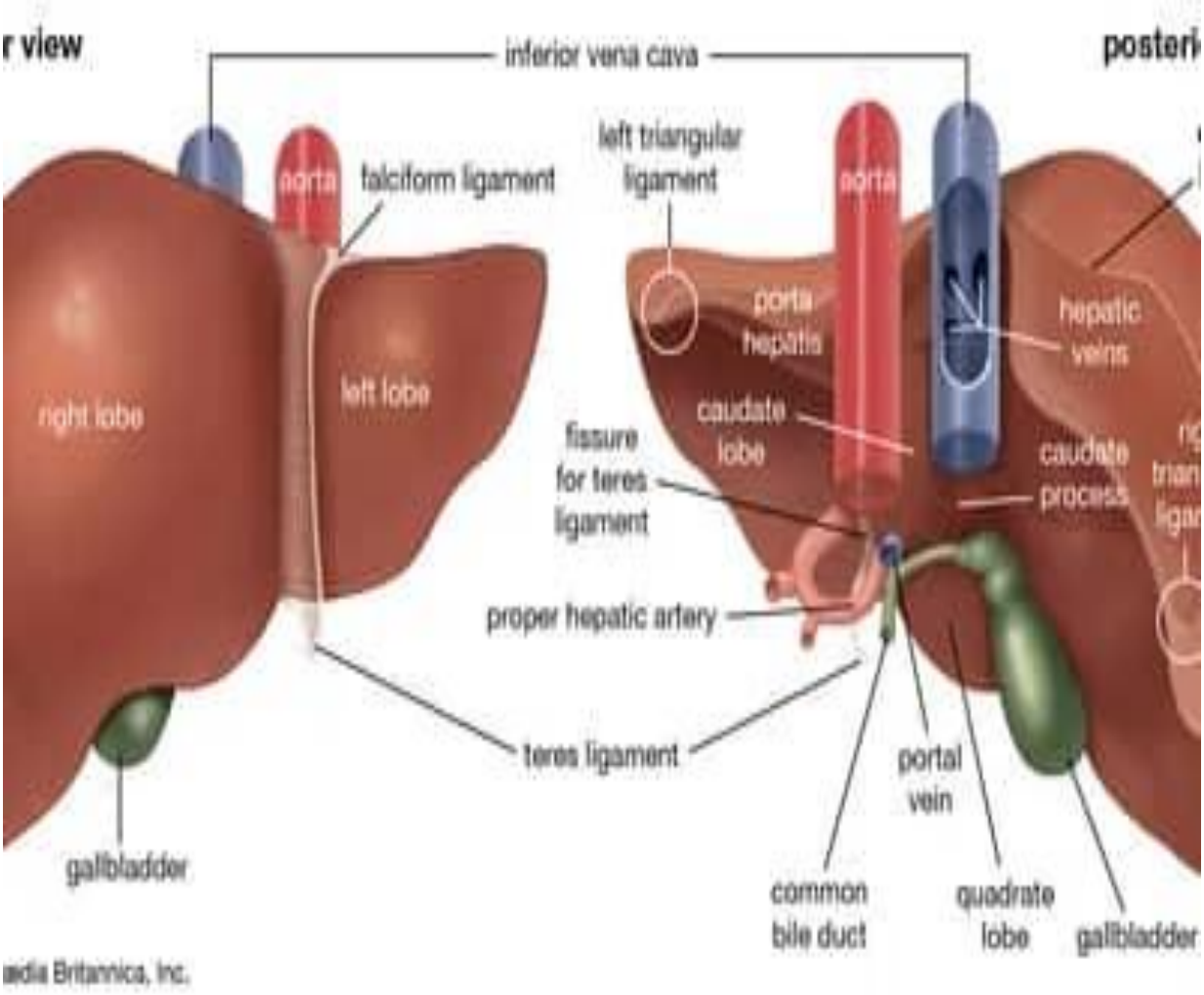


**Figure 4.5: Anterior and posterior views of the liver (Britannica, 2025).**

### 2.4.2 Pathological Features and Protective Effects

#### 1. Histopathological Changes Induced by Toxicity

The liver is susceptible to a range of pathological conditions, notably fibrosis, cirrhosis, and various chemically induced hepatotoxicities. Fibrosis, characterized by excessive accumulation of extracellular matrix components, signifies the liver's reparative response to chronic injury, which could lead to the formation of scar tissue and impaired liver function over time. Cirrhosis represents a more advanced and irreversible state of hepatic injury that may stem from various etiological factors, including alcohol consumption, viral hepatitis, and exposure to hepatotoxins (Zein *et al*., 2023).

Experimental studies have substantiated connections between exposure to hepatotoxins (e.g., carbon tetrachloride and dimethylnitrosamine) and the development of liver pathologies, demonstrating how these agents alter the liver's structural and functional integrity (Oladele *et al*., 2020). Chronic exposure to these substances elicits inflammatory responses, ultimately leading to necrosis, apoptosis, and dysfunction of hepatocytes.

#### 2. *Annona muricata* Leaf Extract Intervention

According to recent research, the ethanolic extract of *Annona muricata* has shown protective effects against hepatotoxicity induced by substances like dimethylnitrosamine. Treatment with *Annona muricata* resulted in a significant reduction of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, indicative of its hepatoprotective properties, as it preserves the integrity of hepatocyte membranes and facilitates the regeneration of damaged cells (Usunobun, 2014).

The potential mechanisms of action for this protective effect include modulation of inflammatory cytokines, antioxidant activities leading to reduced oxidative stress, and stabilization of cellular membranes that bolster structural integrity (Usunobun and Okolie, 2016). Studies have highlighted how pre-treatment with *Annona muricata* improves liver enzyme levels and enhances various hematological parameters, as well as bolsters renal function in contexts where hepatotoxic agents are administered (Usunobun and Okolie, 2016).

Research has also indicated the ability of *Annona muricata* to attenuate increases in biomarkers associated with liver damage, including total bilirubin levels and specific enzyme activities (Fakunle *et al*., 2024). Overall, *Annona muricata* represents a vital area of study for its promising applications in the prevention and treatment of liver-related pathologies, particularly in contexts where chemical insult poses a significant threat to hepatic health.

### 2.5 Kidneys and Other Organs

#### 2.5.1Kidneys

The kidneys are essential organs responsible for filtering blood, excreting waste products, maintaining electrolyte balance, and regulating blood pressure. Renal anatomy comprises several key structures including the renal cortex, medulla, and pelvis, each performing specialized functions in urine formation and blood filtration. The nephron, the functional unit of the kidney, plays a critical role in glomerular filtration, tubular reabsorption, and secretion. This intricate organization of renal tissues allows the kidneys to respond dynamically to various physiological demands and toxic insults. Understanding renal histology is paramount when assessing the impact of toxic agents, as it provides insights into structural changes and functional impairment resulting from exposure to harmful substances Fakunle *et al*. (2024).

Research has demonstrated the nephroprotective effects of *Annona muricata* extracts, particularly concerning heavy metal toxicity, such as that induced by cadmium. Following the administration of cadmium, significant renal histopathological changes can occur, including tubular dilation, necrosis, and glomerulosclerosis (Ehiremen *et al*., 2024). *Annona muricata* extracts have been shown to mitigate these toxic effects, demonstrating the ability to restore normal histological architecture in renal tissues. In studies involving experimental models, treatment with *Annona muricata* has resulted in attenuated levels of renal biomarkers such as urea and creatinine, which are critical indicators of renal function ((Westerhoff and Lamps, 2022; Oladele *et al*., 2019). These findings highlight the potential of *Annona muricata* in promoting kidney health, particularly in mitigating damage induced by adverse chemical exposures.

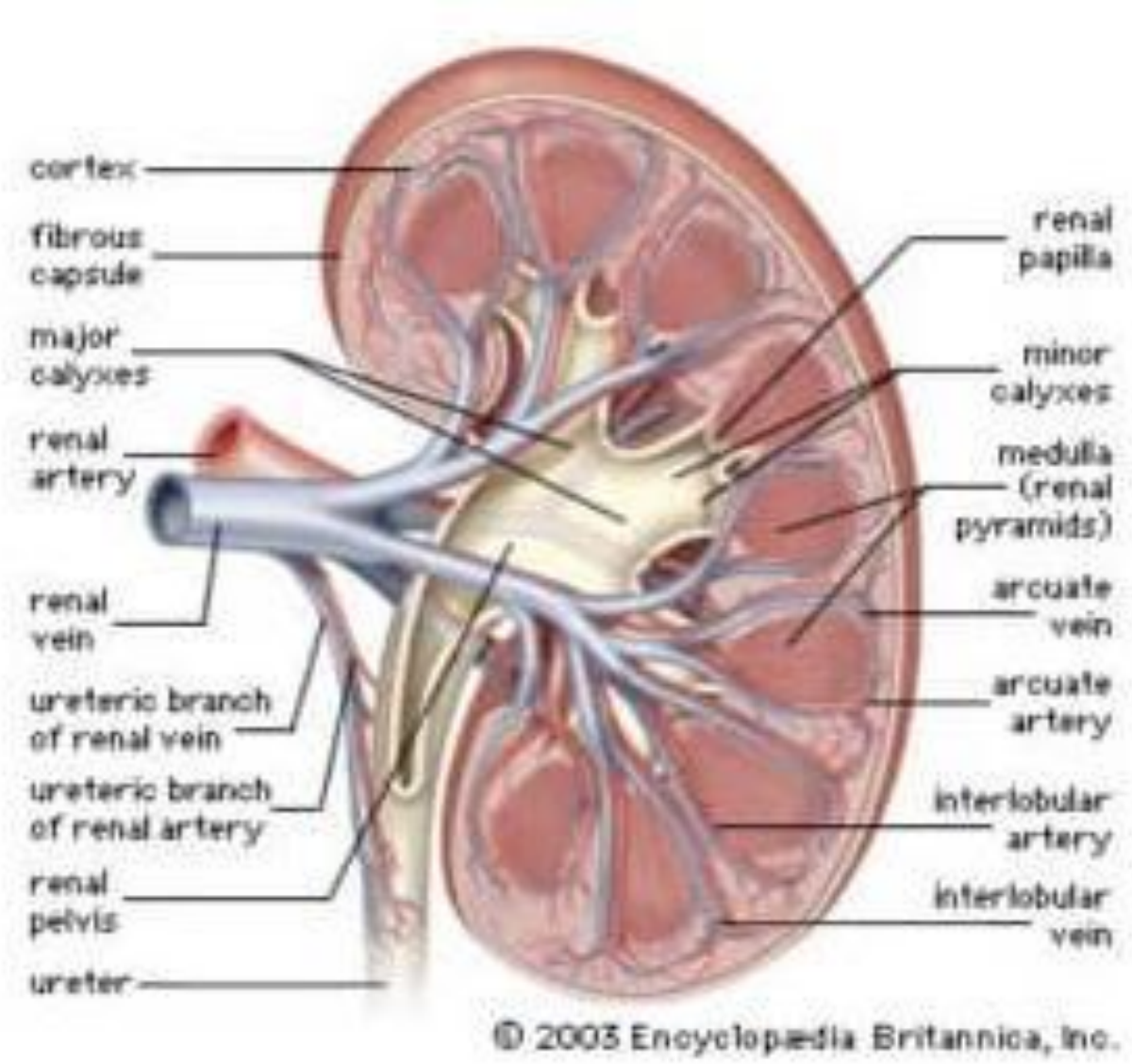


**Figure 4.6: Diagram of the kidney (Britannica, 2025).**

Additionally, the extract's benefits extend to maintaining electrolyte balance, which is vital for overall physiological homeostasis. By potentially stabilizing renal function and protecting against oxidative stress, *Annona muricata* may play an integrative role in the context of multi-organ defensive responses against toxigenic agents (Westerhoff and Lamps, 2022).

### 2.5.2 Comparative Organ Studies

Research on the effects of *Annona muricata* has not been limited to renal tissues; its protective properties have also been investigated in other organs such as the testis. Comparative studies emphasize that the histopathological alterations observed in these organs often mirror those seen in the kidneys, highlighting a systemic response to toxicological stresses (Ehiremen *et al*., 2024).

For instance, testicular histopathology in models of cadmium toxicity has shown increased oxidative stress and apoptosis within the tissues. Similar to its protective role in kidney histology, *Annona muricata* extracts have demonstrated the ability to enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in testicular tissues, effectively reducing oxidative damage and preserving tissue integrity (Ehiremen *et al*., 2024). The commonality in protective mechanisms across different organ systems suggests that *Annona muricata* may possess multi-organ protective properties, essential for its application in holistic therapies.

