SUB-ACUTE TOXICOLOGICAL STUDY AND PHYTOCHEMICAL EVALUATION OF THE ETHANOL EXTRACT OF *CARICA PAPAYA* LINN (CARICACEAE) IN FEMALE WISTAR RATS

BY

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CERTIFICATION

We hereby certify that this project work titled "Phytochemical analysis and toxicological study of the ethanol extracts of *Carica papaya* (Caricaceae) in female Wistar rats" was carried out by ISAAC OSEMUDIAMEN CYPRIAN from the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, done in partial fulfillment of the requirements for the award of Bachelor of Pharmacy and Doctor of Pharmacy degree of the University of Benin, Benin City.

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DEDICATION

I dedicate this work to Almighty God for His guidance and blessings, to my family for their love and support, to Mr. Patrick Okonobo for his financial assistance, to my mentor, Dr. Godday Aghedo, for his guidance, and to my unique family on campus, Scripture Union Campus Fellowship, UNIBEN/UBTH, for their spiritual encouragement throughout my academic journey.

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ABSTRACT

Carica papaya is a widely recognized medicinal plant commonly employed in traditional medicine for the management of diverse health conditions. This study evaluated the phytochemical composition and sub-acute toxicological effects of *C. papaya* leaf ethanol extract in female Wistar rats. The leaves were collected, authenticated, and subjected to qualitative phytochemical screening, which revealed the presence of alkaloids, carbohydrates, saponins, cyanogenic glycosides, and anthraquinones, while tannins, cardiac glycosides, steroids, and triterpenes were absent.

For the toxicological assessment, rats were orally administered *C. papaya* leaf extract at 100 mg/kg and 200 mg/kg daily for 28 days. Hematological analysis indicated mild modulation of immune parameters, with dose-dependent decreases in WBC and lymphocyte counts and increased neutrophils, while RBC indices remained stable. Biochemical assays revealed no significant alterations in renal (urea, creatinine) and liver (AST, ALT, ALP, bilirubin) markers, and serum protein levels were unaffected. Electrolyte profiles showed minor changes in potassium and bicarbonate levels without evidence of overt toxicity.

Histopathological evaluation of major organs, including liver, kidney, spleen, lungs, heart, and uterus, demonstrated preserved architecture, with only minor adaptive cellular changes, such as slight hepatocyte enlargement and increased lymphoid follicle size in the spleen. Overall, sub-acute administration of *C. papaya* leaf ethanol extract at the tested doses exhibited no severe toxicity, suggesting that it is generally safe and may confer protective effects on multiple organ systems. These

findings provide scientific support for the traditional use of *C. papaya* and warrant further pharmacological investigation.

CHAPTER ONE

INTRODUCTION

1.1 Background

Herbal medicine, the therapeutic use of plants or plant-derived preparations is one of the world's oldest forms of healthcare and remains widely used today. Interest has resurged as patients seek treatments perceived as natural, better tolerated, and complementary to conventional care (Salm *et al*., 2023).

As demand grows, modern challenges include inconsistent product composition, contamination risk, unclear dosing, and potential herb–drug interactions. Improving quality control, standardization, authentication is essential to ensure safety, reproducibility and wider clinical acceptance. Integrating traditional knowledge with modern scientific methods offers a pathway toward evidence-based phytotherapy and safer herbal products for public health (Wang *et al*., 2023).

Herbal medicine represents a vital component of primary healthcare for a large portion of the global population, particularly in developing countries where access to conventional medicine may be limited (Wang *et al*., 2023). The World Health Organization (WHO) estimates that up to 80% of people worldwide rely on plant-based remedies for some aspect of primary healthcare (WHO, 2022).

Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids, terpenoids, and saponins, which contribute to their therapeutic potential (Salm *et al*., 2023). These compounds have inspired the development of many modern drugs, highlighting the close relationship between traditional and conventional medicine.

However, despite their perceived safety, herbal products are not without risks. Misidentification of plant species, contamination with heavy metals or microbes, and unregulated processing can lead to adverse effects or herb–drug interactions (Wang *et al*., 2023). Therefore, scientific validation, standardization, and toxicological evaluation of herbal preparations are crucial to ensure quality, safety, and efficacy.

1.2 The need for toxicity evaluation

The effective management of the steadily growing number of chemical substances used in modern society is becoming more important than ever. Toxicity testing is central to the regulatory decisions of governments and agencies seeking to safeguard public health and the environment from the potentially harmful or adverse effects of these numerous chemicals. Therefore, there is a critical need for reliable toxicity-testing methods to identify, assess and interpret the hazardous properties of any substance (Fischer, *et al*., 2020).

Although herbal medicines are often perceived as safe due to their natural origin, several studies have reported cases of toxicity resulting from inappropriate use, contamination, or interactions with conventional drugs (Wang *et al*., 2023). The chemical composition of herbal preparations can vary depending on factors such as plant part used, harvesting season, extraction method, and storage conditions, which may alter their safety profile (Salm *et al*., 2023).

Therefore, systematic toxicity evaluation of herbal extracts is essential to determine safe dosage ranges, identify possible target organs for toxicity, and detect any delayed or cumulative effects (Oluwaseun *et al*., 2023). Such studies not only ensure consumer protection but also provide scientific evidence that supports the standardization and regulatory acceptance of herbal medicines for therapeutic use.

1.3 *Carica papaya*

Carica papaya is a fast-growing, herbaceous plant that can reach heights of 5 to 10 meters. The trunk is usually unbranched and features a soft.hollow structure. The leaves are large, palmate, and deeply lobed, typically measuring 50 to 70 cm in diameter. They are arranged spirally at the top of the stem and have a glossy green appearance (Jadhav *et al*., 2023). The plant produces both male and female flowers. Male flowers are borne in long clusters, while female flowers grow singly or in small groups. The fruit is a large berry, typically oval or pear-shaped, with a smooth, yellow or orange skin when ripe. The flesh is juicy and sweet, containing numerous small black seeds (Mishra *et al*., 2022).

Carica papaya is a plant that belongs to the family Caricaceae and is commonly referred to as papaya. It is also called papaw, paw-paw, kapaya, lapaya, tapaya, papayao, papaia, papita, lechosa, fruta bomba, mamon, mamona, mamao, and tree melon in various regions of the world. Notably, another plant, Asimina triloba, which belongs to the Annonaceae family, is likewise known as pawpaw. Papaya is native to the Caribbean Coast of Central America and is grown in tropical and subtropical parts of the world (Babalola, 2019). Among the 31 species that make up the botanical family Caricaceae and the genus Carica, the papaya species is the most economically significant and frequently grown species (Biswal *et al*., 2022).

During the early years of growth, papaya develops a single stem, which can develop into heavy lateral branches in highly fertile soil, promoting favorable growth conditions (Sagonoy, 2022). The leaves of a mature papaya plant are palmate with deep lobes and are supported by smooth, hollow petioles (Babalola, 2019). Ripe papaya fruits have a slight resemblance to melons, and they are rich in retinol and ascorbic acid. Every part of the plant has been found to be important. They have been reported to be used in the treatment of diseases just like every other plant (Adetobi *et al*., 2022, Otunba *et al*., 2022, Otunba *et al*., 2021).

1.3.1 Geographic distribution

C. papaya, is native to the tropical regions of the Americas but has been widely cultivated across the globe due to its economic significance and nutritional value. According to Kumar *et al*. (2023), *C. papaya* is originally from southern Mexico and Central America. The natural habitat of papaya includes lowland tropical forests, and its cultivation has expanded significantly outside its native range (Kumar *et al*., 2023). Research by Oluwaseun *et al*. (2024) emphasizes that *C. papaya* is now cultivated in various tropical and subtropical regions worldwide, including parts of Asia, Africa, and the Caribbean. The authors note that countries like India, Indonesia, Nigeria, and Brazil are among the leading producers of papaya (Oluwaseun *et al*., 2024).

Table 1.1: Taxonomical classification of *Carica papaya*.

Classification	Taxonomy
Domain	Flowering plant
Kingdom	Plantae
Sub kingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Dilleniidae
Superdivision	Spermatophyta
Phyllum	Steptophyta
Order	Brassicales
Family	Caricaceae
Genus	Carica
Species	*C. papaya*

Figure 1.1: Picture of *Carica papaya* tree.

Figure 1.2: Picture of *Carica papaya* leaves.

1.3.2 Leaf of *Carica papaya*

The leaves of *Carica papaya*, commonly known as papaya, are not only an essential part of the plant but also possess significant nutritional and medicinal properties.

The leaves are typically large and palmate (fan-shaped) with deep lobes, usually having 5 to 7 lobes. Each leaf can grow up to 3 feet (about 1 meter) in diameter. They are bright green, sometimes with a glossy surface, and the color can vary slightly depending on the plant's health and environmental conditions. The leaves are smooth and somewhat leathery, with a prominent network of veins that radiate from the central stem (petiole). The leaf stalk (petiole) is long and may reach up to 3 feet in length. It is sturdy and holds the large leaf blade upright. The veins are prominent and create a striking pattern, contributing to the leaf's overall aesthetic appeal.

1.4 Phytochemical analysis

The medicinal properties of *C. papaya* are attributed to its rich phytochemical content, which has been the focus of numerous studies. According to Kumar *et al*. (2024), *C. papaya* contains a range of bioactive compounds, including flavonoids, alkaloids, and phenolic acids, which contribute to its therapeutic effects.

Recent studies have identified various bioactive compounds in *C. papaya*. Oluwaseun *et al*. (2023) conducted a detailed phytochemical analysis revealing high concentrations of flavonoids, alkaloids, tannins, and saponins, which are known for their health benefits. The study utilized chromatographic techniques to achieve accurate identification and quantification of these compounds (Oluwaseun *et al*., 2023). The leaves of *C. papaya* are rich in vitamins, minerals, and phytonutrients. A study by Ghosh *et al*. (2022) reported that papaya leaves contain substantial amounts of vitamins A, C, and E, along with essential minerals like calcium, magnesium, and potassium (Ghosh, *et al*., 2022).

The antioxidant potential of *C. papaya* has been explored in several studies. Kumar *et al*. (2022) reported significant antioxidant activity in papaya extracts, attributing this effect to the presence of phenolic compounds. Their findings suggest that *C. papaya* could play a role in combating oxidative stress, which is linked to various chronic diseases (Kumar *et al*., 2022).

Ethnomedicinal uses of *Carica papaya*

C. papaya, holds significant cultural and medicinal value among various tribes in Nigeria. Its ethnomedicinal applications are rooted in traditional practices and beliefs. A study by Oluwaseun *et al*. (2022) highlights the diverse traditional uses of *C. papaya* across several Nigerian tribes, including the Yoruba, Igbo, and Hausa. The study documents its application in treating ailments such as malaria, gastrointestinal disorders, and skin infections, emphasizing its role as a common folk remedy (Oluwaseun *et al*., 2022). Nwodo *et al*. (2023) explored the cultural significance of *C. papaya* in the healing practices of various Nigerian ethnic groups. The study revealed that papaya is often utilized in rituals and traditional medicine, showcasing its importance in health practices and cultural identity (Nwodo *et al*., 2023). Bello *et al*. (2023) examined the health benefits associated with the consumption of *C. papaya* in Nigerian communities. The findings indicated that the fruit and leaves are commonly used to boost the immune system, improve digestive health, and manage diabetes (Bello *et al*., 2023).

Different tribes in Nigeria have unique practices regarding the use of *C. papaya* for medicinal purposes.

- Yoruba Tribe: In a study by Ibrahim *et al*. (2022), the Yoruba tribe's use of *C. papaya* for treating various ailments such as stomach ulcers and respiratory issues was highlighted. The study noted that papaya leaves are often prepared as infusions or decoctions for medicinal use (Ibrahim *et al*., 2022).

