PHYTOCHEMICAL ANALYSIS AND TOXICOLOGICAL STUDY OF THE ETHANOL LEAF EXTRACTS OF *VERNONIA AMYGDALINA* DELILE (ASTERACEAE) AND *SPONDIAS MOMBIN* LINN (ANACARDIACEAE) IN FEMALE WISTAR RATS

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CERTIFICATION

We hereby certify that this project work titled "Phytochemical analysis and toxicological study of the ethanol extracts of *Vernonia amygdalina* delile (asteraceae) and *Spondias mombin* linn (anacardiaceae) in female wistar rats" was carried out by ENIAFE, AYOMIDE EMMANUEL from the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, done in partial fulfillment of the requirement 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 God Almighty, the very One who has preserved and kept me through this journey. To my family, for their love and immense support throughout the course of this program. To the Body of Christ in Pharmacy School and my lovely family, Light of Christ Community, UNIBEN.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Vernonia amygdalina Delile (bitter leaf) and *Spondias mombin* Linn (hog plum) are medicinal plants widely used in traditional medicine for treating various ailments. This study aimed to evaluate the safety profile, phytochemical constituents and toxicological effects of these extracts in Female Wistar rats. Ethanol extract of both plants were collected, authenticated and prepared.

Qualitative phytochemical screening was conducted revealing the presence of alkaloids, anthraquinones, phenolic compounds, steroids/triterpenes, saponins and cardiac glycosides, with a notable absence of cyanogenic glycosides. Sub-acute assessment on Female Wistar rats were carried out following oral gavage of the extracts at 100mg/kg and 200mg/kg doses for 28 days.

The *V. amygdalina* extract demonstrated high systemic tolerance, with all doses maintaining stable blood, liver, and kidney functions. It exhibited beneficial immunomodulatory effects, specifically activating the spleen and mobilizing lung defense mechanisms. Meanwhile, a mild liver inflammation (portal hepatitis) was observed exclusively at the 200 mg dose. Conversely, *S. mombin* extract presented a safety paradox: standard blood tests suggested it was systemically protective (reduced AST/ALT and Urea), yet histopathology study revealed toxicity. This toxicity manifested as destructive localized damage, including ulceration of the coronary arteries and bronchioles, making the extract critically unsafe for internal consumption.

This research validates *V. amygdalina* as a safe, systemic immunomodulator within the tested dose range. While *S. mombin* presented a safety paradox. The findings strongly necessitate the mandatory integration of detailed histopathology into regulatory safety screening protocols for traditional plant medicines to detect latent, life-threatening organ toxicity that standard blood tests can miss.

CHAPTER ONE

INTRODUCTION

1.1 Herbal medicine

Herbal medicine, also known as phytotherapy, is a branch of traditional medicine that uses plant-based remedies for therapeutic purposes. It is rooted in centuries-old practices and involves the use of various plant parts like roots, leaves, seeds, or flowers to treat health conditions or maintain well-being. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations (WHO, 2022).

Medicinal plants form the fulcrum of folk medicine due to their numerous economic, pharmacological, nutritional, and tremendous health benefits to humans. The utilization and relevance of medicinal plants, particularly in the drug development and food industries, cannot be overemphasized. Their therapeutic and medicinal effects are ascribed to phytoconstituents-bioactive compounds that can elicit specific physiological responses in humans. (Atolani *et al.,* 2024). Since ancient practice, medicinal plants have been adopted ethnobotanically for medicine, food, clothing, hunting, and, in some religious ceremonies, even though their primary use has been for health care. Different parts of medicinal plants have been used to cure specific ailments. In recent times, there has been a renewed and growing interest in the use of medicinal plants, especially in developing countries. This resurgence is largely due to reports suggesting that, when properly prepared, herbal medicines are safer and have fewer adverse side effects compared to conventional drugs. (Atolani *et al*., 2024).

Traditional and alternative medicine, which includes plant-based remedies and spiritual practices, has been in use for centuries, long before the advent of conventional medicine (Afolaranmi *et al*., 2022). Complementary and alternative medicine (CAM) encompasses a wide range of therapies and practices that differ significantly from conventional medical approaches, often rooted in holistic and cultural perspectives on health (Fjær *et al*., 2020). Unlike conventional medicine, which is grounded in modern scientific principles, CAM includes diverse treatments with historical origins outside mainstream practices and is frequently used alongside traditional medical methods (Fjær *et al*., 2020). In Africa, traditional medicine (TM) has been widely practiced and accepted for centuries, particularly in rural areas where it is often seen as a more affordable healthcare option. African traditional medicine (ATM) involves indigenous methods and treatments that use plant and animal-based remedies to diagnose, manage, or treat various illnesses. Herbal therapies, the most common form of TM in Africa, are reportedly used by up to 80% of the population (WHO, 2022).

According to the World Health Organization (WHO), CAM has been a vital health resource for households and communities for centuries. According to the World Health Organization (WHO), 170 countries have reported the use of traditional and complementary medicine. Acupuncture is notably prevalent, being practiced in 113 countries. Many developed nations are also recognizing and integrating traditional medicine into their healthcare systems. The WHO estimates that a significant portion of the global population relies on traditional medicine for their primary healthcare needs, especially in rural areas of Africa, Asia, and Latin America. While conventional antibiotics are available to treat various diseases, the rise in drug-resistant infections has become a global concern. This trend has prompted increased interest in exploring phytochemicals—natural

compounds found in plants—as potential sources of new pharmacological agents to combat these resistant pathogens (Aisheikh *et al*., 2020, Álvarez-Martínez *et al*., 2020).