Furthermore, studies have documented the use of *Annona muricata* extracts in managing cardiovascular health, highlighting their role in mitigating oxidative stress and inflammation associated with heart disease. However, literature on its specific effects on the cardiovascular system, while suggestive of antioxidant benefits, may require further investigation to establish a more comprehensive understanding of its influence on lipid metabolism and cardiovascular function (Zein *et al*., 2023).

Comparative analysis across these studies illustrates the interconnectedness of organ health, underscoring the importance of exploring the systemic effects of *Annona muricata* in a multifaceted manner. This integrative approach enriches our understanding of how plant-derived extracts can support various physiological systems, enhancing their relevance in preventive and therapeutic frameworks.

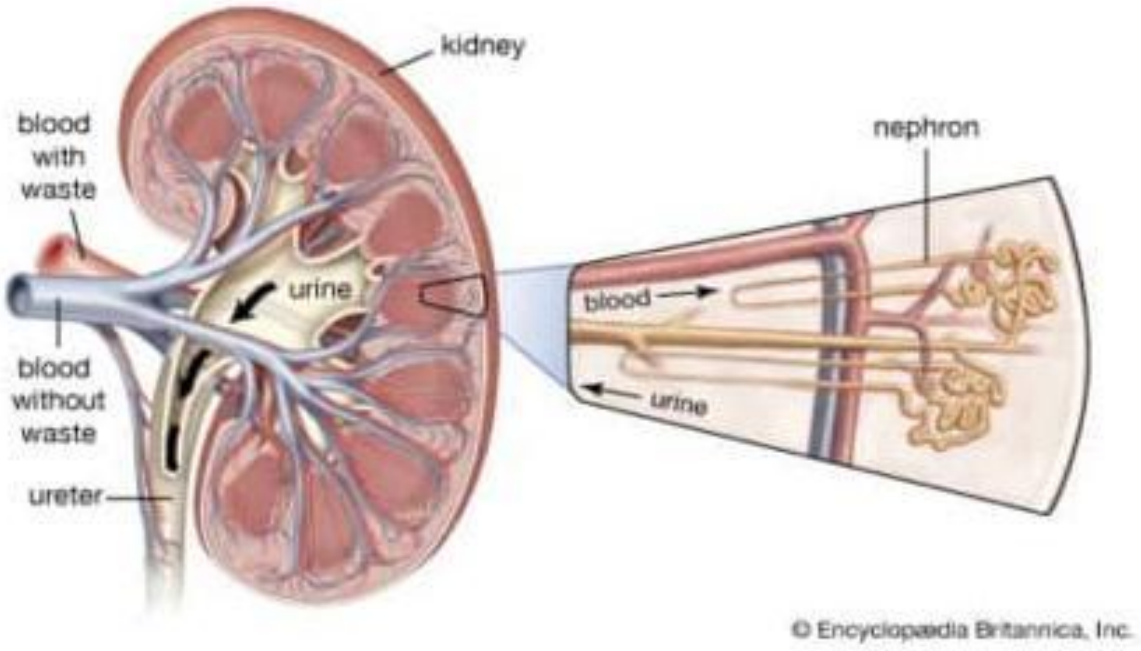


**Figure 4.7: Kidney blood supply (Britannica, 2025).**

The histopathological effects of *Annona muricata* on the kidneys and other organs emphasize its significant potential as a natural protective agent against chemical-induced toxicity. The comprehensive understanding of its protective properties across multiple organ systems bolsters its credibility as an important subject of investigation in the field of medicinal plants and traditional therapies.

## 2.6 TESTES

### 2.6.1 Anatomical and Histological Overview

The testes serve as the principal male reproductive organs, responsible for the production of sperm and synthesis of testosterone. Anatomically, the testes are composed of seminiferous tubules—highly coiled structures where spermatogenesis takes place—and interstitial tissue that contains Leydig cells responsible for androgen production. Histologically, the seminiferous tubules are lined with germinal epithelium arranged in several layers that progress from spermatogonia to mature spermatozoa, supported by Sertoli cells. The vascular network, essential for hormonal exchange and nutrient supply, and the presence of specialized connective tissue, further underscore the complexity of testicular architecture (Ehiremen *et al*., 2024).

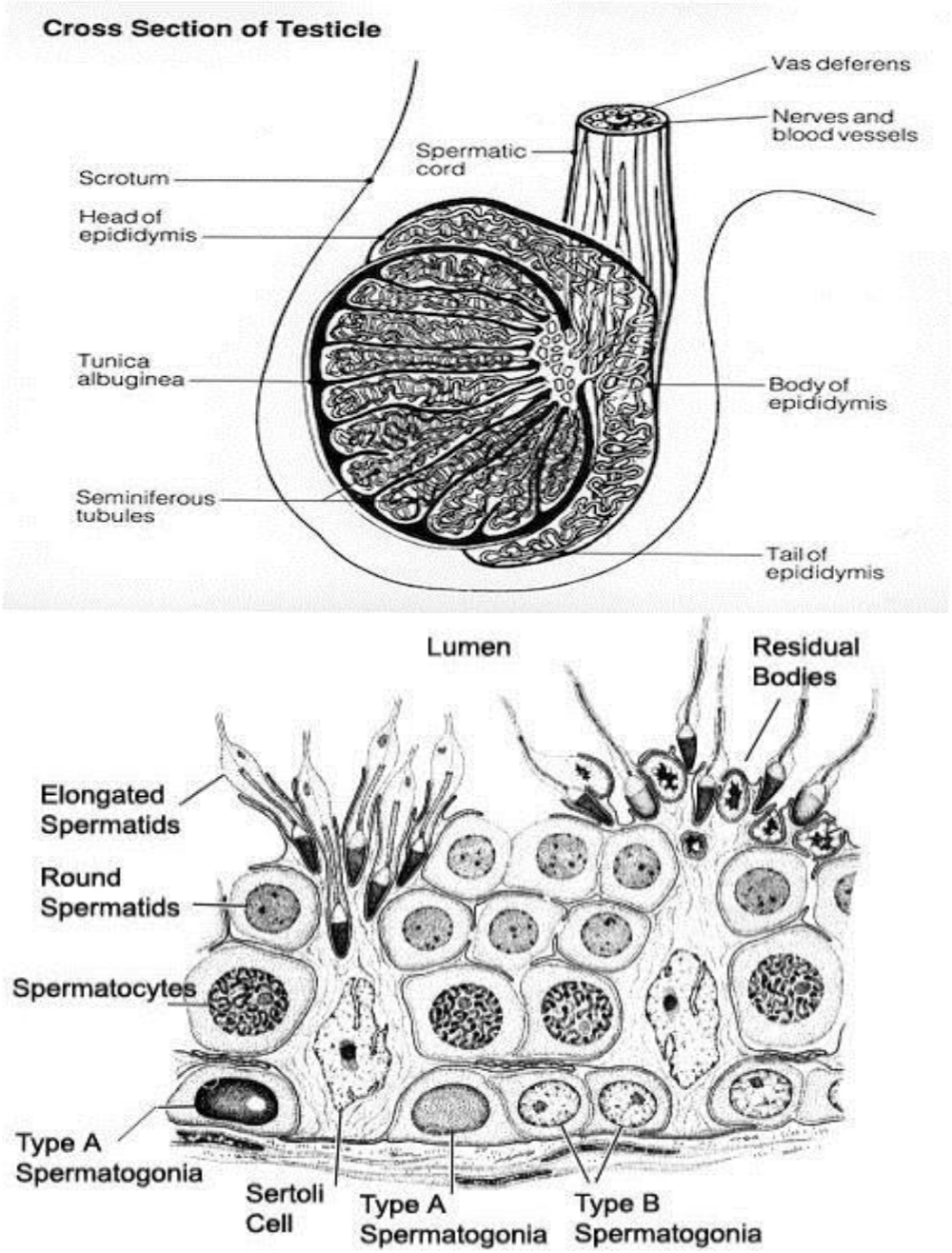


**Figure 4.8: a) Schematic representation of a mammalian testis, b) Cross-section of a seminiferous tubule (Scott, 1997; Klinikapapic, 2025).**

### 2.6.2 Histopathological Changes Induced by Toxic Insults

Exposure to toxic agents, such as heavy metals (e.g., cadmium) or chemical inducers of oxidative stress, has been documented to disrupt the architectural integrity of the testes. Such alterations may include disorganization of seminiferous tubules, degeneration of the germinal epithelium, and decreased sperm quality due to apoptosis and oxidative damage (Hasmila *et al*., 2019). In experimental animal models, toxic insults have led to features such as interstitial edema, reduced Leydig cell density, and sloughing of germ cells into the tubular lumen. These changes collectively undermine not only spermatogenesis but also the endocrine function of the testes.

### 2.6.3 Protective Effects of *Annona muricata* Leaf Extract

Studies report that pre-treatment with *Annona muricata* extract mitigates many of the toxicological changes observed in the testes. The antioxidant properties of the extract, believed to be primarily conferred by its rich flavonoid and phenolic content, significantly reduce oxidative stress and subsequent cellular injury in testicular tissue (Ehiremen *et al*., 2024). Enhanced activity of endogenous antioxidant enzymes—including superoxide dismutase (SOD) and catalase (CAT) has been observed following administration of *Annona muricata* extract in animal models. This protective action appears to stabilize the histoarchitecture of the seminiferous tubules, restore Leydig cell function, and maintain a favorable spermatogenic environment by reducing free radical-induced damage and apoptosis.

## 2.7 OVARIES

### 2.7.1 Anatomical and Histological Overview

The ovaries are the primary female reproductive organs, responsible for oocyte development and the synthesis of estrogens and progesterone. Each ovary contains a outer cortex, rich in primordial follicles at various stages of development, and an inner medulla that is highly

vascular and connective. Histologically, the ovarian cortex is characterized by the presence of follicles that progress from primordial, primary, and secondary stages to mature (Graafian) follicles. The cyclic changes in follicular development and subsequent ovulation are reflected in dynamic morphological alterations that are indispensable for reproductive function (Smith *et al*., 2020).

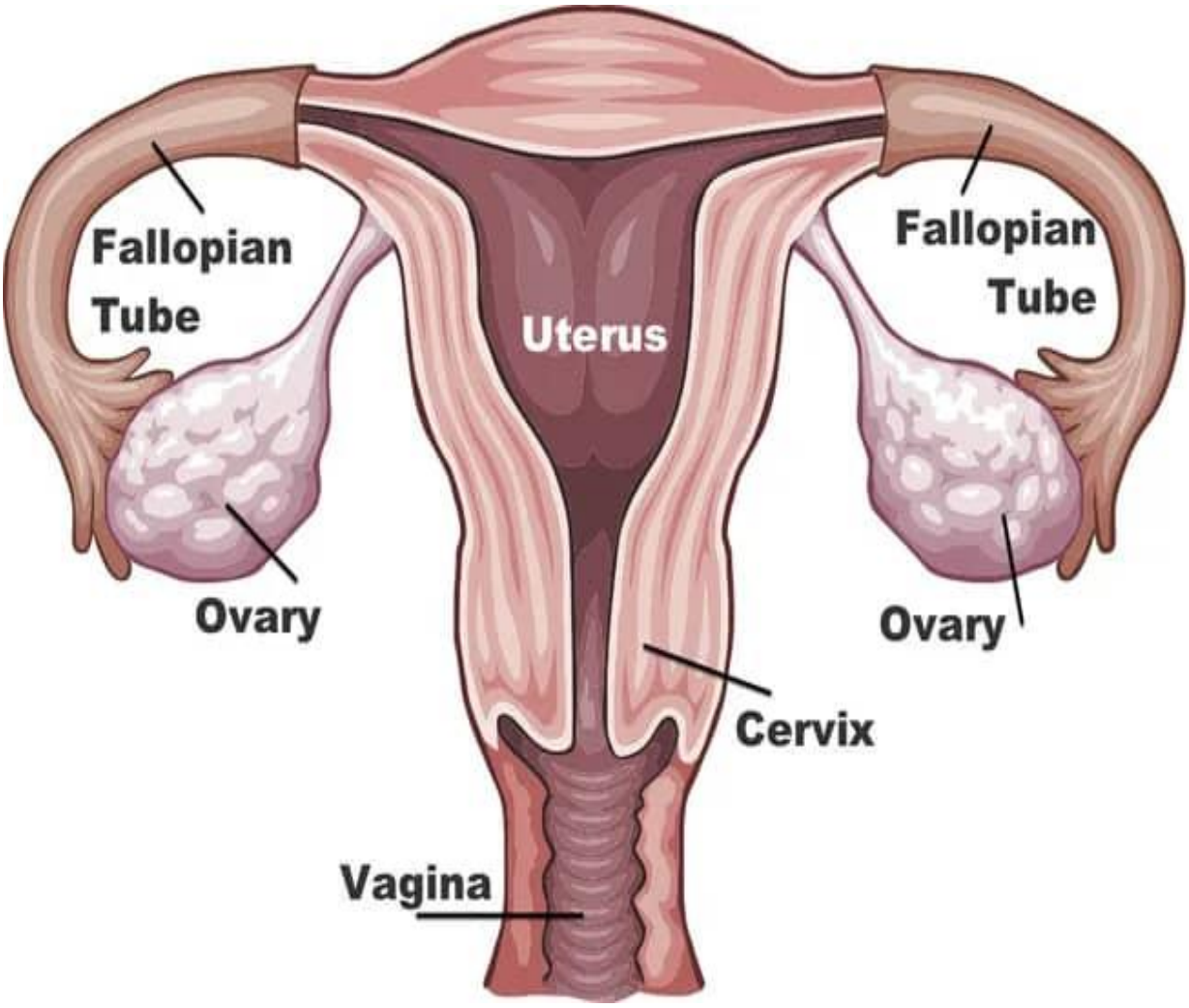


**Figure 4.9: Diagram of the female reproductive system (Alina, 2016).**

### 2.7.2 Histopathological Changes Induced by Toxic Insults

Exposure to toxins can disrupt normal ovarian histology, leading to alterations such as follicular atresia, stromal fibrosis, and compromised vascularization. Toxic agents may induce oxidative stress that results in the degeneration of follicular cells and an increase in apoptotic markers within the ovarian tissue. Additionally, chemical insults can lead to hormonal imbalances that further aggravate structural and functional deterioration in the ovaries, manifesting as a reduced number of viable follicles and aberrant stromal organization (Oladele *et al*., 2019).