- Igbo Tribe: Research by Ajayi *et al*. (2025) focused on the Igbo tribe's use of *C. papaya* in traditional medicine, particularly for its anti-parasitic properties. The study found that the leaves and seeds are commonly used to treat infections and as a remedy for intestinal worms (Ajayi *et al*., 2025).

- Hausa Tribe: Nwodo *et al*. (2024) investigated the Hausa tribe's application of *C. papaya*, particularly in pediatric medicine. The study revealed that papaya is used to treat fevers and digestive problems in children, often in conjunction with other herbal remedies (Nwodo *et al*., 2024).

C. papaya plays a vital role in the ethnomedicine of various Nigerian cultures, with a wide array of traditional applications supported by emerging scientific evidence. Continued research and documentation are essential to preserve its cultural significance and validate its medicinal properties.

1.4 Pharmacological uses of *Carica papaya*

Beyond safety, *C. papaya* has been investigated for its therapeutic potential. Nwodo *et al*. (2025) demonstrated that *C. papaya* leaf extracts exhibit antimicrobial activity against a range of pathogens, suggesting its potential use as a natural antimicrobial agent (Nwodo *et al*., 2025). Bello *et al*. (2022) explored the anti-inflammatory effects of *C. papaya* in an experimental rat model. Their study showed that papaya extracts significantly reduced inflammatory markers, indicating its therapeutic potential in managing inflammatory conditions (Bello *et al*., 2022). In a study by Bello *et al*. (2023), the antimicrobial and antiparasitic properties of *C. papaya* were examined. The findings indicated that extracts from the leaves and seeds exhibited significant activity against various pathogens, supporting its traditional use in treating infections (Bello *et al*., 2023).

A study by Sinha *et al*. (2022) demonstrated that papaya extracts significantly reduce oxidative stress in cellular models, suggesting potential therapeutic applications in conditions associated with oxidative damage (Sinha *et al*., 2022). The anti-inflammatory properties of papaya have been extensively researched. A recent study by Kumar *et al*. (2023) reported that *C. papaya* leaf extracts inhibit the production of pro-inflammatory cytokines in vitro, indicating its potential for managing inflammatory diseases (Kumar *et al*., 2023). *C. papaya* has shown promising antimicrobial activity against various pathogens. A study by Patel *et al*. (2024) investigated the antimicrobial effects of papaya seeds and found that they exhibited significant activity against both Gram-positive and Gram-negative bacteria (Patel *et al*., 2024). The potential anticancer effects of papaya have been a focal point in recent research. A systematic review by Yadav *et al*. (2025) synthesized data from various studies and concluded that *C. papaya* extracts possess cytotoxic properties against several cancer cell lines, making it a candidate for further research in cancer therapy (Yadav *et al*., 2025). Research has also indicated that *C. papaya* may have antidiabetic properties. A clinical trial by Ahmed *et al*. (2023) reported that the consumption of papaya leaf extract led to a significant decrease in blood glucose levels in patients with type 2 diabetes (Ahmed *et al*., 2023).

1.5 Justification of study

C. papaya has gained considerable interest in herbal medicine because of its various pharmacological benefits. However, thorough toxicity assessment are essential to confirm its safety for human use. This study intends to perform phytochemical analysis and assess the safety profile of *C. papaya* using Wistar rats, offering valuable information about its potential therapeutic uses.

1.6 Aim of study

This study aims to identify the phytochemical constituents and evaluate the safety profile of *C. papaya* leaf extract using Wistar rats.

1.7 Objectives of study

The objectives of this study are as follows:

1. To identify the phytochemical constituents of *C. papaya* leaf.

2. To assess the sub-acute effects of *C. papaya* leaf in Wistar rats through the following evaluations:

- Liver function tests to determine possible hepatic effects.
- Lipid profile analysis to assess alterations in serum lipid levels.
- Kidney function tests to evaluate renal safety.
- Haematological indices to examine effects on blood parameters.

3. To assess histopathology of lung, kidney, uterus, spleen, liver and heart.

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 Equipment and apparatus

This includes: milling machine, Soxhlet apparatus, Condenser, round bottom flask, heating mantle, water-bath, microcentrifuge, semi-auto analyser/Spectrophotometer (Mindray BA-88A Reagent system), micropipettes (50µL, 100µL, 1000µL), test tubes and test tube racks, automated hematology analyzer, refrigerator, freezer, porcelain dishes, stirrer, thimble, glass jars, spatula, cages, scrapper, digital weighing balance, universal bottles, plain bottles and EDTA bottles.

2.1.1 Reagents and chemicals

This includes: absolute ethanol (99.5%), 10% neutral buffered formalin solution, distilled water, chloroform, total cholesterol kit, total protein kit, Dragendorff's reagent, Mayer's reagent, Hager's reagent, Picric acid solution, diethyl ether, ferric chloride solution, Fehling's solution A and Fehling's solution B, diethyl ether, ferric chloride solution, sodium picrate paper, sodium potassium tartrate, 33% acetic acid, glacial acetic, concentrated sulfuric acid and dilute hydrochloric acid.

All the reagents used in this study are of proven analytical quality and were sourced from reputable vendors.

2.1.2 Consumables

The materials included hand gloves, face masks, hand sanitizer, detergent, cotton wool, surgical scissors, syringes, cage scrapers, cage beddings, a cage, and commercial Chikun feed.

2.2 Methodology

2.2.1 Plant material collection and Authentication

C. papaya leaves were collected within the school premises of University of Benin, Benin City, Nigeria. It was identified by a plant taxonomist Professor Akinnibosun as *Carica papaya* Linn (Caricaceae). A

voucher specimen was deposited at the Herbarium, Department of Plant biology and biotechnology, Faculty of Life Sciences, University of Benin, verified by Prof. Akinnibosun and given a voucher number UBH-C505.

2.2.2 Preparation and extraction of plant material

The leaves of *C. papaya* plant were collected and dried at room temperature. The plant was pulverised using a milling machine. 1000g of the pulverised powder was exhaustively extracted with 99.5% absolute ethanol, using soxhlet extraction method at an operating temperature of 80°C. The extract was reduced to dryness using a water bath at 60°C. The obtained plant extract was weighed, and the final weight was recorded.

2.3 Animal study

2.3.1 Source of laboratory animals

Female Wistar rats weighing between 124g – 152g were obtained from Department of Anatomy and acclimatized in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were kept in separate plastic cages and housed at room temperature and humidity and allowed free access to dry rodent pellet feeds and water. They were placed in groups of five (5) with a wire screen top for proper ventilation and wood shavings as the bedding material to collect urine and excreta. The processes applied in animal handling were in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals.

2.3.2 Ethical clearance

Approval for the use of laboratory animals was granted by the Ethical Committee of the Faculty of Pharmacy at the University of Benin (Ethical Clearance Number: EC/FP/025/07). All experimental procedures followed the committee's guidelines and conformed to internationally recognized standards for the ethical treatment of animals in research.

2.4 Phytochemical analysis

The ethanol extract of *C. papaya* leaves was subjected to qualitative phytochemical screening to identify the various phytochemical constituents present using the following methods and were referenced from Evans 2009:

Alkaloids

- Wagner's Test: A few drops of Wagner's reagent were introduced into a portion of the extract. The appearance of a whitish precipitate signified the presence of alkaloids.
- Mayer's Test: The extract was treated with a few drops of Mayer's reagent (a solution of mercuric chloride in potassium iodide). Formation of a creamy precipitate confirmed the presence of alkaloids.
- Hager's Test: Addition of Hager's reagent to the extract produced a yellow precipitate, indicating the presence of alkaloids.
- Dragendorff's Test: A few drops of Dragendorff's reagent (bismuth nitrate in potassium iodide) were added to the extract, and the development of an orange or reddish precipitate confirmed the presence of alkaloids.

Carbohydrates

- Molisch's Test: One milliliter (1 mL) of Molisch's reagent was added to 1 mL of the extract filtrate in a test tube, followed by the careful addition of 1 mL of concentrated sulfuric acid along the side to form a distinct lower layer. The appearance of a reddish coloration at the interface of the two layers confirmed the presence of carbohydrates.
- Benedict's Test: To 1 mL of the extract filtrate, 1 mL of Benedict's reagent was added and the mixture was heated in a boiling water bath for approximately 2–5 minutes. A color change from

blue to green, yellow, or red indicated the presence of reducing sugars, with the color intensity corresponding to the concentration of the sugars.

- Fehling's Test: One milliliter (1 mL) each of Fehling's solution A (copper (II) sulfate) and Fehling's solution B (alkaline sodium tartrate) was added to 1 mL of the extract filtrate and the mixture was gently heated. Formation of a brick-red precipitate confirmed the presence of reducing sugars.

Tannins

- Ferric Chloride Test: A few drops (3–5) of ferric chloride solution were added to a portion of the extract. The formation of a greenish-black precipitate indicated the presence of condensed tannins, whereas a blue or brownish-blue coloration suggested the presence of hydrolysable.
- Iron Complex Test: Five milliliters (5 mL) of the plant extract were mixed with 5 mL of 0.5% ferric ammonium citrate solution and 0.5 g of sodium acetate in a test tube. The mixture was boiled and then allowed to cool. The development of a purple-violet or blackish bulky precipitate, insoluble in hot water or yielding a blue solution, confirmed the presence of gallic acid or pseudo-tannins.
- Modified Iron Complex Test: To 5 mL of the plant extract, one drop of 33% acetic acid and 1 g of sodium potassium tartrate were added in a test tube. The mixture was boiled, cooled, and filtered, after which 0.25% ferric ammonium citrate solution was added to the filtrate. The formation of a purple or blackish precipitate, insoluble in hot water, alcohol, or dilute ammonia, indicated the presence of pyrogallol tannins.

Steroids and triterpenes

- Liebermann–Burchard Test: E qual volumes of acetic anhydride and chloroform and the plant extract were mixed together in a test tube. Subsequently, 1 mL of concentrated sulfuric acid was carefully added along the side of the tube to form a separate lower layer. The appearance of an initial color change, followed by further color transitions, signified the presence of steroids and triterpenes. A red, pink, or purple coloration indicated triterpenes, whereas a blue or green coloration confirmed the presence of steroids.

Cardiac glycosides

- Keller–Killiani Test: A portion of the extract was dissolved in 1 mL of glacial acetic acid containing a trace amount of ferric chloride solution. The resulting mixture was transferred into a dry test tube, and 1 mL of concentrated sulfuric acid was carefully added along the side to form a distinct lower layer. The formation of a purple-brown ring at the interface indicated the presence of deoxy sugars, while the appearance of a pale green coloration in the upper acetic acid layer confirmed the presence of cardiac glycosides.
- Salkowski Test: Approximately 0.5 g of the extract was dissolved in 2 mL of chloroform, after which two drops of concentrated sulfuric acid were carefully added to form a lower layer. The appearance of a reddish-brown coloration at the interface signified the presence of a steroidal nucleus (aglycone portion of cardiac glycosides).

Cyanogenic glycosides

- Sodium Picrate Test: A small quantity of the plant extract was distributed into three labeled test tubes (A, B, and C). Water was added to the extracts in tubes A and B, after which a strip of yellow sodium picrate paper was inserted into each tube and the tubes were immediately

stoppered. Tube B was then placed in a boiling water bath for about 5 minutes, while tubes A and C were kept at room temperature. After approximately 30 minutes, the appearance of a brick-red coloration or precipitate (sodium isopurpurate) indicated the presence of cyanogenic glycosides.

Saponin

- Frothing Test: About 10 mL of distilled water was added to a portion of the extract and the mixture was vigorously shaken for approximately 30 seconds. The test tube was then allowed to stand in an upright position for 30 minutes. The formation of a stable, honeycomb-like froth that persisted for 10–15 minutes indicated the presence of saponins.

Anthraquinones

- Borntrager's Test: Five milliliters (5 mL) of chloroform were added to a portion of the extract in a dry test tube and the mixture was shaken vigorously for about 5 minutes. After filtration, 1 mL of ammonia solution was added to 5 mL of the filtrate. The development of a bright pink coloration in the upper aqueous layer indicated the presence of free anthraquinones.

2.5 Dosing of experimental animals

The doses of *C. papaya* leaf extracts used in this study were determined based on their LD50 values, which exceeded 5000 mg/kg (Timothy *et al*., 2022). Female Wistar rats received the extracts orally through gavage. The animals were administered the doses once daily, with the volume adjusted according to their weekly recorded body weight.

2.6 Sub-acute toxicity test

Wistar albino rats were divided into Three groups comprising 5 animals each. Group A received 0.5 mL distilled water (control). Groups B and C, were orally administered different doses of *Carica papaya*

extract (100 and 200 mg/kg), respectively, daily for 28 days. Body weights of the rats were taken on day 0, 7, 14, 21 and 28. Every day, the animals were closely monitored for any changes in their clinical symptoms. Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were the main areas of focus (Imade *et al*., 2024). On the last day of gavage, the rats were fasted for a period of 12hrs before being sacrificed using a chloroform chamber. The blood was drawn by cardiac puncture into two distinct kinds of bottles: Ethylenediaminetetraacetic acid (EDTA) bottles were used to collect the blood's hematological parameters, while plain bottles were used to acquire serum for the analysis of biochemical parameters. Organs for histological analysis were obtained, including the kidney, liver, heart, lungs, spleen and uterus.

2.6.1 Haematological analysis

Hematological parameters were assessed through a blood count using an automated hematology analyzer, with blood samples collected in EDTA bottles (Dymind 2000, China). The hematological parameters analyzed include white blood cell (WBC) count, red blood cell (RBC) count, red cell distribution width (RDW), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), granulocyte count (GRAN), platelet count (PCT), and hematocrit (HCT).

2.6.2 Biochemical analysis

Blood samples were collected in plain bottles and allowed to sit at room temperature for 45 minutes before being centrifuged for 10 minutes at 3400 rpm. The resulting serum, stored at −25 °C, was used to evaluate lipid levels and renal and liver function tests. These parameters were analyzed using an automated chemistry analyzer (Selectra Pro S, Germany). The electrolyte assay was performed with an ion-selective electrode (SFRI 4000, France). The parameters measured included creatinine (Cr), urea (Ur), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase

(ALP), total serum proteins (Tp), total bilirubin (Tb), triglycerides (TG), total cholesterol (T-CH), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and serum electrolytes (Na+, K+, Cl−, HCO3−).

2.6.3 Histopathology analysis of organs

For histological analysis, the liver, heart, kidneys, lungs, spleen and uterus from the sacrificed animals were preserved in a 10% neutral buffered formalin solution. These tissues were then dehydrated through a series of alcohol concentrations (70%, 90%, 96%, and 100%), cleared with xylene, and embedded in molten paraffin wax before being sliced into slides. The sections, 4–5 µm thick, were stained with hematoxylin after being dewaxed with xylene and hydrated through descending alcohol concentrations (100%, 96%, 70%) and water. Differentiation was performed using 1% acid alcohol, and the sections were counterstained with eosin. After rehydration through ascending alcohol concentrations, the sections were cleared in xylene and mounted with dibutyl phthalate polystyrene using cover slips for microscopic examination.

2.6 Statistical analysis

The results from the experiments were reported as mean ± standard error of the mean (S.E.M). Comparison between the control and treatment groups, was conducted using an ordinary one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test. Data analysis and presentation were performed using GraphPad Prism version 8.4.3, with results deemed significant at $P<0.05$.

CHAPTER THREE

RESULTS

3.1 Percentage Yield

The weight of the pulverized leaves of *Carica papaya* was 1000g. The weight of the absolute ethanol extract obtained was 55.00 g. Therefore, the percentage yield of the extract was calculated to be 5.50%.

3.2 Phytochemical analysis

Table 3.1: Qualitative screening of ethanol extract of *C. papaya*

S/N	TEST	OBSERVATION	INFERENCE
1.	ALKALOIDS		
	• Wagner's Test	Formation of a reddish-brown precipitate.	
	• Dragendorff's Test	Formation of a reddish-brown precipitate.	
			Alkaloids present
	• Hager's	Formation of a yellow precipitate.	
	• Mayer's test	Formation of an off-white precipitate.	
2.	CARBOHYDRATES		
	• Molisch's Test	Distinct reddish-violet ring at the acid-extract interface	
	• Benedict's Test	Blue-green colouration formed	Carbohydrates present
	• Fehling's tests	Reddish precipitate was formed	

3	TANNINS (PHENOLIC COMPOUNDS)		
	• Ferric chloride Test	Solution remained pale yellow (no precipitate)	Tannins absent
	• Iron complex/Modified iron complex tests	Solution appeared slightly brown with no precipitate formed.	
4	STEROIDS AND TRITERPENES • Liebermann-Burchard test	mixture stayed colourless	Steroids and triterpenes absent
5	CARDIAC GLYCOSIDES		
	• Keller-Killiani Test	No purple-brown ring at the interface; upper acetic layer remained colourless.	Cardiac glycosides absent
	• Salkowski Tests	A faint yellow interface observed	
6	CYANOGENIC GLYCOSIDES • Sodium picrate test	The yellow sodium picrate paper turns deep orange after about 30 minutes, indicating release of hydrogen cyanide gas.	Cyanogenic glycosides present
7	SAPONINS • Frothing test	Formation of a stable, persistent froth that remains for 10–15 minutes after shaking	Saponin Present
8	ANTHRAQUINONES • Borntrager's test	Development of a bright pink, coloration in the ammoniacal upper layer after shaking the filtrate with ammonia	Anthraquinones present

3.3 Sub-acute toxicity results

3.3.1 Effect of *C. papaya* leaf on hematological parameters

C. papaya leaf extract caused significant decreases in total WBC count, lymphocytes, and platelet count, alongside a significant increase in neutrophils and platelet distribution width at 100 and 200 mg/kg doses. Platelet also decreased significantly at 200 mg/kg. Other hematological parameters showed no significant changes and remained generally stable across all groups (Table3.2).

Dose (mg/kg)	0	100	200
WBC (10^3/μL)	14.64 ± 1.50	11.06 ± 1.38	10.86 ± 1.57
LYM (%)	83.00 ± 1.66	72.90 ± 4.85	68.24 ± 3.61[a]
MON (%)	2.06 ± 0.33	2.34 ± 0.59	1.26 ± 0.18
NEU (%)	10.56 ± 1.65	18.90 ± 3.57	24.58 ± 2.95[b]
EOS (%)	0.84 ± 0.37	0.64 ± 0.19	1.78 ± 0.57
BAS (%)	3.50 ± 0.42	5.22 ± 1.17	4.14 ± 0.58
RBC (10^6/μL)	6.38 ± 0.24	6.36 ± 0.12	6.61 ± 0.20
HGB (g/dL)	13.50 ± 0.51	13.60 ± 0.14	13.84 ± 0.49
HCT (%)	40.20 ± 1.56	40.00 ± 0.55	40.60 ± 1.25
MCV (μm^3)	50.28 ± 0.42	51.92 ± 0.87	50.94 ± 0.65
MCH (ρg)	21.16 ± 0.10	21.40 ± 0.36	20.92 ± 0.29
MCHC (g/dL)	42.12 ± 0.37	41.22 ± 0.37	41.04 ± 0.15
RDWC (%)	16.28 ± 0.88	15.12 ± 0.44	15.08 ± 0.30
RDWS (μm^3)	26.96 ± 1.65	25.42 ± 0.71	24.30 ± 0.43
PLT (10^3/μL)	741.20 ± 98.38	603.60 ± 61.55	618.80 ± 61.57
MPV (μm^3)	6.54 ± 0.27	7.04 ± 0.21	7.02 ± 0.14
PCT (%)	0.49 ± 0.08	0.40 ± 0.02	0.35 ± 0.03[a]
PDW (%)	24.98 ± 1.43	18.90 ± 0.22[a]	26.46 ± 1.89
PLCR (%)	5.96 ± 2.29	6.90 ± 0.35	7.50 ± 0.91

Table 3.2: Results of hematological assay after 28 days administration

The data are presented as mean ± SEM (n = 5). In the test groups, values without a letter are not significantly different from the control, based on the Turkey-Kramer multiple comparison test. Letters (a and b) indicate varying levels of significance compared to the control: $p<0.05$ (a) and $p<0.01$ (b). The parameters analyzed include white blood cells (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), and various red blood cell and platelet indices.

3.3.2 Effect of *C. papaya* leaf on serum biochemical parameters

Effect on kidney function test parameters

Potassium levels increased significantly at both 100 mg/kg (5.10 ± 0.26, $p<0.05$) and 200 mg/kg (5.14 ± 0.38, $p<0.01$) doses of *C. papaya* leaf extract. Bicarbonate levels significantly decreased at 200 mg/kg (17.4 ± 1.33, $p<0.05$). Urea, creatinine, sodium, and chloride levels showed no significant changes across all groups (Table3.3).

Table 3.3: Kidney function test parameters after 28 days of administration

		Carica papaya	
Dose (mg/kg)	0	100	200
Urea (mg/dL)	45.40 ± 2.25	42.40 ± 3.54	43.60 ± 3.25
Creatinine (mg/dL)	0.68 ± 0.06	0.84 ± 0.07	0.86 ± 0.07
Na^+ (mmol/L)	140.60 ± 1.47	138.40 ± 0.81	139.60 ± 1.29
K^+ (mmol/L)	4.12 ± 0.10	5.10 ± 0.26[a]	5.14 ± 0.38[b]

HCO_3^- (mmol/L)	21.40 ± 0.28	17.80 ± 0.92	17.4 ± 1.33[a]
Cl^- (mg/dL)	104.80 ± 0.73	104.8 ± 0.73	105.00 ± 0.84

The data are presented as mean ± SEM (n = 5). In the test groups, values without a letter are not significantly different from the control, based on the Turkey-Kramer multiple comparison test. Letters (a and b) indicate varying levels of significance compared to the control: $p<0.05$ (a) and $p<0.01$ (b).

Effect on lipid profile tests

Total cholesterol (T-CH) levels demonstrated a dose-dependent decrease. High-Density Lipoprotein (HDL - "Good Cholesterol") levels decreased significantly with increasing doses. Low-Density Lipoprotein (LDL - "Bad Cholesterol") levels showed a notable decrease with increasing doses. Triglyceride levels decreased at 100 mg/kg but returned near baseline at 200 mg/kg (Table 3.4).

Table 3.4: Lipid profile parameters after 28 days of administration

	Carica papaya		
Dose (mg/kg)	0	100	200
T-CH (mg/dL)	82.20 ± 4.03	69.80 ± 2.85	60.00 ± 1.92[a]
HDL (mg/dL)	25.40 ± 0.98	21.60 ± 0.98	18.60 ± 0.87[a]
LDL (mg/dL)	36.60 ± 2.94	31.60 ± 2.04	21.80 ± 1.16
TG (mg/dL)	100.60 ± 2.89	82.20 ± 4.69[b]	98.00 ± 1.00

The data are presented as mean ± SEM (n = 5). In the test groups, values without a letter are not significantly different from the control, based on the Turkey-Kramer multiple comparison test. Letters (a and b) indicate varying levels of significance compared to the control: $p<0.05$ (a) and $p<0.01$ (b). The parameters measured include total cholesterol (T-CH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG).