### 2.7.3 Protective Effects of *Annona muricata* Leaf Extract

*Annona muricata* extract has demonstrated potential in ameliorating ovarian damage induced by toxic substances. Although less extensively studied than its effects on the testes, available evidence suggests that its antioxidant and anti-inflammatory properties may help preserve the histological integrity of ovarian tissue. Experimental studies indicate that treatment with *Annona muricata* extract helps to reduce oxidative damage, attenuate follicular atresia, and maintain overall ovarian morphology by reducing the expression of pro-apoptotic markers and enhancing the activity of antioxidant enzymes (Ehiremen *et al*., 2024). Such protective mechanisms might help in stabilizing the hormonal environment and ensuring the proper progression of follicular development.

### 2.7.4 Comparative Organ Analysis

Comparative studies of the reproductive organs indicate that although both the testes and ovaries are vulnerable to similar oxidative and toxic insults, the nature and extent of histopathological alterations may differ due to inherent physiological differences. In the testes, disruptions in seminiferous tubule organization and Leydig cell function primarily affect spermatogenesis and testosterone synthesis. Conversely, in the ovaries, toxic exposures primarily result in increased

follicular atresia and stromal disruption, which can compromise ovulation and estrogen production. *Annona muricata* extract appears to exhibit a multi-organ protective profile, with its antioxidant constituents providing a common protective benefit that is critical in both male and female reproductive tissues. This systemic response underscores its potential as an adjunct therapy in conditions of reproductive toxicity (Ehiremen *et al*., 2024)

## 2.8 MECHANISMS OF ACTION AND THERAPEUTIC IMPLICATIONS

### 2.8.1 Molecular Pathways and Bioactivities

*Annona muricata*, commonly known as soursop, exhibits a diverse range of biological activities attributed to its phytochemical composition, which includes alkaloids, flavonoids, and unique compounds known as annonaceous acetogenins. These mechanisms, particularly concerning anti-inflammatory and antioxidant properties, are pivotal in mediating the plant's therapeutic effects.

1. **Anti-inflammatory and Antioxidant Mechanisms**

The anti-inflammatory properties of *Annona muricata* have been documented across various studies, where extracts have been shown to suppress the activity of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) Chikwana *et al*. (2021). The ability of the plant extracts to inhibit the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is particularly notable, as this signaling pathway is deeply involved in inflammatory responses and is commonly associated with the pathogenesis of chronic diseases (Roduan *et al*., 2019). Additionally, the capacity of *Annona muricata* to enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase

(CAT) contributes to the mitigation of oxidative stress, a key player in tissue damage and inflammation (Ehiremen *et al*., 2024).

Furthermore, the inhibitory effects of *Annona muricata* on inflammasomes underscore its importance as an anti-inflammatory agent. Recent studies have suggested that certain bioactive compounds in *Annona muricata* can modulate the activation and effector pathways of inflammasomes, consequently reducing the production of inflammatory mediators and systemic inflammation (Nsor *et al*., 2024).

**2. Modulation of Apoptotic Pathways**

The bioactive constituents of *Annona muricata* are also implicated in the modulation of apoptotic pathways. Research has indicated that extracts from the leaves of this plant actively enhance the expression of p53, a critical tumor suppressor gene that regulates the cell cycle and promotes apoptosis in damaged cells (Adelina *et al*., 2013). The induction of apoptosis by *Annona muricata* has been observed in various cancer cell lines, where it triggers mitochondrial pathways and activates caspases, leading to programmed cell death (Astuti *et al*., 2021; Moghadamtousi *et al*., 2014). This property presents a promising avenue for utilizing *Annona muricata* as a potential chemotherapeutic agent against certain malignancies, reinforcing its role in reversing tissue damage and inhibiting cancer progression.

The combined effect of these mechanisms—anti-inflammatory activity, antioxidant support, and induction of apoptosis—positions *Annona muricata* as a multifaceted therapeutic agent, capable of addressing a variety of pathological conditions ranging from chronic inflammatory diseases to cancer.

### 2.8.2 Integration with Traditional and Modern Therapeutic Practices

The use of *Annona muricata* in traditional medicine illustrates its long-standing role in the treatment of various ailments, including infections, diabetes, and hypertension. Its incorporation into modern pharmacotherapy, particularly for liver and kidney disorders, shows promise based on emerging scientific evidence. Ethnobotanical studies have documented the traditional use of *Annona muricata* leaves, roots, and fruit in managing health complaints, providing a cultural context that informs contemporary research efforts (Leite *et al*., 2020).

Recent studies supporting the efficacy of *Annona muricata* as a natural therapeutic agent indicate its potential integration into standard protocols for treating liver conditions, especially in mitigating the hepatotoxic effects of agents like dimethylnitrosamine (DMN) (Westerhoff and Lamps, 2022) and cadmium (Oladele *et al*., 2019). Such integration is supported by evidence illustrating the extract's ability to prevent oxidative stress and restore enzyme levels associated with liver function. The hepatoprotective effects observed affirm the traditional claims and add empirical backing to its therapeutic utility.

In terms of kidney health, the antioxidative and anti-inflammatory effects of *Annona muricata* reinforce its inclusion as a complementary treatment. Its potential to attenuate oxidative stress and inflammation associated with renal dysfunction positions it as a valuable adjunct in the management of conditions such as diabetic nephropathy and acute kidney injury (Oladele *et al*., 2019).

The biochemical properties of *Annona muricata* harnessed from its natural origin provide a foundation for the development of new pharmacological formulations. The exploration of its bioactive compounds in clinical settings promises to make a significant impact on how modern medicine can utilize traditional resources to enhance patient care. As studies continue to unveil

the multifactorial benefits of *Annona muricata*, the plant will likely play a more prominent role in integrative approaches to healthcare, marrying traditional practices with contemporary scientific insights (Leite *et al*., 2020).

The mechanisms through which *Annona muricata* exerts its health benefits present a valuable opportunity for further investigation and application, highlighting the significance of combining traditional knowledge with modern pharmacological frameworks in addressing a spectrum of health challenges.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Collection of the *Annona muricata* leaves

Fresh *Annona muricata* leaves were obtained from Oluku market. The leaves were 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 *Annona muricata*. Following that a sample was placed in the departmental herbarium, and a voucher number (UBH-A356) was given for referral

### 3.1.1 Extraction of *Annona muricata* Leaves

The extraction was conducted at the Department of Pharmacology, University of Benin, Benin City. The leaves were washed in distilled water to remove any dirt or contaminants and subsequently air-dried in a shaded area to prevent degradation of phytochemicals. After drying, the leaves were ground into a fine powder using a laboratory grinder. Fifty grams of the powdered soursop leaves were soaked in 500 ml of ethanol for 48 hr at room temperature. The mixture was stirred continuously with a magnetic stirrer to facilitate optimal extraction. The mixture was filtered using a fine mesh cloth to separate the liquid extract from the solid remnants. The filtrate was concentrated using a rotary evaporator to yield a viscous extract, which was stored in a cool, dark place until use (Chemat *et al*., 2012)

### 3.1.2 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.

## 3.2 Animals

Twenty (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.

### 3.2.1 Acute Toxicity Test

The lethal dose (LD50) of *Annona muricata* (soursop) 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 soursop 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.

### 3.2.2 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.

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

All animals were administered orally via oral gavage.

### 3.2.3 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, with reference number MAFSAEC: 025-07/28/0040. The rats were handled following the Guidelines for the Care and Use of Laboratory Animals.

## 3.3 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.

**Processing of Specimen**

### 3.3.1 Biochemical Analyses

Blood samples were analyzed for serum levels of liver function tests (Albumin, Bilirubin, ALT, AST, ALP), kidney function tests (Creatinine and Urea) and reproductive hormones, including testosterone, progesterone, LH, and FSH, using enzyme-linked immunosorbent assay (ELISA) kits.

### 3.3.2 Liver Function Tests (LFTs)

Liver function tests primarily include measuring serum levels of Albumin, Bilirubin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP). The process was as follows:

**1. Sample Collection:** Blood samples were collected into serum tubes and allowed to clot for 30 minutes at room temperature. They were then centrifuged at 2000 rpm for 10 minutes to obtain serum.

**2. Preparation Using ELISA Kits**: Commercially available ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA) specific for these liver biomarkers were utilized (Dita, 2021).

**3. Assay Protocol:**

a) The serum samples were dilute as per the kit instructions.

b) The samples and control solutions were added to designated wells of the 10ul ELISA plate.

c) The plate were incubated according to the manufacturer's recommendations, at room temperature for 30 minutes

d) The wells were washed properly to remove unbound substances in a 3 step procedure using 250ul washing solution. Then substrate solution was added and incubated until color developed after 15 minutes.

e) The absorbance was measured using a microplate reader (Biotek Synergy LX800, Biotek Instruments, Inc.) at the specified wavelength of 450 nm for the enzyme-substrate reaction to evaluate enzyme activity (Dita, 2021).

**LFT Bio-markers Normal Range:**

**1. Aspertate Aminotransferase (AST)**

Normal Range: 5-40U/L (McPherson and Pincus, 2011).

**2. Alkaline Phosphatase (ALP)**

Adult Range: 44-147 U/L (Ravel, 2007)

**3. Alanine Aminotransferase (ALT)**

Adult Male: 7-56 U/L

Adult Female: 7-45 U/L (Cooper *et al*., 2015)

**4. Total Bilirubin**

Adult Range: 0.3-1.2 mg/dL (McPherson and Pincus, 2011).

**5. Albumin**

Normal Range: 3.4-5.4g/dL (Rifai *et al*., 2018)

### 3.3.3 Kidney Function Tests (KFTs)

Kidney function is generally evaluated by measuring Creatinine and Urea levels. The procedures were as follows:

**1. Sample Collection:** Similar to LFTs, blood samples were collected into serum tubes and seperated through centrifugation.

**2. Assay with ELISA Kits:** Kits specifically assaying for Creatinine and Urea (Renalcheck kidney marker Elisa kit, NephroScience Diagnostics, USA) were used for analysis of renal biomarkers, All reagent were kept at room temperature (25 C) (Kasama *et al*., 2021).

**3. Assay Protocol:**

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

b) The serum samples were placed into 25ul ELISA wells using a clean west tune 10ml automatic micro pipette, and transferred into an incubator at 37 C for 60 minutes.

c) Post-incubation proceeded with washing four times with 300ul washing solution and substrate addition for 20 minutes.

d) The colorimetric results were analyzed via a microplate reader (Axiom Microplate reader Urit-660) at 450nm, ensuring calibration with standards included in the kit (Dita, 2021).

**KFT Markers Normal Ranges**

**1) Urea (Serum)**

Adult Range: 7-20mg/dL (2.5-7.1 mmol/L).

**2) Creatinine (Serum)**

Adult Male: 0.6-12mg/dL

Adult Female: 0.5-1.1 mg/dL (McPherson and Pincus, 2011).

### 3.3.4 Reproductive Hormone Assays

Assessment of reproductive hormones such as Testosterone, Progesterone, Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) were carried out using ELISA techniques (Ehiremen *et al*., 2024).

**1. Sample Collection:** Blood samples were collected through cardiac puncture into anticoagulant-free containers and left to clot to obtain serum.

**2. Use of Dedicated ELISA Kits:** Kits designed for hormonal assays (EndoCheck Multi-Hormone Elisa Kit) available from suppliers RandD Systems (Minneapolis, MN, USA). The reagents used were equilibrated to room temperature of 25 C. (Gao *et al*., 2020).

**3. Assay Protocol:**

a) The serum samples were diluted as instructed by the kits manufacturer and pipette into 100ul ELISA wells using a clean west tune 10ml automatic micro pipette.

b) The Elisa wells were transferred into an incubator for 60 minutes at 37 C and followed by washing 5 times using 300ul washing solution each time.

c) The specific substrate solution were added and left for 15 minutes away from sunlight. Stop solution were added to stop reaction and optical density measured after color development using an ELISA plate reader at 450nm (Thermo Fisher's Multiskan GO) (Lee *et al*., 2023).

**Hormone specific range:**

a) Male: 1-18 mIU/mL

b) Female (Follicular phase): 3-20 mIU/mL

c) Postmenopausal: 25-150 mIU/mL (Handelsman *et al*., 2018)

**Testosterone:**

a) Male: 300-1000 ng/dL

b) Female: 15-70 ng/dL (Handelsman *et al*., 2018)

**Progesterone**

a) Male: <1 ng/mL

b) Follicular Phase (Female): <1 ng/mL

c) Luteal Phase: 1.5-6.0 pg/mL (Speroff and Fritz, 2005)

**Follicle-Stimulating Hormone (FSH)**

a) Male: 1.5-12.4 mIU/mL

b) Follicular Phase (Female): 3.5-12.5 mIU/mL

c) Postmenopausal Female: 26.7-133.6 mIU/mL (Legro *et al*., 2013)

**Luteinizing Hormone (LH):**

a) Male: 1.5-9.3 mIU/mL

b) Follicular Phase (Female): 2.4-12.6 mIU/mL

c) Postmenopausal Female: 14.2-52.3 mIU/mL (Weiss *et al*., 2010)

**Estradoil:**

a) Follicular Phase: 20-150 pg/mL

b) Luteal Phase: 100-350 pg/mL

c) Postmenopausal: <20 pg/Ml (Legro *et al*., 2013)

### 3.3.5 Histopathological investigation

The fixed 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. Embedded 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 for 10 min, rinsed in water, and differentiated in 1% acid alcohol. Sections were counterstained in 1% aqueous eosin for 3 min, 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.

## 3.4 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 *Annona muricata* leaf extract on FBC analytes in the Control Group

*Annona muricata* had no significant effect on the FBC analyte results in the Control group

**Table 4.1: ANOVA of FBC analytes in the Control Group**

| | Mean ± SD | F | p-value |
|---|---|---|---|
| **WBC** | 8.2 ± 3 | 1.017 | 0.419 |
| **LYM** | 7.2 ± 2.5 | 1.103 | 0.386 |
| **LYM (%)** | 88 ± 2.9 | 0.844 | 0.496 |
| **MID** | 0.8 ± 0.4 | 0.502 | 0.688 |
| **MID (%)** | 8.5 ± 2.4 | 0.884 | 0.477 |
| **GRA** | 0.3 ± 0.2 | 0.324 | 0.808 |
| **GRA (%)** | 3.5 ± 0.7 | 0.718 | 0.56 |
| **HGB** | 14.1 ± 1.2 | 0.107 | 0.954 |
| **MCH** | 18.7 ± 0.7 | 1.431 | 0.282 |
| **MCHC** | 35.5 ± 0.5 | 0.705 | 0.567 |
| **RBC** | 7.5 ± 0.4 | 0.271 | 0.845 |
| **MCV** | 52.9 ± 1.9 | 1.712 | 0.217 |
| **HCT** | 39.8 ± 3.5 | 0.429 | 0.736 |
| **RDW-SD** | 33.1 ± 2.2 | 0.709 | 0.565 |
| **RDW-CV** | 15.6 ± 0.8 | 0.235 | 0.87 |
| **PLT** | 484.8 ± 79.1 | 0.2 | 0.895 |
| **MPV** | 7.5 ± 0.1 | 0.866 | 0.485 |
| **PDW** | 9.3 ± 0.4 | 0.428 | 0.736 |
| **PCT** | 0.4 ± 0.1 | 0.082 | 0.969 |
| **P-LCR** | 2.9 ± 2.4 | 0.506 | 0.685 |

### 4.2 Effect of *Annona muricata* leaf extract on FBC analytes in Group B

*Annona muricata* had no significant effect on the FBC analyte results in Group B.

**Table 4.2: ANOVA of FBC analytes in Group B**

| | Mean ± SD | F | p-value |
|---|---|---|---|
| **WBC** | 9.4 ± 2.7 | | |
| **LYM** | 8.3 ± 2.1 | | |
| **LYM (%)** | 89.2 ± 3.5 | | |
| **MID** | 0.8 ± 0.5 | | |
| **MID (%)** | 8 ± 2.8 | | |
| **GRA** | 0.3 ± 0.2 | | |
| **GRA (%)** | 2.8 ± 0.7 | | |
| **HGB** | 14.2 ± 1.3 | | |
| **MCH** | 18.8 ± 0.6 | | |
| **MCHC** | 34.7 ± 1.7 | | |
| **RBC** | 7.5 ± 0.5 | | |
| **MCV** | 54.4 ± 2.9 | | |
| **HCT** | 40.8 ± 2.6 | | |
| **RDW-SD** | 34.2 ± 5.2 | | |
| **RDW-CV** | 15.7 ± 1.7 | | |
| **PLT** | 461.8 ± 153.6 | | |

| | |
|---|---|
| **MPV** | 7.9 ± 0.4 |
| **PDW** | 9.8 ± 2 |
| **PCT** | 0.4 ± 0.1 |
| **P-LCR** | 6.5 ± 7.5 |

### 4.3 Effect of *Annona muricata* leaf extract on FBC analytes in Group C

*Annona muricata* had no significant effect on the FBC analyte results in Group C.

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

| | Mean ± SD | F | p-value |
|---|---|---|---|
| **WBC** | 8.4 ± 0.7 | | |
| **LYM** | 7.2 ± 0.8 | | |
| **LYM (%)** | 85.7 ± 3.1 | | |
| **MID** | 0.9 ± 0.2 | | |
| **MID (%)** | 10.4 ± 1.7 | | |
| **GRA** | 0.3 ± 0.1 | | |
| **GRA (%)** | 3.9 ± 1.4 | | |
| **HGB** | 14.2 ± 0.7 | | |
| **MCH** | 19.2 ± 1.1 | | |
| **MCHC** | 35.5 ± 0.8 | | |
| **RBC** | 7.4 ± 0.4 | | |
| **MCV** | 54.3 ± 3.9 | | |
| **HCT** | 39.9 ± 2.2 | | |
| **RDW-SD** | 33.1 ± 2.8 | | |
| **RDW-CV** | 15.2 ± 0.5 | | |
| **PLT** | 490.8 ± 71.7 | | |

| | |
|---|---|
| **MPV** | 7.7 ± 0.6 |
| **PDW** | 9.1 ± 1.1 |
| **PCT** | 0.4 ± 0.1 |
| **P-LCR** | 5 ± 6.5 |

### 4.4 Effect of *Annona muricata* leaf extract on FBC analytes in Group D

*Annona muricata* had no significant effect on the FBC analyte results in Group D.

**Table 4.4: ANOVA of FBC analytes in Group D**

| | Mean ± SD | F | p-value |
|---|---|---|---|
| **WBC** | 11.3 ± 3.8 | | |
| **LYM** | 9.9 ± 3.5 | | |
| **LYM (%)** | 87.2 ± 3.1 | | |
| **MID** | 1.1 ± 0.4 | | |
| **MID (%)** | 9.5 ± 2 | | |
| **GRA** | 0.4 ± 0.1 | | |
| **GRA (%)** | 3.3 ± 1.3 | | |
| **HGB** | 13.8 ± 0.7 | | |
| **MCH** | 18.1 ± 0.5 | | |
| **MCHC** | 35.8 ± 1.2 | | |
| **RBC** | 7.6 ± 0.3 | | |
| **MCV** | 50.6 ± 1.6 | | |
| **HCT** | 38.6 ± 2.4 | | |
| **RDW-SD** | 31 ± 1.2 | | |
| **RDW-CV** | 15.2 ± 0.6 | | |
| **PLT** | 529.5 ± 168.8 | | |
| **MPV** | 7.6 ± 0.2 | | |
| **PDW** | 8.9 ± 0.9 | | |
| **PCT** | 0.4 ± 0.1 | | |
| **P-LCR** | 2.4 ± 2.8 | | |

### 4.5 Effect of *Annona muricata* leaf extract on Liver Function in control group

*Annona muricata* had no significant effect on the liver function results in the Control group

**Table 4.5: ANOVA of comparison of liver function in Control Group**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Liver** | 5.9 ± 0.7 | 0.125 | 0.943 |
| **Urea** | 33 ± 4.7 | 0.607 | 0.623 |
| **Creatinine** | 1 ± 0.1 | 0.911 | 0.465 |
| **AST** | 81.3 ± 18.1 | 0.633 | 0.608 |
| **ALT** | 51.5 ± 7.1 | 0.683 | 0.579 |
| **ALP** | 49.5 ± 14.9 | 0.691 | 0.575 |
| **TB** | 0.3 ± 0.1 | 0.571 | 0.644 |
| **CB** | 0.1 ± 0 | 0 | 1 |

### 4.6 Effect of *Annona muricata* leaf extract on Liver Function in group B

*Annona muricata* had no significant effect on the liver function results in group B

**Table 4.6: ANOVA of liver function in Group B**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Liver** | 6.2 ± 0.7 | | |
| **Urea** | 31.5 ± 7.6 | | |
| **Creatinine** | 1.2 ± 0.2 | | |
| **AST** | 82.8 ± 15.5 | | |
| **ALT** | 49 ± 9.8 | | |
| **ALP** | 51.3 ± 12.5 | | |
| **TB** | 0.3 ± 0.1 | | |
| **CB** | 0.1 ± 0 | | |

### 4.7 Effect of *Annona muricata* leaf extract on Liver Function in group C

*Annona muricata* had no significant effect on the liver function results in group C

**Table 4.7: ANOVA of liver function in Group C**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Liver** | 5.9 ± 0.7 | | 0.943 |
| **Urea** | 46.3 ± 39 | | 0.623 |
| **Creatinine** | 1.1 ± 0.4 | | 0.465 |
| **AST** | 112.5 ± 78 | | 0.608 |
| **ALT** | 68.5 ± 45.9 | | 0.579 |
| **ALP** | 75.5 ± 63.2 | | 0.575 |
| **TB** | 0.3 ± 0.1 | | 0.644 |
| **CB** | 0.1 ± 0 | | 1 |

### 4.8 Effect of *Annona muricata* leaf extract on Liver Function in group D

*Annona muricata* had no significant effect on the liver function results across the group D.

**Table 4.8: ANOVA of liver function in Group D**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Liver** | 6 ± 1.2 | | |
| **Urea** | 51.5 ± 30.9 | | |
| **Creatinine** | 1.2 ± 0.2 | | |
| **AST** | 118 ± 52.8 | | |
| **ALT** | 71 ± 27.6 | | |
| **ALP** | 83.8 ± 50.1 | | |
| **TB** | 0.2 ± 0.1 | | |
| **CB** | 0.1 ± 0 | | |

### 4.9 Effect of *Annona muricata* leaf extract on Kidney Function in the control group

*Annona muricata* had significant effect ($p < 0.05$) on the values of Sodium and Chlorine analytes between in the control group.

**Table 4.9: ANOVA of comparison of kidney function in the Control Group**

| | Group | Mean ± SD | F | Sig. |
|---|---|---|---|---|
| **Kidney** | Control | 0.6 ± 0.1 | 1 | 0.426 |
| **Na** | Control | 137.3 ± 1.5 | 4.117 | 0.032 |
| **K** | Control | 3.9 ± 0.6 | 0.977 | 0.436 |
| **Cl** | Control | 103.5 ± 0.6 | 5.53 | 0.013 |
| **HCO3** | Control | 20.8 ± 3.4 | 2.368 | 0.122 |

### 4.10 Effect of *Annona muricata* leaf extract on Kidney Function in Group B

*Annona muricata* had significant effect ($p < 0.05$) on the values of Sodium and Chlorine analytes between in the control group B.

**Table 4.10: ANOVA of comparison of kidney function in Group B**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Kidney** | 0.5 ± 0.1 | | |
| **Na** | 139.8 ± 1.7 | | |
| **K** | 4.7 ± 0.9 | | |
| **Cl** | 105.5 ± 1.7 | | |
| **HCO3** | 17.5 ± 1.9 | | |

### 4.11 Effect of *Annona muricata* leaf extract on Kidney Function in Group C

*Annona muricata* had significant effect ($p < 0.05$) on the values of Sodium and Chlorine analytes between in the group C.