Effect on liver function test parameters

Aspartate Aminotransferase (AST) levels decreased significantly with C.papaya treatment. Alanine Aminotransferase (ALT), showed significant decreases at 100 mg/kg. Alkaline Phosphatase (ALP), remained relatively stable across different doses. Bilirubin levels, including Total Bilirubin (Tb) and Conjugated Bilirubin (Cb), remained unchanged across all doses. No significant changes were observed in Protein Levels (Total Protein, Albumin, and Globulin). There were slight variations but no significant effects in Albumin (ALB). No major changes observed in Globulin (Table 3.5).

Table 3.5: Liver function test parameters after 28 days of extract administration

	Carica papaya		
Dose (mg/kg)	0	100	200
AST (µ/L)	77.40 ± 4.27	50.00 ± 1.52[c]	57.40 ± 2.14[b]
ALT (µ/L)	45.80 ± 2.42	28.20 ± 1.46[d]	40.00 ± 3.38
ALP (µ/L)	59.60 ± 3.54	66.60 ± 2.36	58.60 ± 3.23
Tb (mg/dL)	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.02
Cb (mg/dL)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Tp (g/dL)	6.34 ± 0.12	6.68 ± 0.19	6.24 ± 0.13
ALB (g/dL)	2.82 ± 0.02	2.78 ± 0.09	2.78 ± 0.06
GLo (g/dL)	3.52 ± 0.14	3.90 ± 0.10	3.40 ± 0.11

The data are presented as mean ± SEM (n = 5). According to the Turkey-Kramer multiple comparison test, test group values without a letter are not significantly different from the control. Letters (a, b, c, d) indicate significance levels compared to the control: $p<0.05$ (a), $p<0.01$ (b), $p<0.001$ (c), and $p<0.0001$ (d). The analyzed parameters include alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (Tb), conjugated bilirubin (Cb), total protein (Tp), albumin (ALB), and globulin (GLo).

3.4 Histological evaluation

Adult female Wistar rats were treated with graded doses (100mg and 200mg/kg body weight) of *C. papaya* plant extracts for a specified period of time, after which they were sacrificed and the spleen, liver, kidneys, lungs, heart and uterus were harvested. The tissues were subsequently subjected to histological analysis for possible reactions.

Sections of the spleen taken from rats given baseline feed and water freely show normal tissue architecture, with well-defined splenic arterioles, white pulp (comprising the lymphoid follicles), which provides the local immune system of the spleen; red pulp, where the red blood cells are sequestered in the spleen (trapping and holding of both viable and non-viable erythrocytes) and the splenic sinuses, which constitute the lymphatic channels(Figure 3.1). Sections treated with graded doses of *C. papaya* showed varying degrees of activation (boosting) of the lymphoid follicles and increase in the sequestration of red blood cells in the splenic red pulp(Figure 3.2 and 3.3). Thus, *C. papaya* caused increase in two of the major activities of the spleen (activation of the immune system and trapping and storage of erythrocytes, as well as destruction and excretion of effete red blood cells), it also boosted the activity of the local immune system of the spleen.

Sections taken from the liver of adult female Wistar rats given baseline feed and water freely show normal architecture, with well-defined hepatocytes, sinusoids and portal triad (hepatic portal vein, artery and bile duct) as seen in figure 3.4. The same was also observed in sections taken from rats treated with graded doses of C. papaya (Figure 3.5 and 3.6). Thus, *C. papaya* appear to preserve the normal liver architecture and have some added beneficial effects.

Sections of the kidney taken from rats given baseline feed and water freely (Figure 3.7), as well as sections taken from rats treated with graded doses of all the plant extracts (Figure 3.8 and 3.9). show normal histological architecture, with well-defined tubules, glomeruli, interstitial space and arcuate

blood vessels. However, sections taken from rats treated with graded doses of C.papaya show added beneficial haemodynamic and vasoactive effects of increased blood circulation in the kidneys and vasodilatation.

Sections of the uterus taken from rats given baseline feed and water show normal histological architecture with well-defined uterine cavity, surrounded by the endometrial lining (membrane), and endometrium containing the glands embedded in the stroma (Figure 3.10). Sections taken from the uterus of rats treated with graded doses of *C. papaya* show normal histological architecture (Figure 3.11 and 3.12). The endometrial membrane ulceration observed in the sections was not taken to be a toxic effect since it was also observed in the control group. It was thus considered to be artefactual (error introduced during tissue processing) and should be discountenanced.

Sections of the lungs taken from rats given baseline feed and water freely show normal histological architecture, with well-defined alveolar sacs, interstitial space, bronchioles and bronchial blood vessels. In the interstitial space were also found cells of the mononuclear phagocyte system, which constitute part of the local immune system of the lungs (Figure 3.13). Sections taken from rats given graded doses of *C. papaya* show normal alveolar sacs (Figure 3.14 and 3.15). There were also some added beneficial changes of bronchiolar dilation in the rats. Moreover, the *C. papaya* plant extracts boosted the local immune system of the lungs (activation of the bronchiolo-alveolar lymphoid aggregates and mobilization of cells of the mononuclear phagocyte system).

Sections of the heart taken from rats given baseline feed and water freely (Figure 3.16), and sections taken from rats given *C. papava* plant extracts (Figure 3.17 and 3.18) show normal histological architecture, with well-defined bundles of myocardiac fibres, interstitial space, coronary arteries and cardiac veins. Sections of the heart taken from rats treated with graded doses of *C. papaya* show the

beneficial haemodynamic and vasogenic changes of increased blood circulation (active vascular congestion) and vasodilatation.

SPLEEN

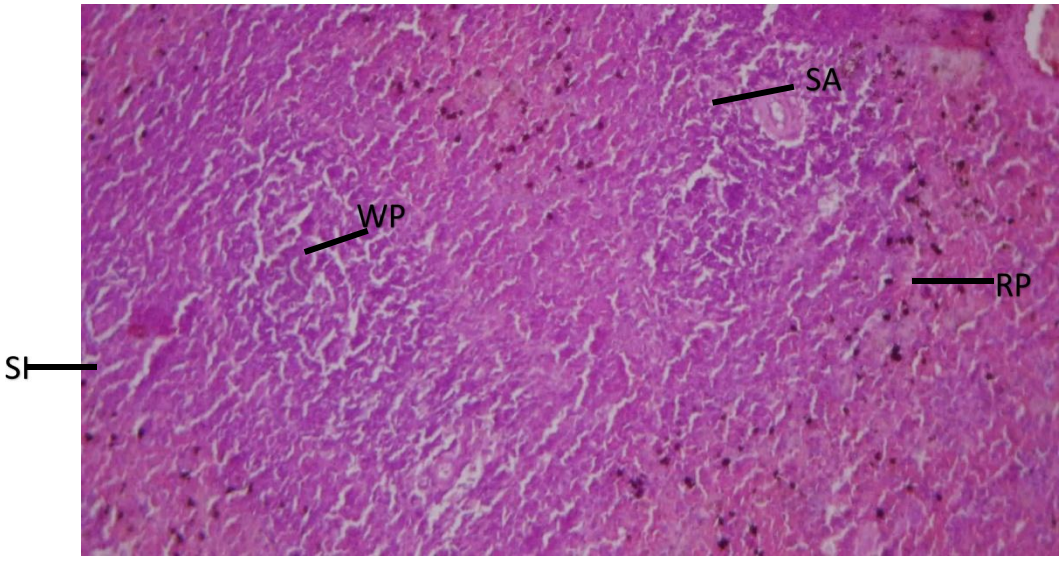

Figure 3.1: Rat spleen control, showing: normal architecture: white pulp (WP), sinuses (SI), splenic arterioles (SA) and red pulp (RP): H&E 400 X

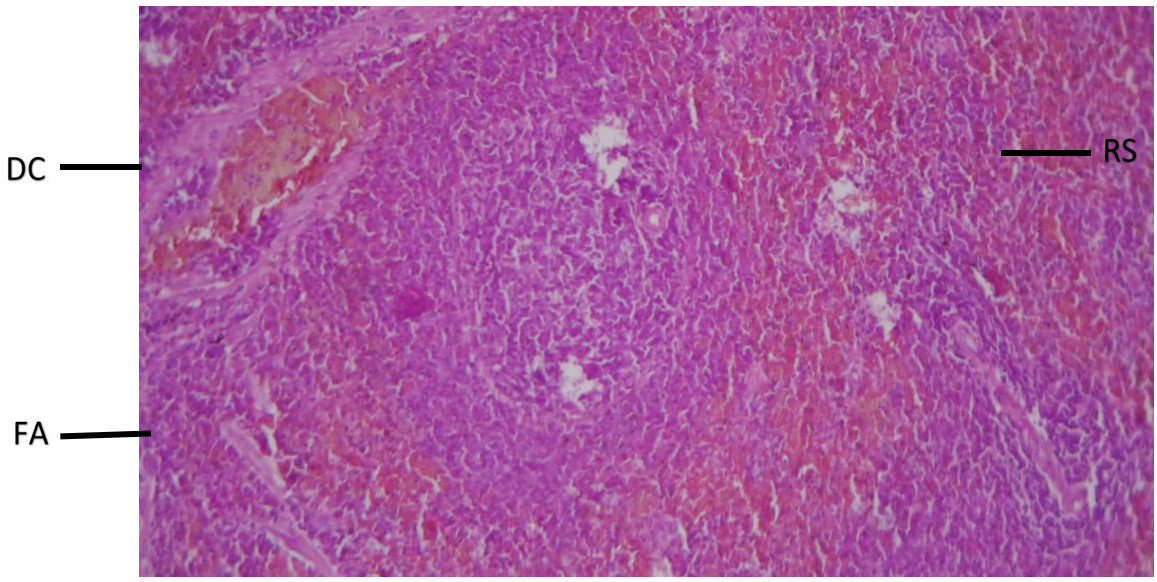

Figure 3.2: Rat spleen given 100mg/kg C. papaya showing: vasodilatation and active congestion (DC), follicular activation (FA), increased red cell sequestration (RS): H&E 400 X

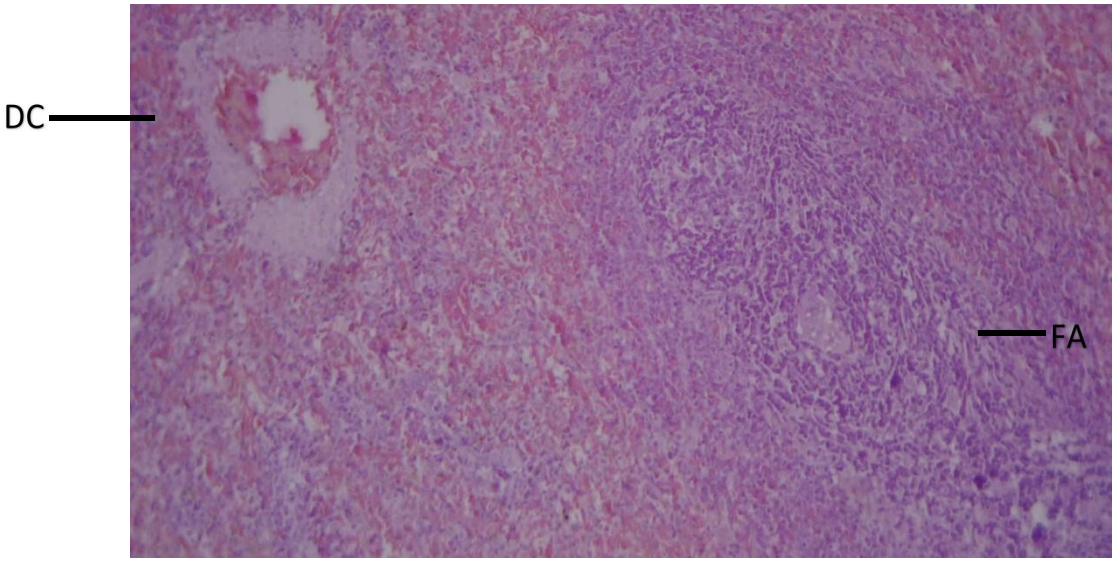

Figure 3.3: Rat spleen given 200mg/kg C. papaya showing: vasodilatation and active congestion (DC), increased red cell sequestration and follicular activation (FA): H&E 400 X