**Table 4.11: ANOVA of comparison of kidney function in Group C**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Kidney** | 0.5 ± 0.1 | | |
| **Na** | 139.3 ± 1.5 | | |
| **K** | 4.8 ± 1.5 | | |
| **Cl** | 105 ± 1.8 | | |
| **HCO3** | 17.8 ± 3.3 | | |

### 4.12 Effect of *Annona muricata* leaf extract on Kidney Function in Group D

*Annona muricata* had significant effect ($p < 0.05$) on the values of Sodium and Chlorine analytes between in group D.

**Table 4.12: ANOVA of comparison of kidney function in Group D**

| | Mean ± SD | F | Sig. |
|---|---|---|---|
| **Kidney** | 0.5 ± 0.1 | | |
| **Na** | 143 ± 3.8 | | |
| **K** | 4 ± 0.3 | | |
| **Cl** | 107.3 ± 0.5 | | |
| **HCO3** | 22 ± 2.7 | | |

### 4.13 Effect of Annona *muricata* leaves extract on rat body weight

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

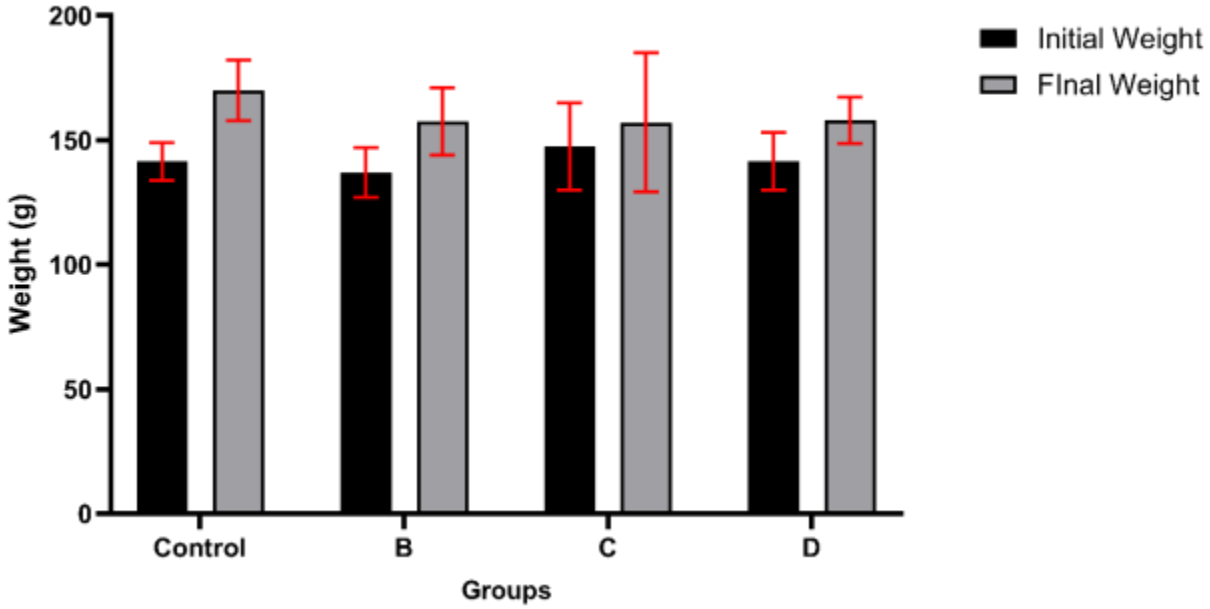


Figure 4.1: Changes in the weight of rats after administration

### 4.14 Effect of *Annona* muricata leaves extract on weight of reproductive organs

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

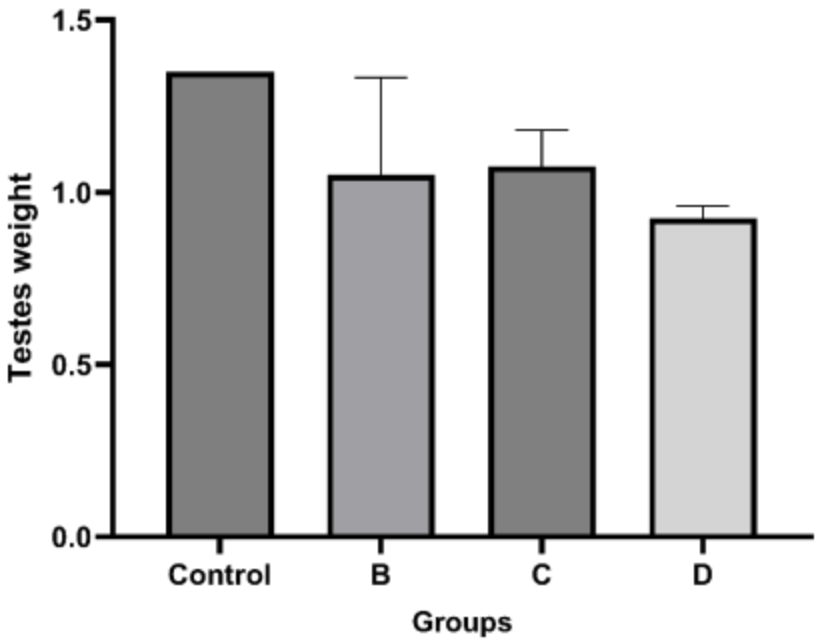


Chart 2: showing effect of *Annona muricata* leaves extract on weight of the testes

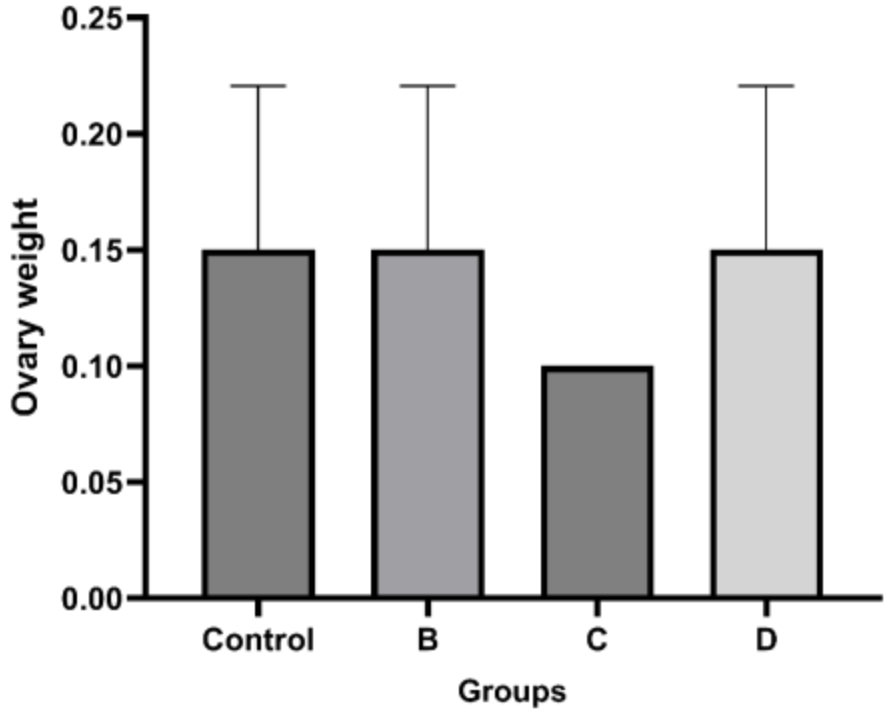


Chart 3: showing the effect of *Annona muricata* leaves extract on weight of the ovaries

### 4.15 Effect of *Annona muricata* leaves extract on reproductive hormones

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

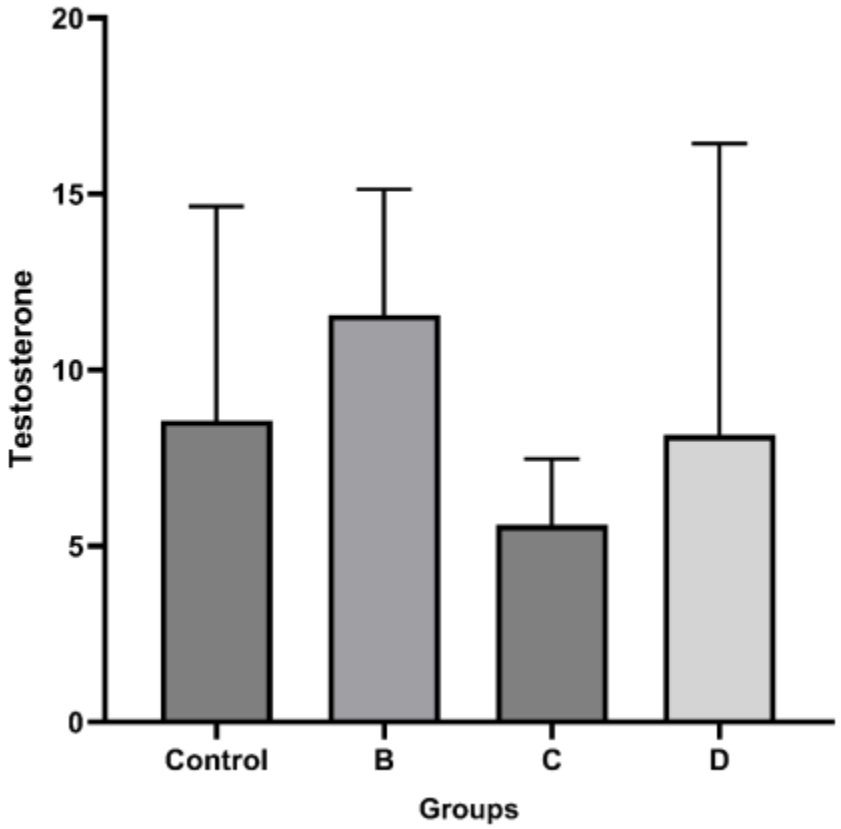


Chart 4: showing effect of *Annona muricata* leaves extract on testosterone

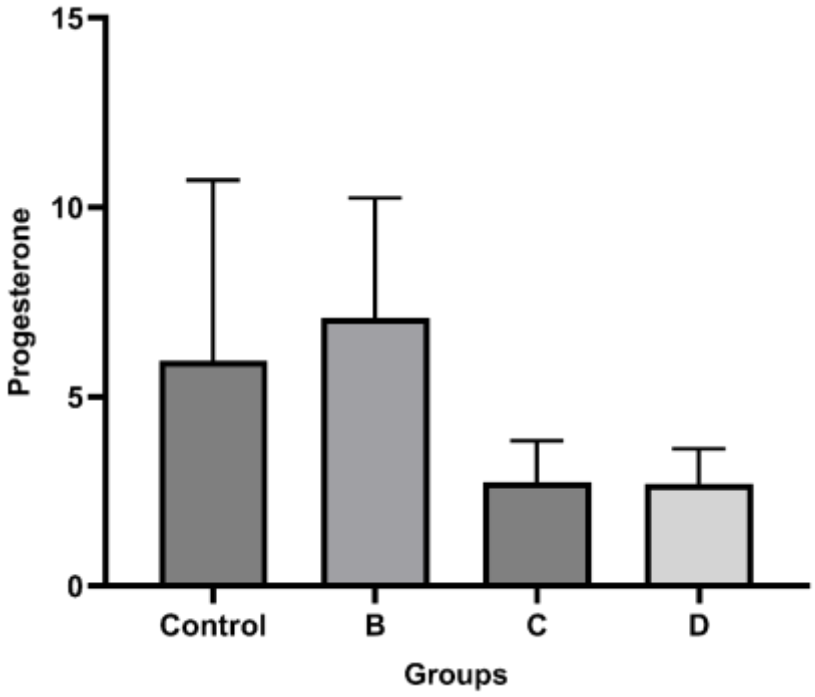


Chart 5: showing effect of *Annona muricata* leaves extract on progesterone

GROUP A2 M LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

GROUP B2 M LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**

GROUP C2 M LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**

GROUP D1 M LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

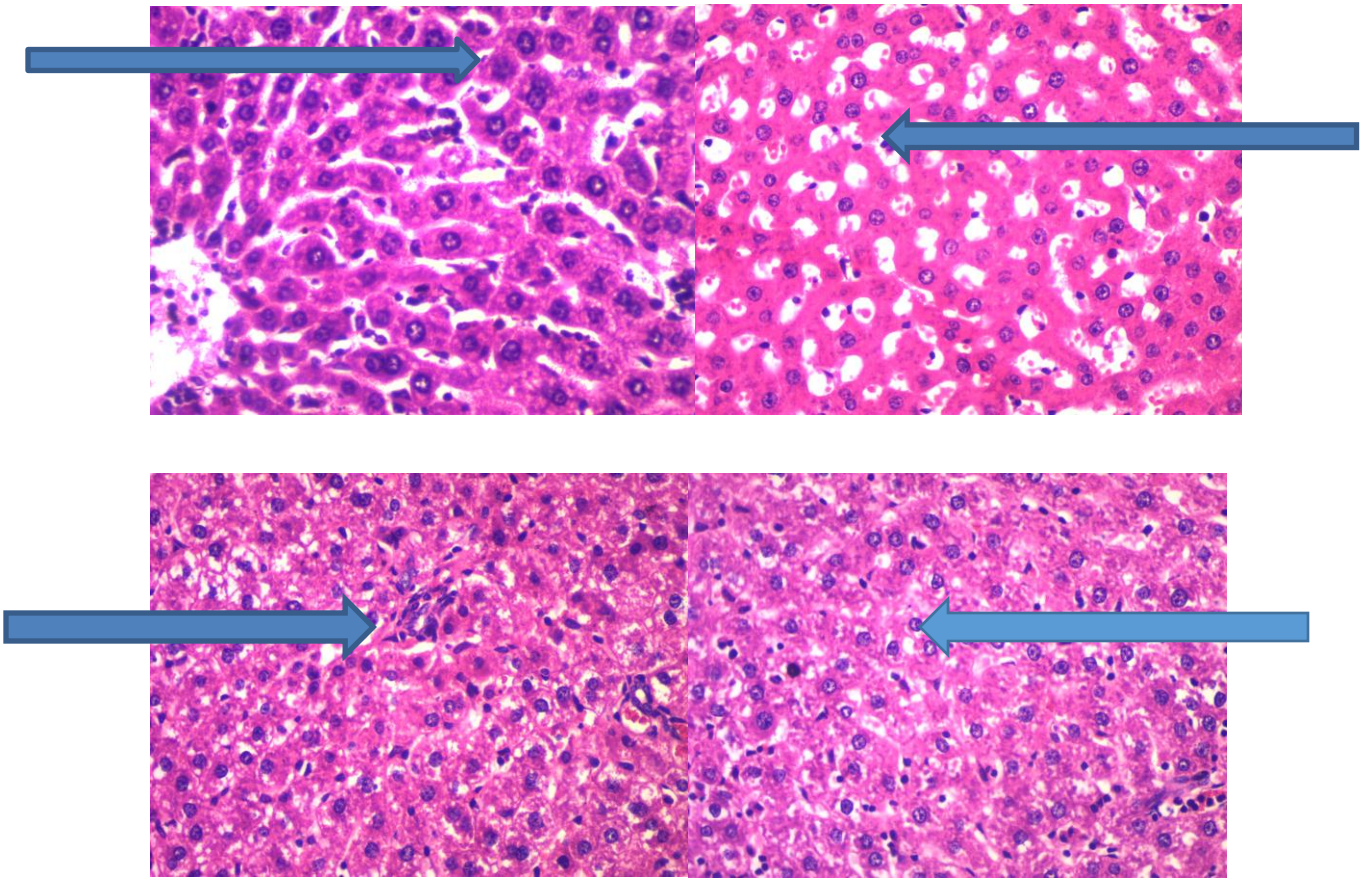

Plate 4.1: Section of liver of male rat for received pellet and distilled water only for 1 month
Plate 4.2: Section of liver of male rat administered 250 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.3: Section of liver of male rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.4: Section of liver of male rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

GROUP A1 F LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding a centrally placed normochromic nuclei with indistinct nucleoli. **FEATURES IN KEEPING WITH NORMAL HEPATOCYTES**

GROUP B2 F LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**

GROUP C2 F LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing **FEATURES IN KEEPING WITH STEATOSIS** microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei.

GROUP D2 F LIVER X400

Section of the liver shows hepatocytes (arrow) with eosinophilic cytoplasm containing microvacuoles (ballooning degeneration), the cytoplasm surrounds a centrally placed nuclei. **FEATURES IN KEEPING WITH STEATOSIS**

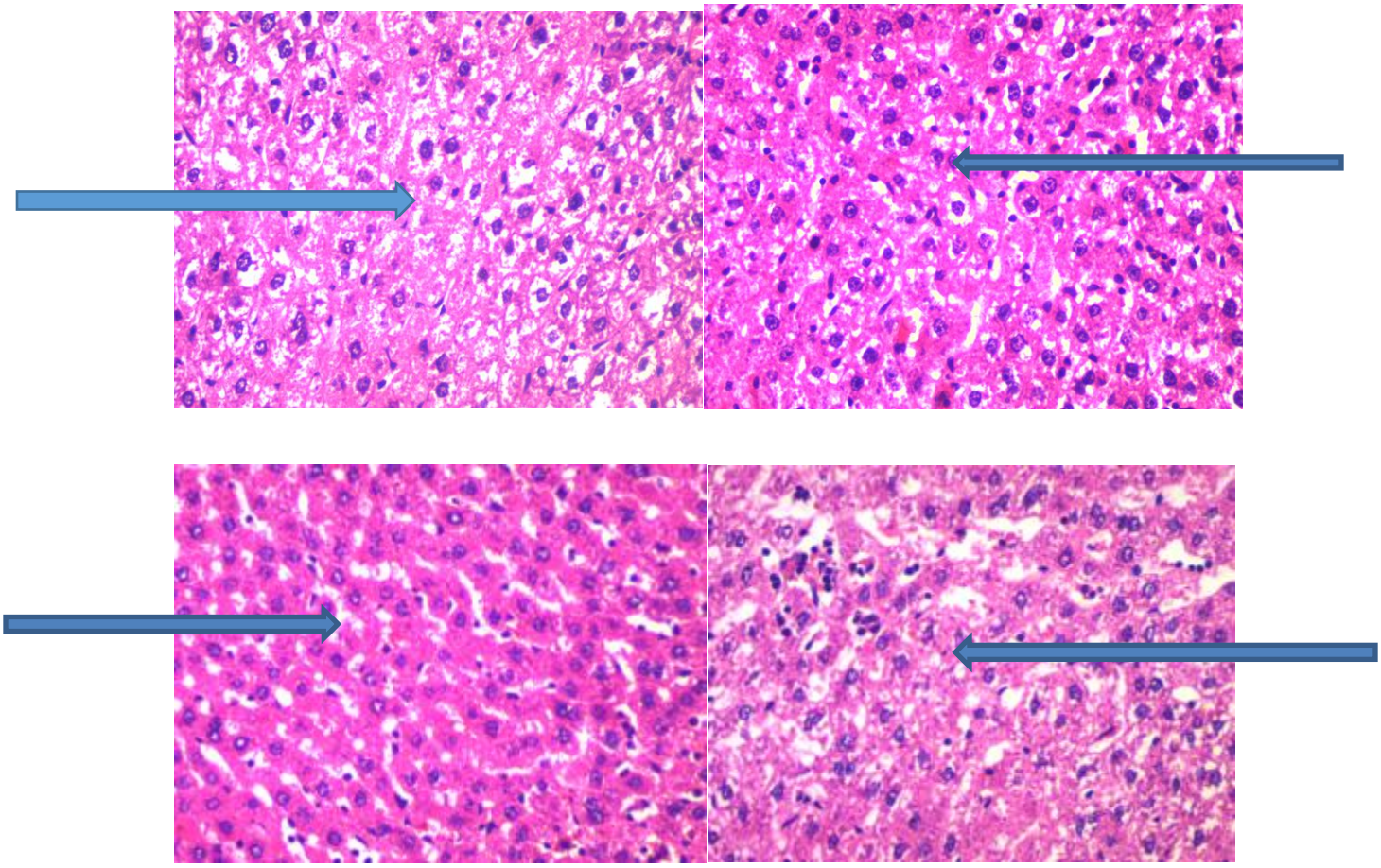

Plate 4.5: Section of liver of female rat received pellet and distilled water only for 1 month

Plate 4.6: Section of liver of female rat administered 250 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.7: Section of liver of female rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.8: Section of liver of female rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

GROUP A1 F KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP A2 M KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP B2 F KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP B2 M KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

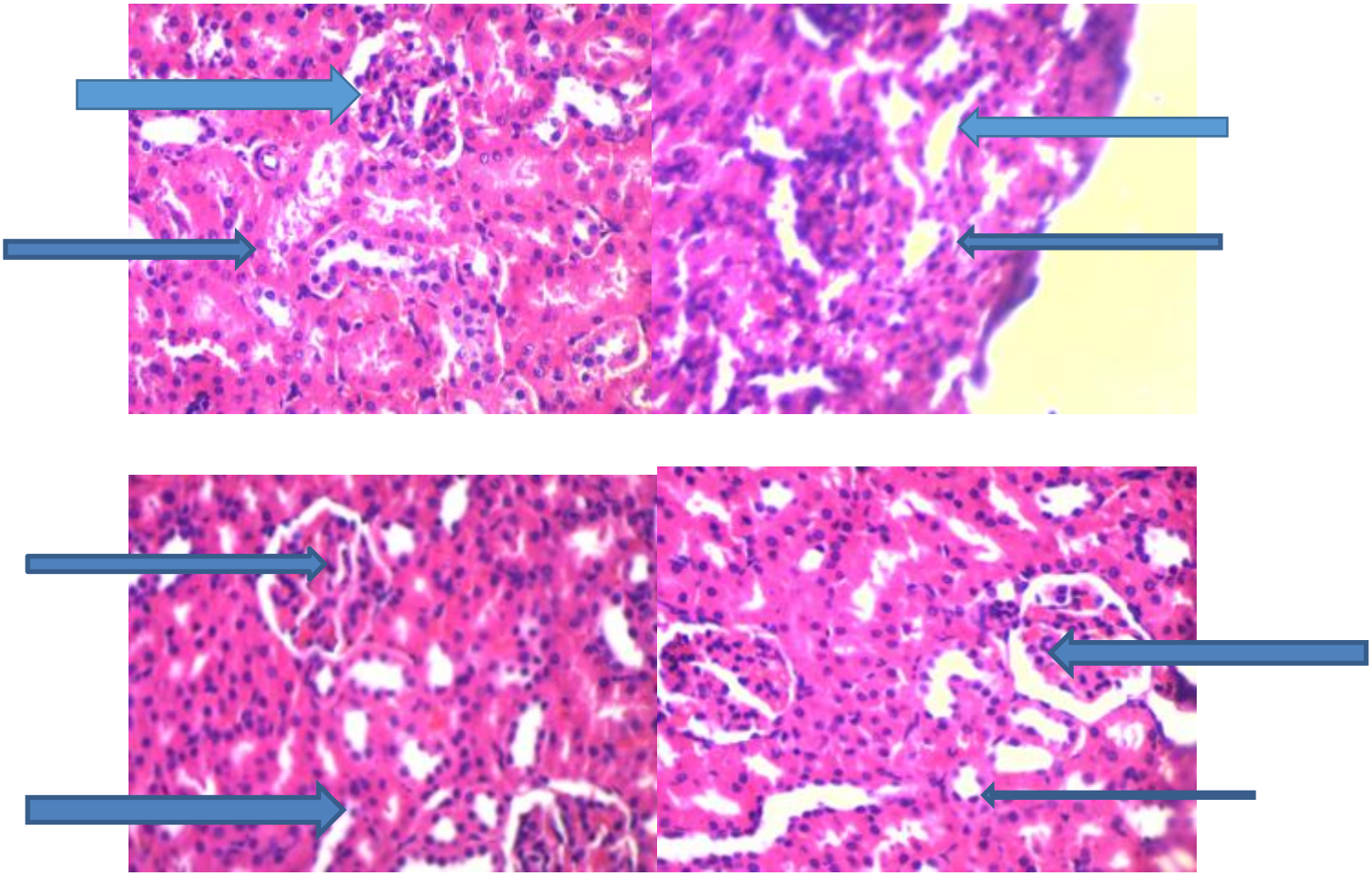

Plate 4.9: Section of kidney of female rat received pellet and distilled water only for 1 month

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

Plate 4.11: Section of liver of female rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.12: Section of kidney of male rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

GROUP C1 M KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP C2 F KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP D1 M KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