LIVER

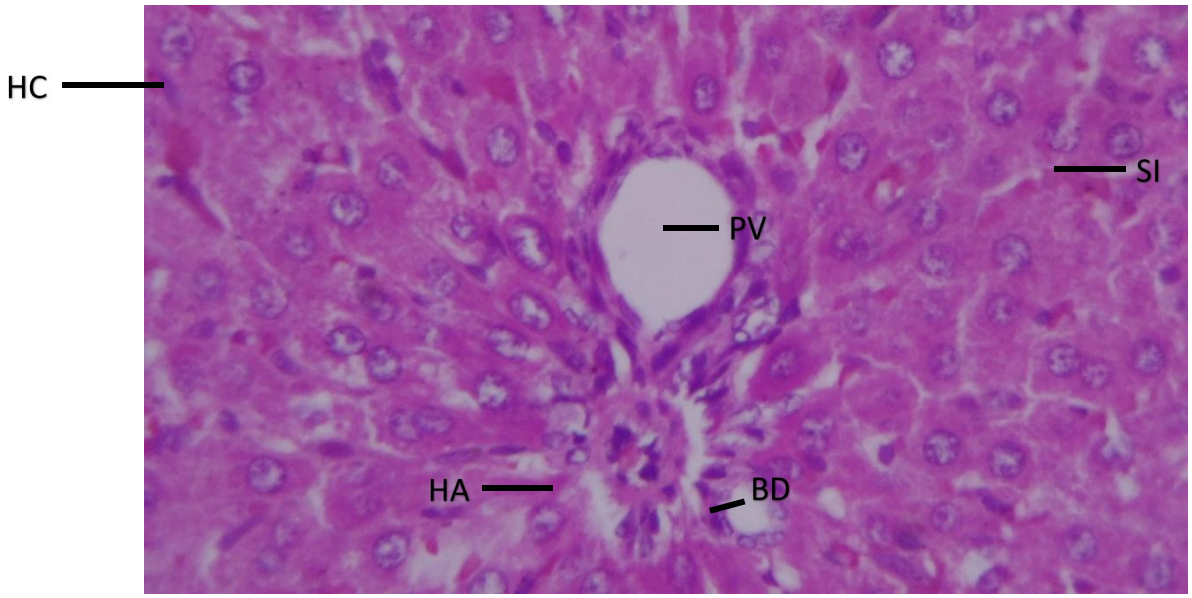

Figure 3.4: Rat liver, control, showing: normal architecture: hepatocytes (HC), sinusoids (SI), portal vein (PV), hepatic artery (HA) and bile duct (BD): H&E 400 X

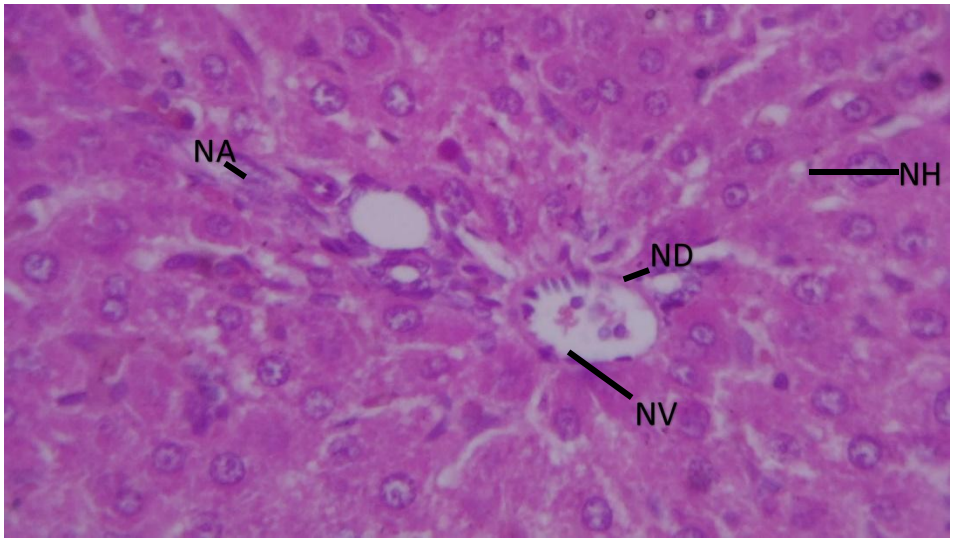

Figure 3.5: Rat liver given 100mg/kg C. papaya showing: normal hepatocytes (NH), hepatic artery (NA), bile duct (ND) and portal vein (NV): H&E 400 X

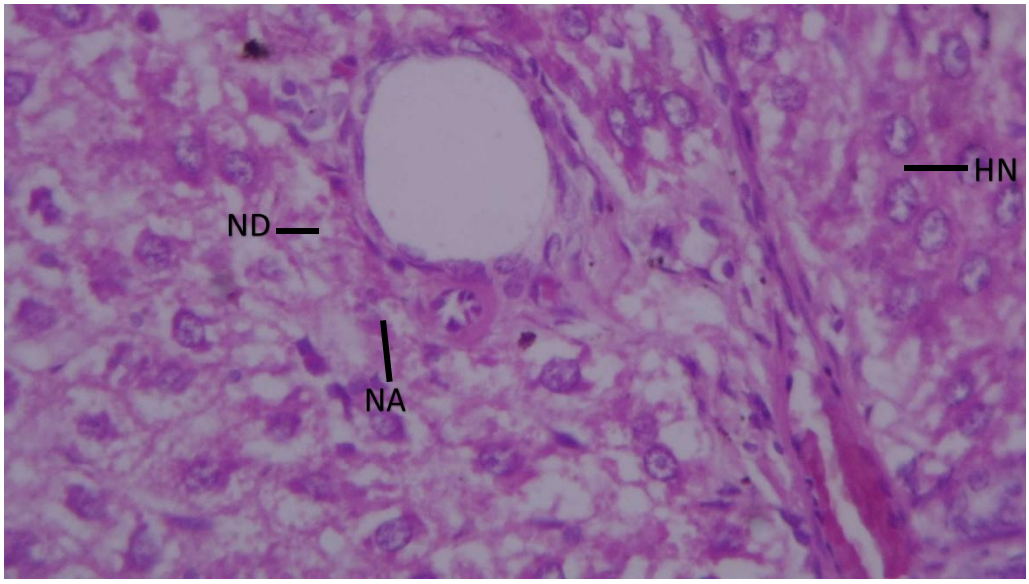

Figure 3.6: Rat liver given 200mg/kg C. papaya showing: normal hepatocytes with conspicuous nucleoli (HN), normal bile duct (ND) and hepatic artery (NA): H&E 400 X

KIDNEY

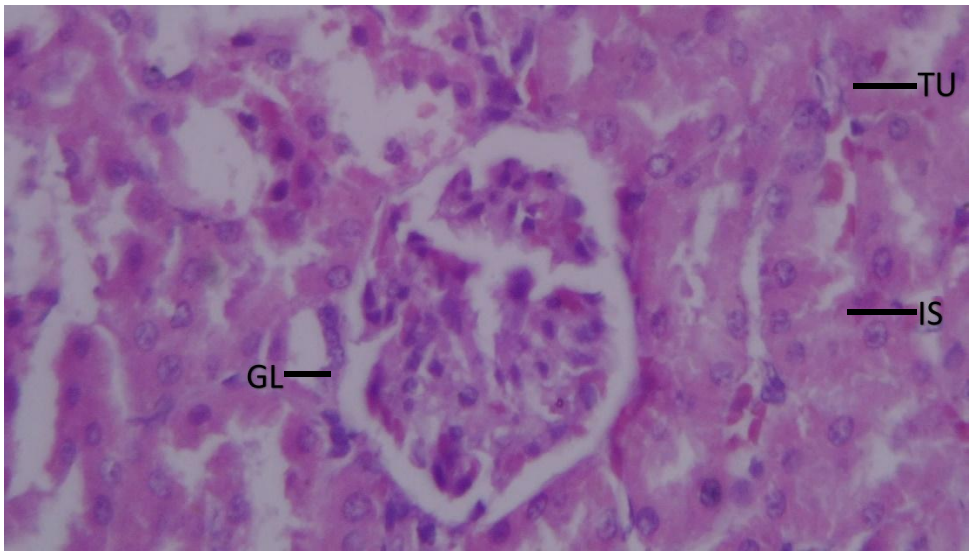

Figure 3.7: Rat kidney, control, showing normal architecture: tubules (TU), glomerulus (GL) and interstitial space (IS): H&E 400 X

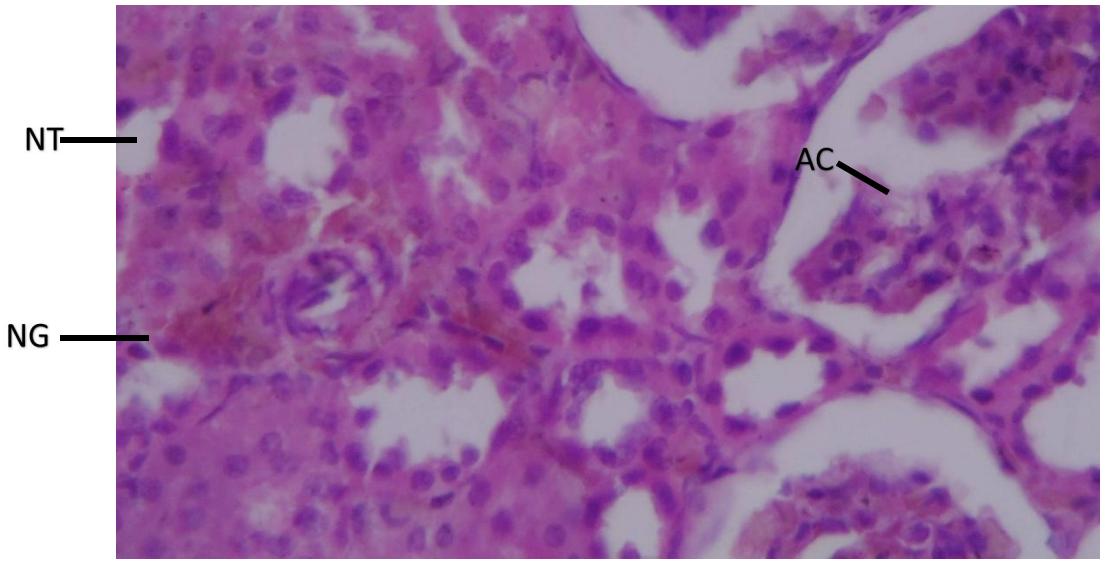

Figure 3.8: Rat kidney given 100mg/kg C. papaya showing: normal tubules (NT), glomeruli (NG) and active interstitial congestion (AC): H&E 400 X

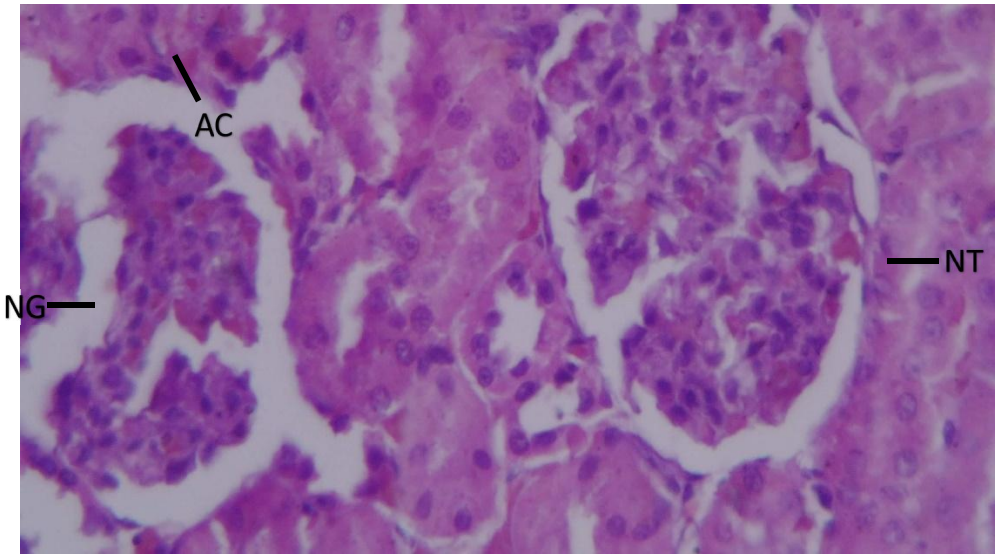

Figure 3.9: Rat kidney given 200mg/kg C. papaya showing: normal tubules (NT), glomeruli (NG) and active interstitial congestion (AC): H&E 400 X

UTERUS

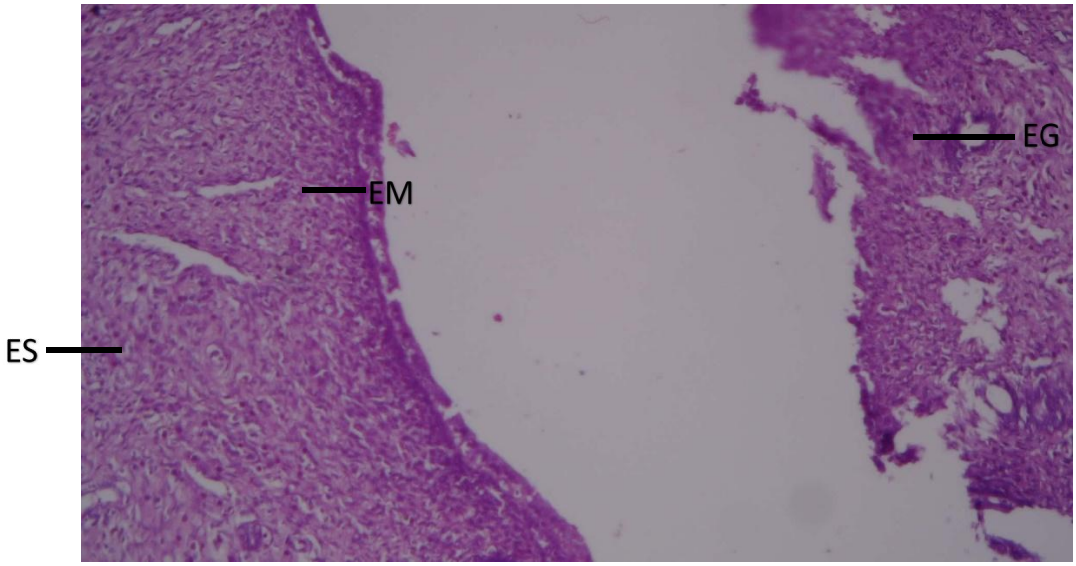

Figure 3.10: Rat uterus, control, showing: normal architecture: endometrial membrane (EM), stroma (ES) and glands (EG): H&E 400 X

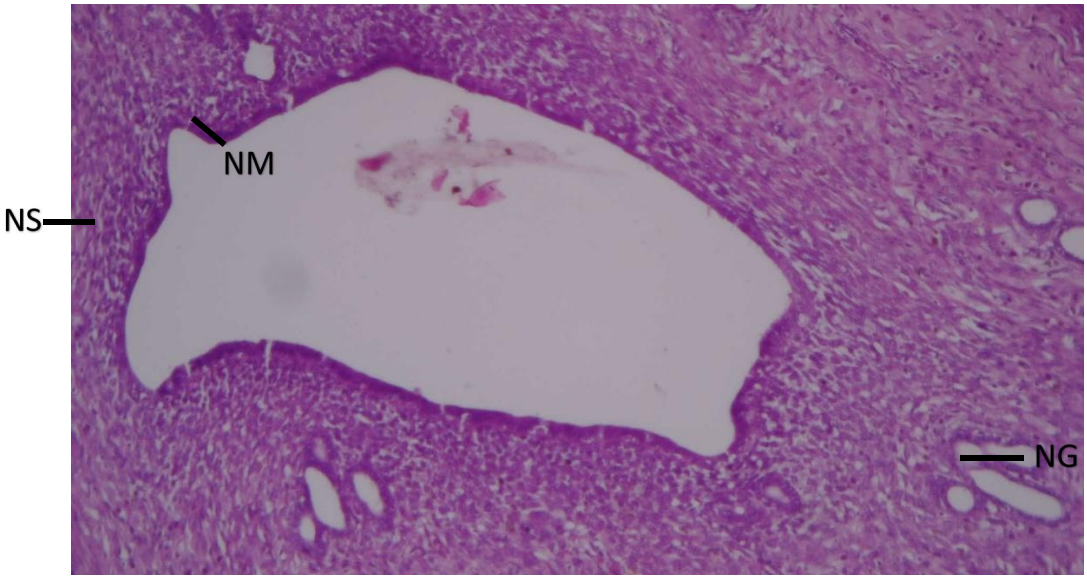

Figure 3.11: Rat uterus given 100mg/kg C. papaya showing: normal endometrial membrane (NM), stroma (NS) and glands (NG): H&E 400 X

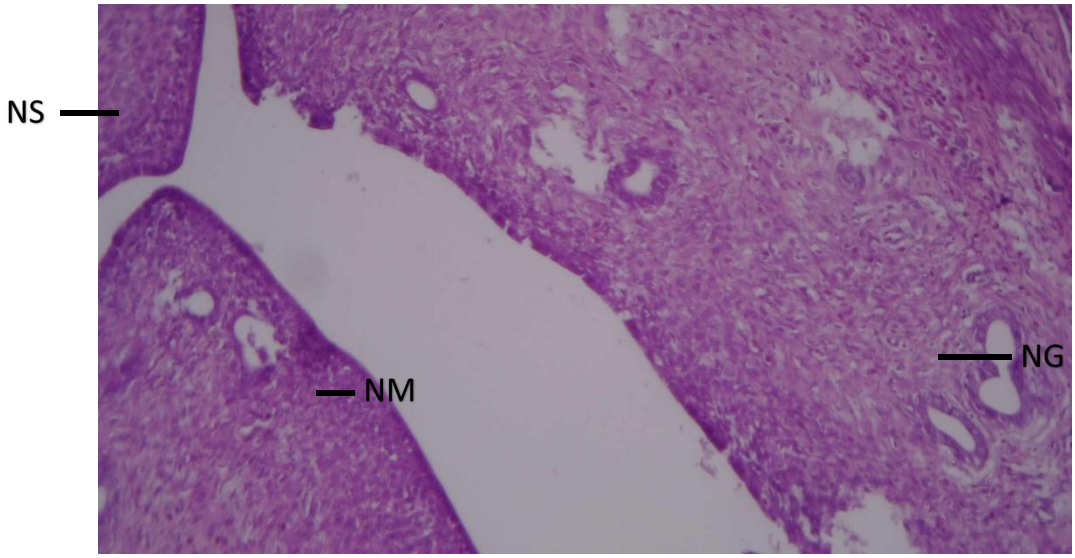

Figure 3.12: Rat uterus given 200mg/kg C. papaya showing: normal endometrial membrane (NM), stroma (NS) and glands (NG): H&E 400 X

LUNG

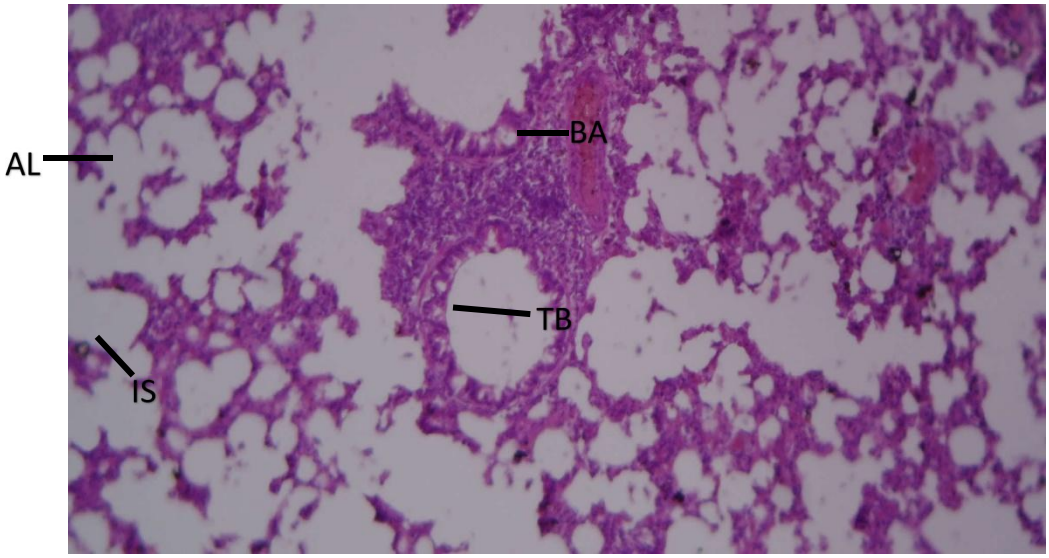

Figure 3.13: Rat lungs, control, showing normal architecture: alveoli (AL), interstitial space (IS), terminal bronchiole (TB) and bronchial artery (BA): H&E 400 X

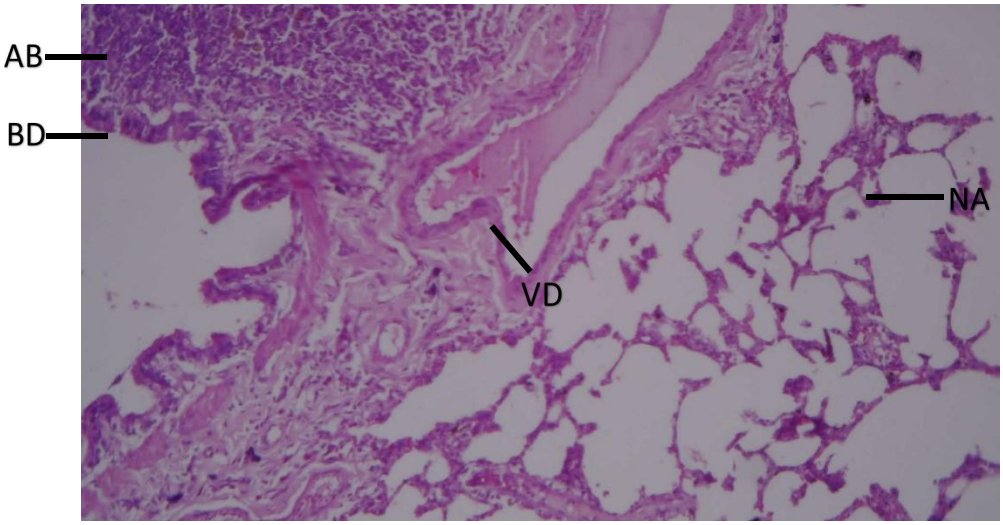

Figure 3.14: Rat lungs given 100mg/kg C. papaya showing: normal alveoli (NA), bronchiolar dilation (BD), activation of bronchiolo-alveolar lymphoid aggregates (AB) and vasodilatation (VD): H&E 400 X