GROUP D2 F KIDNEY X400

Section of the kidney shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

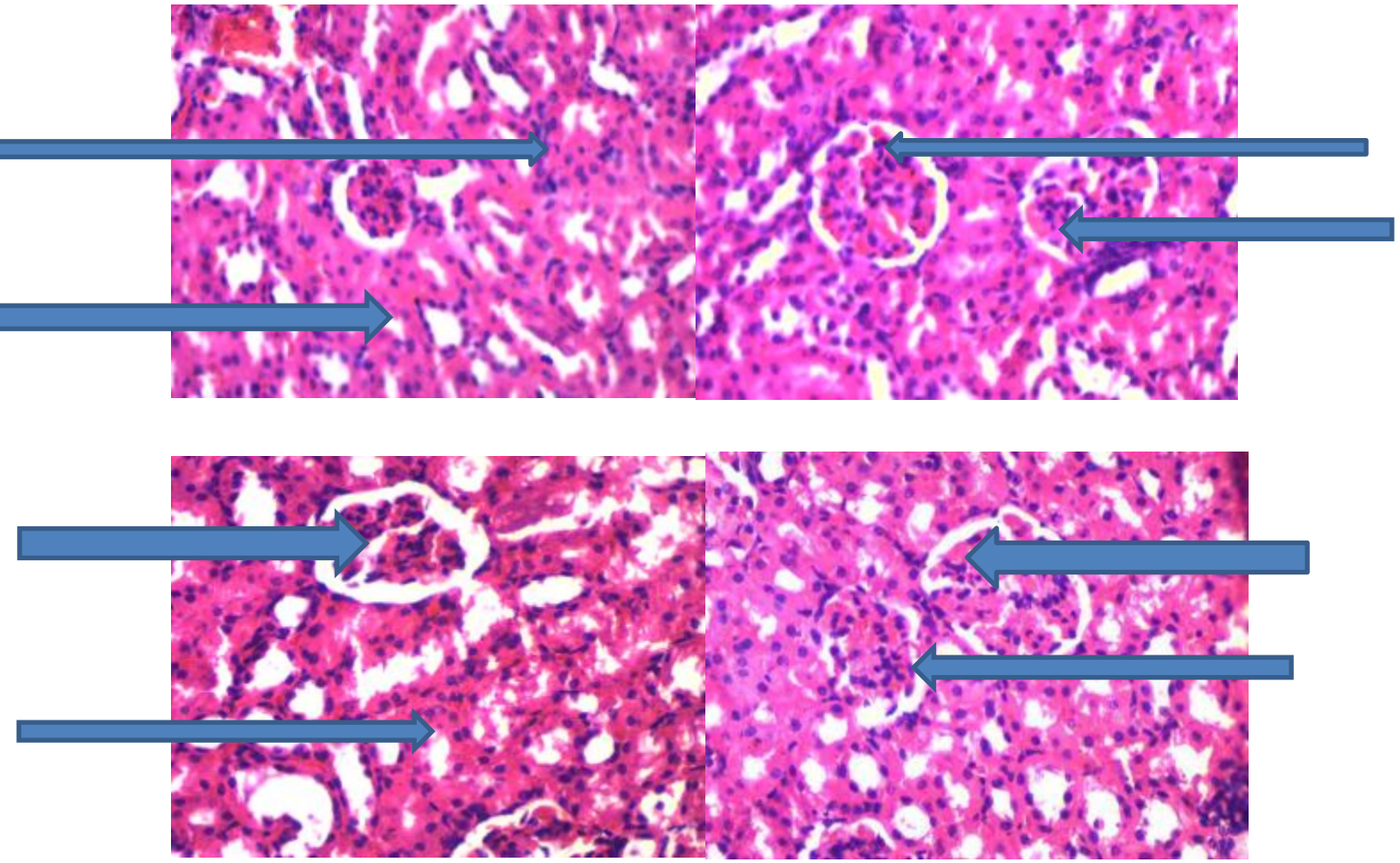

Plate 4.13: Section of kidney of male rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.14: Section of kidney of female rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.15: Section of kidney of male rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.16: Section of kidney of female rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

GROUP A2 M TESTIS X400

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**

GROUP B2 M TESTIS X400

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**

GROUP C2 M TESTIS X400

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**

GROUP D1 M TESTIS X400

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**

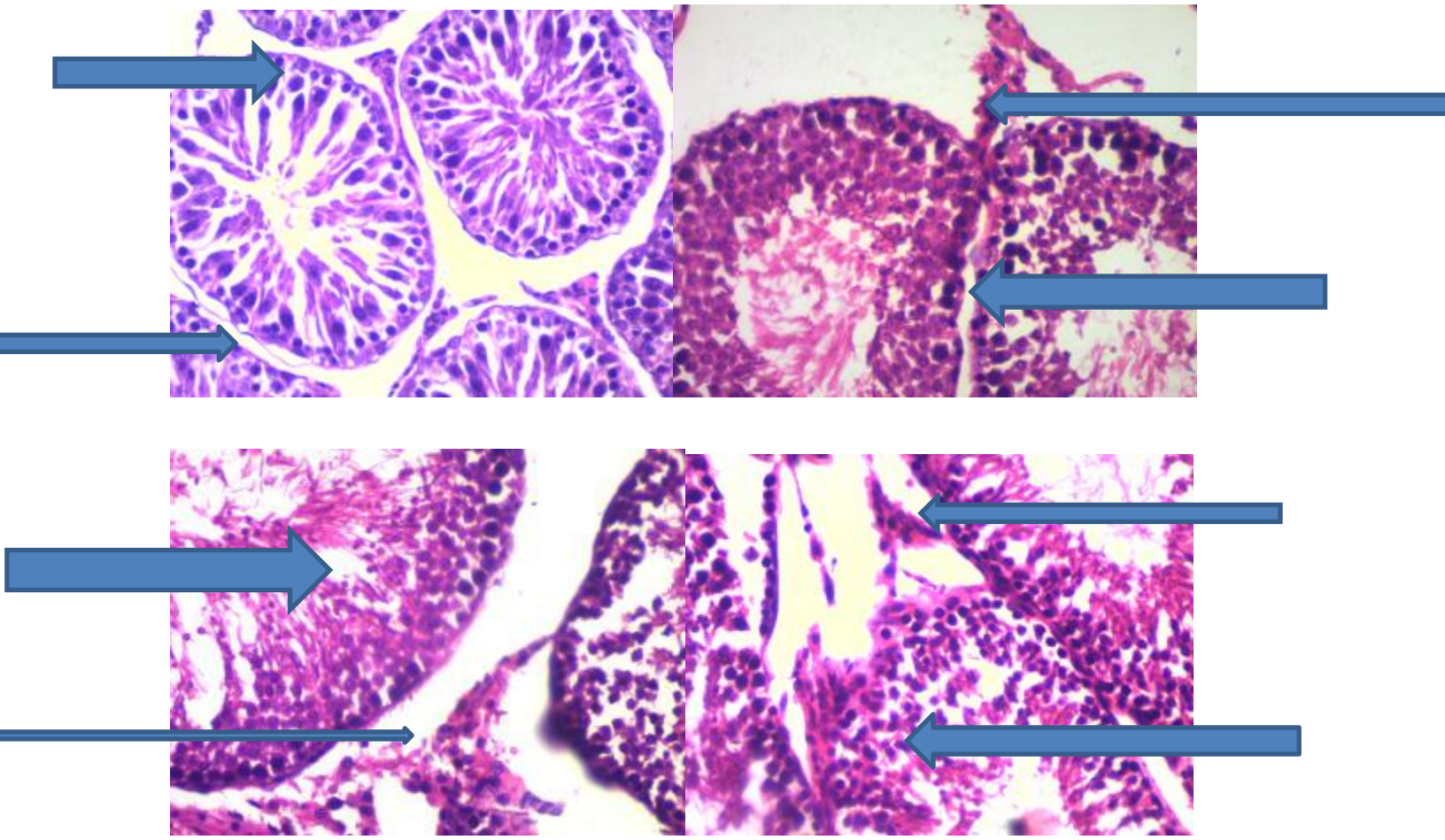

Plate 4.17: Section of testes of male rat received pellet and distilled water only for 1 month

Plate 4.18: Section of testes of male rat administered 250 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.19: Section of testes of male rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.20: Section of testes of male rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

GROUP A1 F OVARY X100

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**

GROUP B2 F OVARY X100

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**

GROUP C2 F OVARY X100

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**

GROUP D2 F OVARY X100

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**

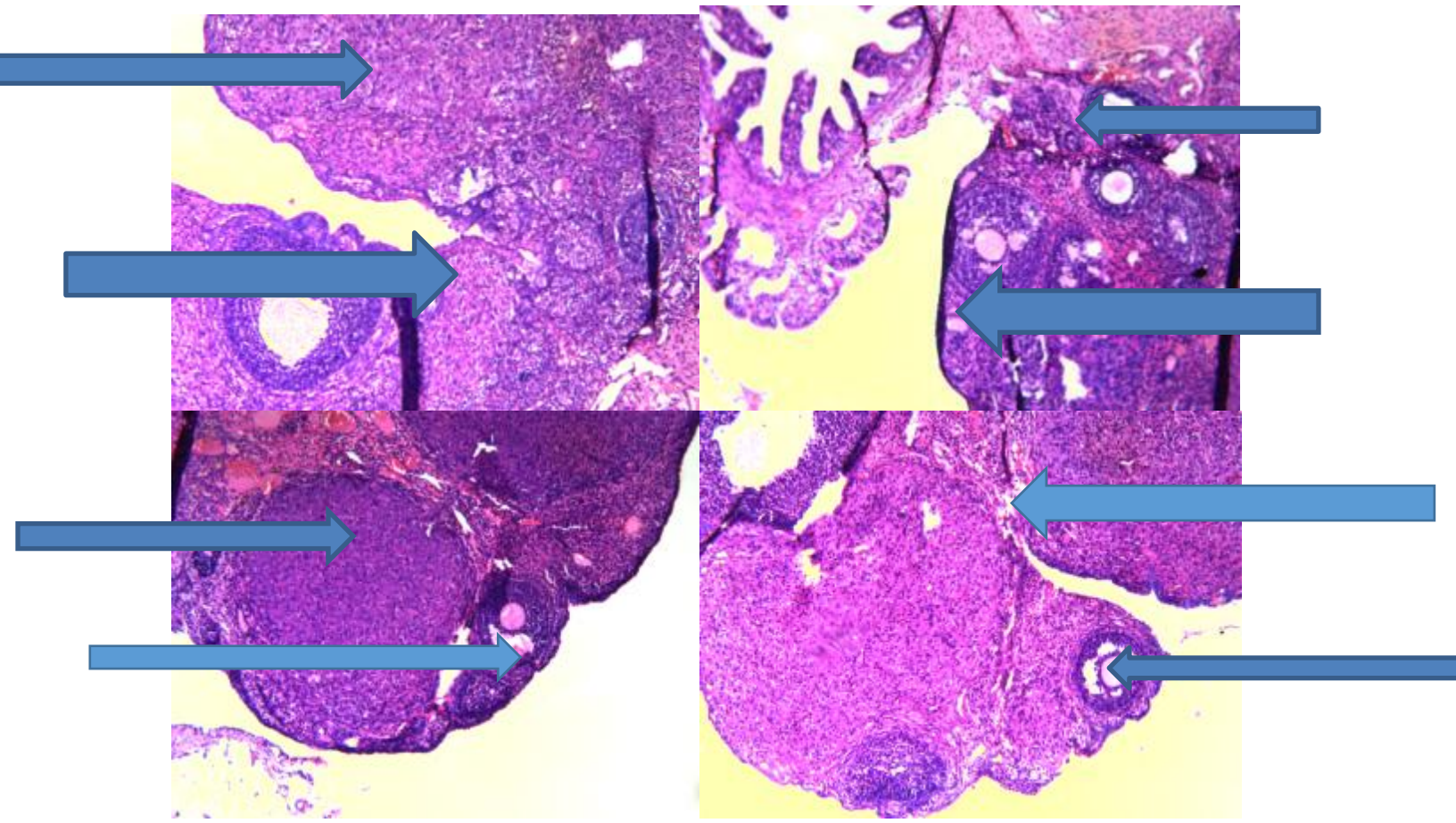

Plate 4.21: Section of ovary of female rat received pellet and distilled water only for 1 month

Plate 4.22: Section of ovary of female rat administered 250 mg/kg body weight of *Annona muricata* for 1 mont

Plate 4.23: Section of liver of female rat administered 500 mg/kg body weight of *Annona muricata* for 1 month

Plate 4.24: Section of liver of female rat administered 1000 mg/kg body weight of *Annona muricata* for 1 month

# CHAPTER FIVE
# 5.0 DISCUSSION AND CONCLUSION

## 5.1 Discussion

The present study investigated the histopathological effects of *Annona muricata* leaf extract on selected organs of albino rats, specifically examining the liver, kidneys, testes, and ovaries across different dosage regimens (250mg/kg, 500mg/kg, and 1000mg/kg) over a one-month period. The findings provide valuable insights into the potential therapeutic applications and safety profile of soursop leaf extract, while revealing both beneficial and concerning effects that warrant careful consideration.