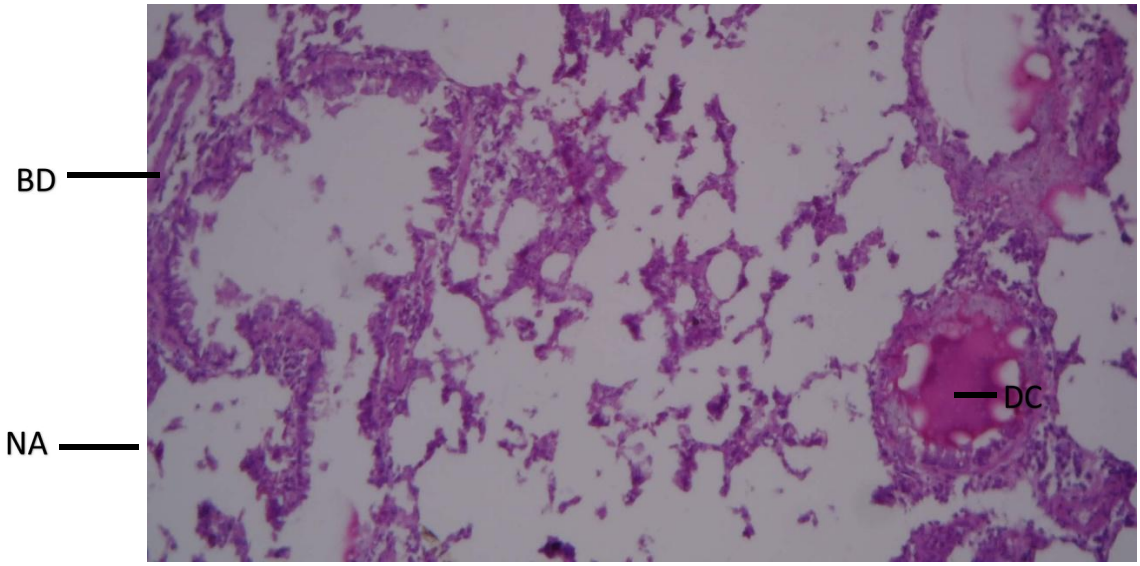

Figure 3.15: Rat lungs given 200mg/kg C. papaya showing: normal alveoli (NA), bronchiolar dilation (BD), vasodilatation and active congestion (DC): H&E 400 X

HEART

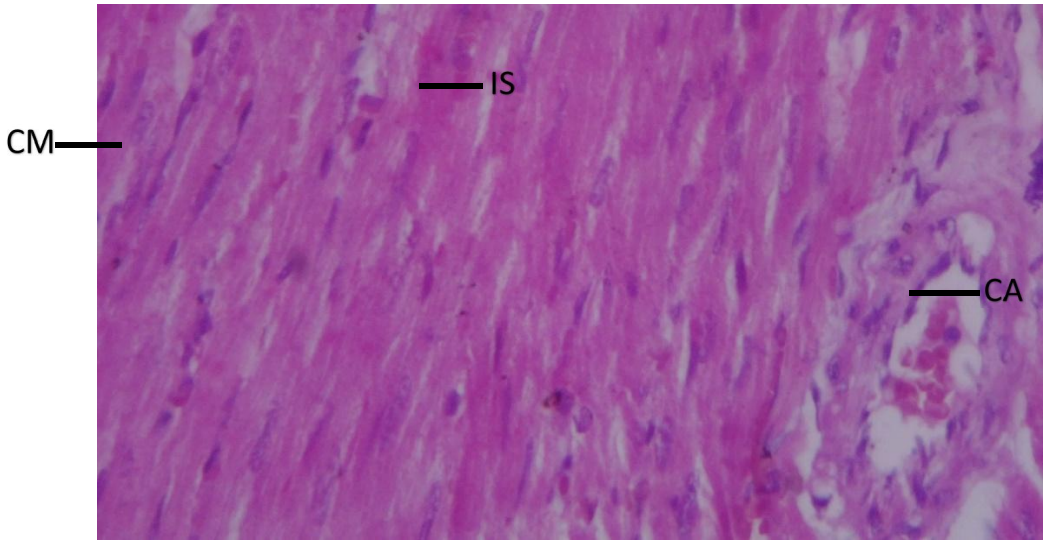

Figure 3.16: Rat heart, control, showing normal architecture: bundles of cardiomyocytes (CM), interstitial space (IS) and coronary artery (CA): H&E 400 X

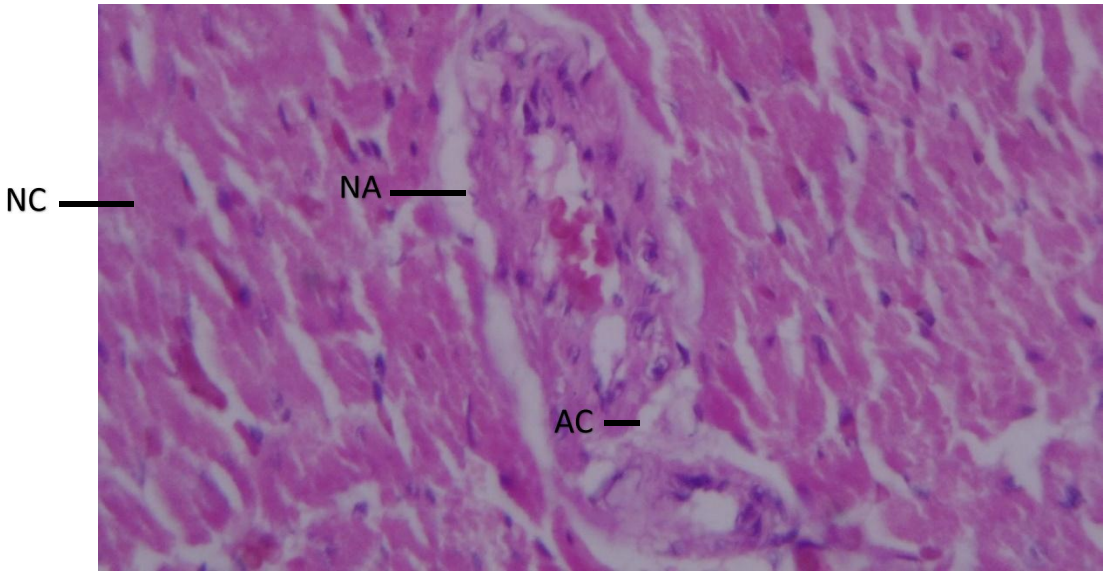

Figure 3.17: Rat heart given 100mg/kg C. papaya showing: normal bundles of cardiomyocytes (NC), coronary artery (NA) and active interstitial congestion (AC): H&E 400 X

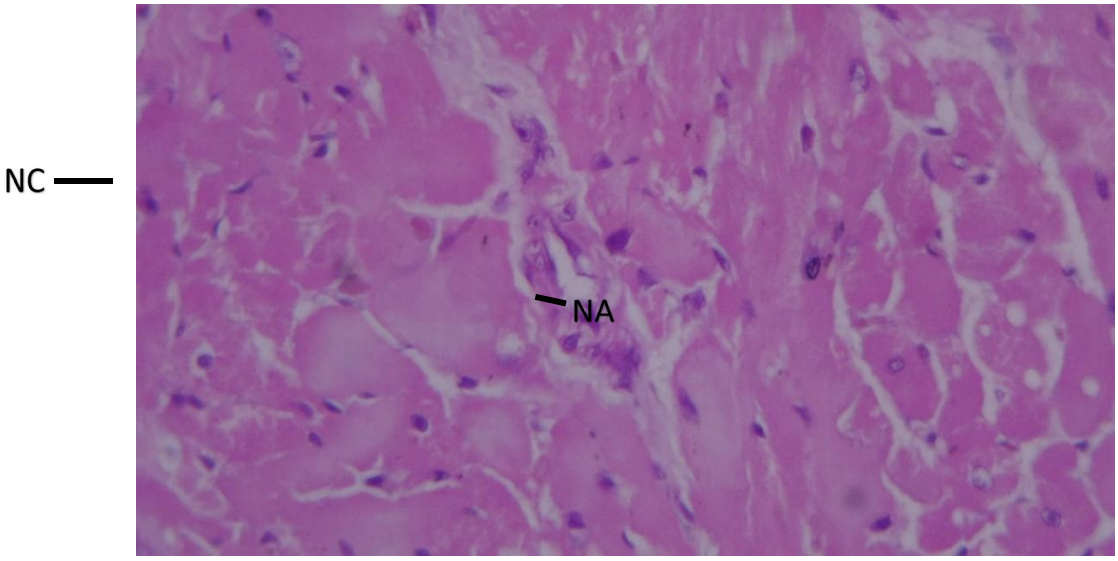

Figure 3.18: Rat heart given 200mg/kg C. papaya showing: normal bundles of cardiomyocytes (NC) and coronary artery (NA): H&E 400 X

CHAPTER FOUR

DISCUSSION

4.1 Discussion

This project investigates the sub-acute toxicity effects of graded doses (100 mg/kg and 200 mg/kg body weight) of ethanolic extract of *C. papaya* leaves administered to adult female Wistar rats. The study evaluates key hematological and biochemical parameters to assess the impact on renal, hepatic, cardiovascular, and lipid profiles over an extended exposure period. Such comprehensive assessment is critical to understanding the safety and potential toxicological risks associated with sustained use of this plant extract in traditional medicine.

The phytochemical analysis of the ethanol extract of *C. papaya* leaves revealed the presence of alkaloids, saponins, carbohydrates, cyanogenic glycosides, and anthraquinones, while tannins, cardiac glycosides, steroids, and triterpenes were absent. The detection of alkaloids supports earlier findings that link *C. papaya* leaves with antimicrobial and anti-inflammatory activities (Susilawati *et al*., 2021). Alkaloids such as carpaine are bioactive compounds known for their therapeutic potential in disease management (Okereke *et al*., 2023).

The presence of saponins aligns with studies suggesting their role in reducing serum cholesterol and enhancing immune responses (Adebayo *et al*., 2024). Saponins are also known for their surface-active properties that contribute to cellular protection.

The positive result for carbohydrates indicates that *C. papaya* leaves contain essential primary metabolites which may serve as an energy source or structural component (Nwankwo *et al*., 2022).

The identification of cyanogenic glycosides may point to the plant's potential defense mechanism, though these compounds can be toxic if not properly detoxified during preparation (Oluwole *et al*., 2023).

The detection of anthraquinones suggests possible laxative and antimicrobial properties, consistent with previous phytochemical evaluations of papaya leaves (Eze *et al*., 2024).

The absence of tannins, cardiac glycosides, steroids, and triterpenes may be attributed to the plant's chemical variation or solvent selectivity during extraction (Olagunju *et al*., 2021).

Overall, the phytochemical results reaffirm that *C. papaya* leaves are rich in bioactive compounds that contribute to their traditional medicinal uses.

Renal function markers, including creatinine and urea, alongside electrolytes and acid-base balance, were analyzed to monitor kidney health and cardiovascular implications. Liver function was assessed through protein indices—total protein, albumin, and globulin—to gauge hepatic synthetic capacity and immunological status. Lipid profile evaluation further contributed to understanding metabolic effects. Histological examination of cardiac tissue provided morphological evidence for any structural alterations induced by the extract.

Using adult female Wistar rats offers a reliable preclinical model for Sub-acute toxicity studies, allowing extrapolation of safety data relevant to female physiology. The graded dosing enabled assessment of dose-dependent effects, ensuring precise determination of a safe therapeutic margin. The findings aim to support evidence-based recommendations for the safe use of *C. papaya* leaf extract in complementary and alternative medicine.

White blood cells (WBCs) and lymphocytes play critical roles in immune defense mechanisms. A reduction in lymphocyte count may signal immune suppression, whereas an elevated neutrophil count often indicates inflammation or physiological stress. In cardiovascular disease, persistent inflammation—manifested by increased neutrophils or decreased lymphocytes—is associated with the progression of atherosclerosis and adverse cardiovascular outcomes (Wang *et al*., 2025; Molla *et al*., 2023). Red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT) are indicative of the blood's oxygen transport capacity. Low levels typically suggest anemia, while elevated values may lead to increased blood viscosity, raising the risk of thrombosis or hypertension, both detrimental to cardiovascular health (Ghorbaninejad *et al*., 2024). Platelets, key contributors to hemostasis, when abnormal, can heighten the likelihood of thromboembolic events such as myocardial infarction and stroke. Mean platelet volume (MPV) and plateletcrit (PCT) have emerged as significant biomarkers of platelet activation and have been directly implicated in the pathogenesis of cardiovascular diseases (Sharma *et al*., 2023).

Administration of the ethanol extract of *C. papaya* at doses of 100 mg/kg and 200 mg/kg for 28 days resulted in dose-dependent changes in several hematological indices. Notably, the 200 mg/kg group exhibited a significant reduction in total white blood cell and lymphocyte counts, suggesting either immune suppression or a diminished inflammatory response. In contrast, neutrophil counts rose significantly with increasing dose, which may indicate a compensatory activation of innate immunity. These results differ from earlier studies showing either increased WBC and lymphocytes at lower doses of *C. papaya* extract (Nwankwo *et al*., 2022) or no significant hematological changes in sub-acute toxicity testing at much higher doses (Ajani *et al*., 2023).

RBC count, hemoglobin (HGB), and hematocrit (HCT) levels showed no significant variation, indicating that the extract did not impair erythropoiesis or red cell stability. This aligns with the findings

of Timothy *et al*. (2022), who observed no alteration in erythrocyte indices in rats treated with ethanol leaf extract of *C. papaya* at 150 mg/kg. Similarly, Taychaworaditsakul *et al*. (2024) reported that sub-chronic exposure to *C. papaya* leaf extract (100–300 mg/kg) preserved hemoglobin and hematocrit values, suggesting a non-toxic effect on red blood cell synthesis and oxygen transport.

Platelet count decreased in both treatment groups, with a marked reduction in plateletcrit (PCT) at 200 mg/kg, suggesting a possible thrombocytopenic response that may arise from mild bone marrow suppression or enhanced peripheral platelet destruction. A similar dose-dependent decrease in platelet indices following ethanol leaf extract of *C. papaya* at 150 mg/kg was reported by Timothy *et al*. (2022), indicating potential modulation of megakaryocytic activity. Interestingly, platelet distribution width (PDW) increased significantly with dose, implying heightened platelet activation or release of morphologically diverse platelets from the marrow. This finding agrees with the report of Shoyshob *et al*. (2024), who observed elevated PDW values in rats exposed to phytochemical-rich extracts, attributing it to systemic oxidative or inflammatory stress.

Creatinine and urea, as waste products filtered by the kidneys, serve as important indicators of renal function. Elevated concentrations of these markers suggest renal impairment, a well-established risk factor for cardiovascular disease. The interrelationship between kidney and heart function is exemplified by cardiorenal syndromes, highlighting the intertwined nature of these organ systems (Wang *et al*., 2025). Potassium is vital for proper cardiac muscle contraction and the maintenance of electrical stability; elevated potassium levels can precipitate arrhythmias. Sodium regulates blood pressure and volume, with excessive sodium intake contributing to hypertension, a principal cardiovascular risk factor (Molla *et al*., 2023). Bicarbonate plays a critical role in maintaining acid-base homeostasis. Chronic acidosis, characterized by reduced bicarbonate levels, is associated with adverse cardiovascular effects, including vascular calcification (Ghorbaninejad *et al*., 2024).

Urea and creatinine levels, the key indicators of renal function, remained within normal limits in both dose groups, indicating no evident nephrotoxicity from the ethanol extract of Carica papaya. Although creatinine showed a slight increase at the higher dose, the change did not reach statistical significance, implying that glomerular filtration rate (GFR) was likely maintained. These findings are consistent with those of Taychaworaditsakul *et al*. (2024), who found no significant alteration in renal biomarkers after 90-day administration of *C. papaya* leaf extract, and with Rani *et al*. (2024), who reported only marginal, non-significant creatinine elevation in rats treated with similar phytochemical extracts.

Electrolyte assessment revealed a significant dose-dependent increase in potassium and a marked decrease in bicarbonate at 200 mg/kg, pointing to potential disruption of renal potassium excretion or increased cellular leakage and a mild metabolic acidosis possibly due to impaired renal acid-base handling. Similar increases in potassium and decreases in bicarbonate were observed in rats treated with aqueous *C. papaya* extract at 500 mg/kg (Okon *et al*., 2023), and a related phytochemical-rich extract study also reported elevated potassium with decreased bicarbonate consistent with tubular dysfunction (Smith *et al*., 2024).

Elevated total cholesterol and low-density lipoprotein (LDL) levels are strongly associated with atherosclerosis, characterised by lipid deposition within arterial walls, thereby increasing the risk of coronary artery disease, stroke, and peripheral artery disease (Zhao *et al*., 2022). High-density lipoprotein (HDL), often referred to as “good cholesterol,” facilitates reverse cholesterol transport and removal of LDL from circulation, providing significant cardiovascular protection (Lee *et al*., 2023). Elevated triglycerides (TG) independently predict cardiovascular risk, particularly when accompanied by low HDL or high LDL concentrations (Martinez *et al*., 2021). Moreover, experimental and clinical evidence indicates that dose-dependent therapeutic interventions can beneficially modulate lipid metabolism, significantly reducing total cholesterol, LDL, and TG levels (Singh *et al*., 2022; Chen and

Wang, 2024). The reduction of LDL is especially advantageous since it is the principal atherogenic lipoprotein whose decrease significantly improves cardiovascular outcomes (Patel *et al*., 2023; Roberts, *et al.,* 2024; Nguyen *et al*., 2022).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are key indicators of liver function. Although these enzymes are not direct markers of cardiovascular disease, hepatic health critically influences lipid metabolism, coagulation factors, and drug metabolism, all of which are essential in cardiovascular management. Additionally, reduced serum albumin levels have been associated with poorer cardiovascular outcomes, including higher mortality rates in patients with heart failure (Singh *et al*., 2023; Martinez, et al., 2024; Zhao *et al*., 2022).

There was a significant, dose-dependent decrease in AST and ALT levels, suggesting preserved hepatocyte integrity and potential hepatoprotective activity. The stability of ALP and bilirubin levels further supports the absence of hepatic toxicity. These findings align with Ehigiamusoe *et al*. (2025), who reported reduced liver enzyme activity following sub-chronic administration of papaya leaf extract in rats. Similarly, Oparaku *et al*. (2024) observed stabilization of AST and ALT with no significant alteration in ALP or bilirubin after ethanol extract treatment, while Taychaworaditsakul *et al*. (2024) documented comparable hepatoprotective effects at 100–300 mg/kg doses.

Serum protein parameters, including total protein, albumin, and globulin, remained stable across both dose groups, indicating preserved hepatic synthetic function and balanced immunoglobulin levels. These findings agree with Taychaworaditsakul *et al*. (2024), who reported no significant alterations in protein profiles following sub-chronic administration of ethanol leaf extract of *C. papaya* in rats at 100–300 mg/kg.

Histological evaluation

Histological examination of spleen sections from rats administered 100 mg/kg and 200 mg/kg of *C. papaya* ethanol leaf extract revealed notable alterations in architecture. The significant enlargement of lymphoid follicles suggests enhanced immune activity, likely driven by the bioactive phytochemicals in C. papaya. These changes, including proliferation of immune cells, indicate a potential immunostimulatory effect, reinforcing the spleen's central role in lymphoid function. Similar immunomodulatory effects were reported by Subramanian *et al*. (2023) following ethanol leaf extract treatment, and Mukherjee *et al*. (2024) observed increased lymphoid follicle size and cellularity in rodents exposed to phytochemical-rich plant extracts.

Histological examination of liver sections revealed normal hepatic architecture, with well-defined hepatocytes and sinusoids. Notably, the slight increase in hepatocyte size may represent an adaptive response, potentially reflecting enhanced metabolic activity or hepatoprotective effects of *C. papaya* phytochemicals. Similar hepatoprotective effects, including preserved liver structure and hepatocyte integrity, have been reported in rodents administered ethanol leaf extract of *C. papaya* (Subramanian *et al*., 2023).

Histological examination of kidney sections showed normal glomeruli and renal tubules, indicating preserved renal structure and function. The presence of well-defined blood vessels may suggest improved renal perfusion, potentially supporting glomerular filtration. The absence of significant histopathological alterations implies that *C. papaya* may confer protective effects on kidney integrity, even at higher doses. Similar nephroprotective effects were reported by Setiawan *et al*. (2023) and Francis *et al*. (2023) in rodents treated with phytochemical-rich plant extracts.

Histological examination of uterine sections revealed normal architecture, with well-defined uterine epithelium and intact surrounding connective tissue. The absence of notable histopathological changes

indicates that *C. papaya* does not adversely affect reproductive tissue, supporting its safety for reproductive health. Similar findings were reported by ResearchGate *et al*. (2023) and Chaganti *et al*. (2023), who observed preserved uterine morphology in rodents treated with phytochemical-rich plant extracts.

Histological examination of lung sections revealed well-defined alveolar sacs, bronchioles, and bronchial blood vessels. Increased vascularization suggests enhanced pulmonary blood flow, which may improve oxygen exchange and overall respiratory efficiency. The presence of immune cells within the interstitial spaces indicates an activated immune response, potentially contributing to the lung's defense against pathogens. Similar pulmonary effects, including enhanced vascularization and immune cell infiltration, were observed by Setiawan *et al*. (2022) in rodents treated with phytochemical-rich herbal extracts.

Histological examination of heart sections showed well-preserved cardiac tissue with normal morphology and vascularization. The treatment appeared to enhance vascular circulation, which may support improved cardiac function. These observations suggest potential cardiovascular benefits of C. papaya, in line with previous reports showing preserved cardiac histology and enhanced vascular perfusion in rodents administered phytochemical-rich plant extracts (Ismail *et al*., 2014; ResearchGate *et al*., 2023).

4.2 Conclusion

The sub-acute administration of ethanol leaf extract of *C. papaya* at 100 mg/kg and 200 mg/kg for 28 days demonstrated a favorable safety profile in Wistar rats. Hematological assessments revealed mild modulation of immune parameters, with decreased WBC and lymphocyte counts and increased neutrophils, without compromising erythropoiesis or oxygen-carrying capacity. Minor alterations in

platelet indices were observed, suggesting potential changes in platelet activation that may warrant further investigation.

Biochemical evaluations showed that renal (urea, creatinine) and liver function markers (AST, ALT, ALP, bilirubin) remained largely within normal limits, indicating preserved organ function. Electrolyte levels were mostly stable, with slight variations in potassium and bicarbonate that did not indicate overt toxicity. Serum protein parameters, including albumin and globulin, were unaffected, reflecting maintained hepatic synthetic capacity and immunoglobulin balance.

Histopathological examinations of major organs—including liver, kidney, spleen, lungs, heart, and uterus—revealed normal architecture, with only minor adaptive cellular changes, such as slight hepatocyte enlargement and increased lymphoid follicle size in the spleen. These findings suggest that *C. papaya* may provide hepatoprotective, immunomodulatory, and cardiovascular-supportive effects, likely due to its rich phytochemical composition.

Overall, the study demonstrates that sub-acute administration of *C. papaya* leaf ethanol extract is generally safe at the tested doses, with no evidence of severe toxicity or organ damage. The extract may even confer protective and supportive effects on multiple organ systems, supporting its traditional medicinal use. These findings provide a scientific basis for further studies to explore its pharmacological potential, including long-term toxicity, reproductive safety, and mechanistic investigations of its bioactive compounds.

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