The study demonstrated that *Annona muricata* leaf extract had no significant effect on full blood count (FBC) analytes across all treatment groups compared to controls ($p > 0.05$). These findings align with previous research by Adegboyega *et al*. (2021), who reported that ethanol extract of *Annona muricata* leaves maintained hematological stability in sodium arsenite-induced toxicity models. The preservation of white blood cell counts, lymphocyte percentages, hemoglobin levels, and platelet counts suggests that the extract does not induce significant hematotoxicity at the tested doses, supporting its potential safety for therapeutic applications.

However, these results contrast with some earlier studies that suggested potential immunomodulatory effects of soursop extracts. Gavamukulya *et al*. (2019) reported alterations in certain hematological parameters following *Annona muricata* treatment, though their study utilized different extraction methods and dosing regimens. The discrepancy may be attributed to variations in extraction protocols, phytochemical concentrations, or duration of treatment, highlighting the importance of standardized preparation methods for consistent therapeutic outcomes.

The biochemical analysis revealed no statistically significant changes in liver function tests, including AST, ALT, ALP, total bilirubin, and albumin levels across treatment groups ($p > 0.05$). This finding is particularly noteworthy given the critical role of the liver in drug metabolism and detoxification. The maintenance of normal enzyme levels suggests that *Annona muricata* extract does not induce acute hepatotoxicity at the administered doses, consistent with reports by Usunobun (2014) and Usunobun and Okolie (2016), who demonstrated hepatoprotective properties of the extract against dimethylnitrosamine-induced liver damage.

However, the histopathological examination revealed a concerning pattern of steatosis (fatty degeneration) in liver sections from treatment groups B and C (250mg/kg and 500mg/kg), characterized by hepatocytes containing microvacuoles and ballooning degeneration. Interestingly, the highest dose group (D - 1000mg/kg) and the control group maintained normal hepatocyte morphology. This paradoxical finding suggests a non-linear dose-response relationship, potentially indicating that while higher doses may activate protective mechanisms or adaptive responses, intermediate doses might induce metabolic stress leading to lipid accumulation.

The steatotic changes observed contradict previous studies by Oladele *et al*. (2020) and Fakunle *et al*. (2024), who reported hepatoprotective effects of *Annona muricata* extracts. This discrepancy may be explained by differences in extraction methods, treatment duration, or the presence of specific phytochemical compounds that vary with preparation techniques. The development of steatosis without corresponding elevation in liver enzymes suggests early-stage metabolic disruption that may not yet manifest in biochemical markers, emphasizing the importance of histopathological evaluation in toxicity studies.

The study revealed significant effects on electrolyte balance, particularly sodium and chloride levels, with group D (1000mg/kg) showing significantly elevated concentrations compared to controls ($p < 0.05$). The post-hoc analysis confirmed that the highest dose resulted in mean increases of 5.75 mEq/L for sodium and 3.75 mEq/L for chloride. These changes suggest potential effects on renal electrolyte handling mechanisms, possibly involving alterations in sodium-potassium ATPase activity or changes in glomerular filtration and tubular reabsorption processes.

Despite these biochemical changes, histopathological examination of kidney sections revealed normal glomerular and tubular architecture across all treatment groups. The preservation of renal morphology while observing electrolyte disturbances indicates functional rather than structural alterations, which may represent early adaptive responses to the bioactive compounds in the extract. This finding partially supports the nephroprotective effects reported by Ehiremen *et al*. (2024), who demonstrated *Annona muricata*'s ability to mitigate cadmium-induced renal damage, though their study focused on protective rather than direct effects.

The maintenance of normal creatinine and urea levels, despite electrolyte changes, suggests preserved overall renal function. However, the electrolyte imbalances warrant careful monitoring in clinical applications, particularly in populations with compromised renal function or those taking medications affecting electrolyte balance.

One of the most significant findings of this study was the absence of adverse effects on reproductive organs and hormones. Both testes and ovaries maintained normal histological architecture across all treatment groups, with no evidence of structural damage, cellular degeneration, or inflammatory changes. The seminiferous tubules showed normal spermatogenesis stages, intact Sertoli cells, and well-preserved Leydig cells in the interstitium.

Similarly, ovarian sections demonstrated normal follicular development stages, healthy theca and granulosa cells, and appropriate stromal organization.

These histological findings were corroborated by stable reproductive hormone levels, with no significant changes in testosterone or progesterone concentrations across treatment groups. This preservation of reproductive function contrasts with some concerns raised about potential reproductive toxicity of certain plant extracts and aligns with traditional uses of *Annona muricata* that do not report reproductive adverse effects.

The results support findings by Ibegbulem *et al*. (2023), who reported beneficial effects of *Annona muricata* on prostate health and hormone balance. Furthermore, the study's findings are consistent with research by Ehiremen *et al*. (2024), who demonstrated protective effects of the extract on testicular histomorphology in cadmium-induced toxicity models. The preservation of reproductive function suggests that *Annona muricata* leaf extract does not interfere with normal gonadal function and may even provide protective benefits against reproductive toxicity.

The varied effects observed in this study likely reflect the complex phytochemical composition of *Annona muricata* leaves, which includes flavonoids, alkaloids, phenolic compounds, and acetogenins. The antioxidant properties attributed to flavonoids and phenolic compounds may explain the protective effects observed in reproductive organs and the maintenance of overall organ function (Aguilar-Hernández *et al*., 2019; Pieme *et al*., 2014).

The development of hepatic steatosis at intermediate doses while maintaining normal liver function at higher doses suggests complex metabolic interactions. This phenomenon may involve the biphasic effects of bioactive compounds, where moderate concentrations induce metabolic stress while higher concentrations activate adaptive protective mechanisms. The acetogenins

present in *Annona muricata* are known to affect cellular metabolism and may contribute to these dose-dependent effects (Rady *et al*., 2018).

The electrolyte changes observed at high doses may result from the extract's effects on membrane transport proteins or hormonal systems regulating fluid and electrolyte balance. The flavonoid content, particularly quercetin and kaempferol, has been associated with effects on renal tubular function and may explain the observed sodium and chloride elevation (Astuti *et al*., 2021).

The study's findings have important implications for the clinical application of *Annona muricata* leaf extracts. The preservation of reproductive function and absence of significant hematological or severe organ toxicity support the potential therapeutic use of the extract. However, the development of hepatic steatosis at intermediate doses and electrolyte disturbances at high doses indicate the need for careful dose optimization and monitoring.

The non-linear dose-response relationship observed, particularly for hepatic effects, suggests that therapeutic applications should focus on either lower doses (below 250mg/kg) or higher doses (around 1000mg/kg), avoiding the intermediate range where steatotic changes were observed. This finding emphasizes the importance of dose-finding studies in herbal medicine development and challenges the common assumption that natural products exhibit linear dose-response relationships.

The electrolyte changes at high doses suggest the need for monitoring sodium and chloride levels in clinical applications, particularly in patients with cardiovascular or renal comorbidities where electrolyte imbalances could have significant consequences.

Several limitations of this study should be acknowledged. The relatively short treatment duration (one month) may not fully capture long-term effects or allow for complete adaptation to the extract. The small sample size (n=2-4 per group) may limit the statistical power to detect subtle effects. Additionally, the study did not investigate the reversibility of observed changes or explore different extraction methods that might yield varying phytochemical profiles.

Future research should focus on longer-term safety studies, investigation of different extraction methods and standardization protocols, exploration of the molecular mechanisms underlying the observed dose-response relationships, and clinical trials to establish safe and effective dosing regimens for human applications. Additionally, studies examining the reversibility of hepatic steatosis and the long-term consequences of electrolyte changes would be valuable.

### 5.2 Conclusion

This comprehensive investigation into the histopathological effects of *Annona muricata* leaf extract on albino rat organs provides valuable insights into both the therapeutic potential and safety considerations of this widely used medicinal plant. The study demonstrates a complex dose-response relationship that challenges conventional assumptions about natural product safety and efficacy.

The preservation of reproductive organ integrity and function across all dose levels, combined with the maintenance of normal hematological parameters, supports the traditional use of *Annona muricata* as a safe herbal remedy. The absence of reproductive toxicity is particularly significant given the widespread use of soursop preparations and addresses important safety concerns for populations of reproductive age.

However, the development of hepatic steatosis at intermediate doses (250-500mg/kg) while maintaining normal liver histology at higher doses (1000mg/kg) reveals a unique dose-response pattern that requires careful consideration in therapeutic applications. This finding suggests that the therapeutic window for *Annona muricata* leaf extract may be narrower than previously assumed and emphasizes the critical importance of precise dosing in herbal medicine.

The significant elevation of sodium and chloride levels at the highest dose, despite preserved renal morphology, indicates functional rather than structural effects on kidney physiology. While these changes did not compromise overall renal function as evidenced by normal creatinine and urea levels, they suggest the need for electrolyte monitoring in clinical applications, particularly in vulnerable populations.

The study's findings contribute significantly to the growing body of evidence supporting the therapeutic potential of *Annona muricata* while highlighting important safety considerations that must be addressed in clinical applications. The research validates some traditional uses of the plant while revealing previously unreported effects that warrant further investigation.

From a broader perspective, this study underscores the complexity of natural product pharmacology and the importance of comprehensive safety evaluation including both biochemical and histopathological assessments. The non-linear dose-response relationships observed challenge the common perception that natural products are inherently safe at any dose and emphasize the need for rigorous scientific evaluation of traditional medicines.

The research provides a foundation for future clinical studies and regulatory considerations for *Annona muricata* preparations. It suggests that with appropriate dose optimization and

monitoring protocols, soursop leaf extracts could be developed into safe and effective therapeutic agents for various applications, particularly those not requiring high-dose regimens.

In conclusion, while *Annona muricata* leaf extract demonstrates significant therapeutic potential with an acceptable safety profile, the complex dose-response relationships observed necessitate careful clinical evaluation and standardized preparation protocols. The study contributes valuable data to support evidence-based use of this important medicinal plant while identifying areas requiring further research to optimize its therapeutic applications safely and effectively.

The implications extend beyond *Annona muricata* to the broader field of ethnopharmacology, reinforcing the importance of scientific validation of traditional medicines and the need for comprehensive safety evaluation that goes beyond simple toxicity testing to include detailed histopathological and physiological assessments. This approach ensures that the rich heritage of traditional medicine can be safely integrated into modern healthcare systems while maintaining the highest standards of patient safety and therapeutic efficacy.

### 5.3 Recommendation

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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.

## APPENDIX IV

### Study Area

This study was carried out at the Department of Medical Laboratory Science, (Histopathology Unit Laboratory), School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The University of Benin is primarily located within Ovia North-East Local Government Area, with its main campus situated around 6.3506°N latitude and 5.6179° E longitude. This study commenced on February 21, 2025, and concluded on August 1, 2025.

### Reagents and chemicals

Sodium hydroxide, Hydrochloric acid, Eosin dye, Haematoxylin dye, distrene plasticizer, Distilled water, Ethanol, Acetic acid, normal saline, 1% acid-alcohol, xylene and 10% neutral buffered formalin including other ingredients.

NB: All the reagents were standardized before use.

### Equipment and Apparatus

Microscope (Olympus England), Embedding Machine (Hesition- E500 Germany), digital electronic balance (Gilbertini, Italy; sensitivity =0.001g), Leuckhart molds, Water bath (Gallenkamp), 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), Digital rotary microtome (Hestion ERM 4000 Germany), Hot plate, Muslin cloth, Staining rack, Forceps and Swifts.

## APPENDIX V

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

# CERTIFICATE
## OF ETHICAL APPROVAL

*This is to certify that*

**OSAUZOU EVWOMAZINO**

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

**HISTOPATHOLOGICAL EFFECTS OF ANNONA MURICATA LEAF EXTRACT ON SOME ORGANS OF ALBINO RATS.**

In accordance with the Animal Disease Control Act, 2022.

**Dr L.I Adebudo**
**Chairman MAFSAEC**

**Approval No.**
**MAFSAEC: 025-07/28/0040**

**Date Of Approval**
**28th July, 2025**

(This Approval is only valid for this study)

## APPENDIX VI

*Prof. Akinnibosun Henry Adewale* (FLS, MRSB; London)
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*University of Benin*

**Department of Plant Biology and Biotechnology**

**Herbarium Unit**

**Faculty of Life Sciences**

**University of Benin, Benin City, Edo State**

**Plant Name:** *Annona muricata* Linn.

**Family**: Annonaceae

**Common Name:** Soursop

**Voucher Number:** UBH-A356

**Student Name:** Osauzou Evwomazino Emmanuel

**Plant Identification and Voucher Number Issued by:**

27/08/2025

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