Vernonia amygdalina (bitter leaf) and *Spondias mombin* (yellow mombin/hog plum) are plants with significant roles in traditional medicine across Africa and tropical regions. *V. amygdalina* is frequently used for its diverse pharmacological properties, including antimalarial, antidiabetic, and antimicrobial effects, which are linked to its complex phytochemical profile (Oboh *et al*., 2021; Igbinosa *et al*., 2023). *S. mombin*, on the other hand, is valued for its anti-inflammatory, antiviral, and wound-healing properties (Silva *et al*., 2022; Kuete *et al*., 2024), attributed to its bioactive compounds.

1.2 Safety profile evaluation

The safety profile of herbal medicines is crucial for their acceptance in modern therapeutics. Several studies have investigated the toxicological effects of *V. amygdalina*. For instance, a study by Akinmoladun *et al*., (2021) assessed the acute toxicity of the plant extract in Wistar rats and found no significant adverse effects at therapeutic doses. However, chronic exposure raised concerns about potential hepatotoxicity, emphasizing the need for careful dosage regulation.

In the case of *S. mombin*, research conducted by Ojo *et al.,* (2023) revealed that while the plant exhibits beneficial effects on metabolic parameters in Wistar rats, excessive consumption could lead to renal impairment. This underscores the importance of understanding the dose-response relationship and long-term safety implications when using these plants for therapeutic purposes.

1.3 Plants under investigation

1.3.1 Vernonia amygdalina

Vernonia amygdalina, a tree or shrub of the family Asteraceae and genus Vernonia, commonly known as bitter leaf, is a perennial shrub native to tropical Africa and Asia. It is characterized by its intensely bitter-tasting leaves, which have been extensively utilized in traditional medicine across the continent. The plant has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron, and vitamins A, C, and E. Scientific and pharmacological studies have revealed the antihyperglycemic action of the roots of *V. amygdalina*. Studies have indicated that *V. amygdalina* contains potent phytochemicals with significant pharmaceutical potential. The therapeutic properties of plants are largely attributed to their phytochemical constituents, which include compounds such as flavonoids, phenolic acids, and sesquiterpene lactones. These bioactive substances contribute to various health benefits, including antimicrobial, antioxidant, and anti-inflammatory effects (Atolani *et al*., 2024).

Local names:

Nigeria: Ewuro (Yoruba), Olugbu (Igbo), Shuwaka (Hausa)

Geographical distribution and taxonomy

V. amygdalina of the family Asteraceae is an African and Asia renowned shrub or tree with valuable medicinal principles. Bitter leaf is well-known in countries such as Cameroon, Nigeria, Egypt, Uganda, and Tanzania, where it is commonly found along water paths, forest zones, and home plantations. (Atolani *et al*., 2024). In Africa, the rough brown bark plant often reaches a height of 7m as the semi-oblong leaves attain an average size of 10 x 4 cm. The plant produces

small, creamy-white to off-white flowers that are sometimes slightly tinged with mauve. These thistle-like flower heads, approximately 10mm in length, are grouped in dense, flat-topped clusters about 15 cm in diameter, and are both axillary and terminal in position. Notably, the flowers emit a sweet fragrance, particularly noticeable at night. (Atolani *et al*., 2024). The taxonomic classification of *V. amygdalina*, commonly known as bitter leaf, is as follows:

Table 1.1: Taxonomical classification of *V. amygdalina*.

Classification	Taxonomy
Kingdom	Plantae
Clade	Angiospermae
Order	Asterales
Family	Asteraceae
Genus	*Vernonia*
Species	*V. amygdalina* Delile

Phytochemical constituents

V. amygdalina contains a variety of bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolic acids. Alkaloids present in the plant have been linked to various pharmacological effects, including analgesic, antimalarial and anti-inflammatory activities (Ibrahim *et al*., 2022). Terpenoids exhibit anti-inflammatory and antimicrobial properties, making them significant in the plant's therapeutic applications (Akinmoladun *et al*., 2023). Phenolic compounds contribute to the antioxidant capacity of the plant, protecting cells from damage (Musa

et al., 2024). Recent studies have highlighted the antioxidant properties of these phytochemicals, suggesting their role in mitigating oxidative stress-related diseases (Okwu *et al.*, 2021).

Ethnomedicinal uses of *Vernonia amygdalina*

In Africa, *V. amygdalina* is adopted for various ethnobotanical and medicinal purposes on;

- Inducing labour and postpartum care**:** Traditional birth attendants in Malawi have utilized bitter leaf decoctions to stimulate uterine contractions, aiding in labor induction and the expulsion of the placenta.
- Antiparasitic applications**:** The leaf extract is employed in traditional medicine to expel parasites, such as ringworms, and to manage various infections when taken orally.
- Respiratory health**:** Leaf decoctions are used to alleviate symptoms of coughs and colds.
- Lactation enhancement**:** In certain Nigerian communities, nursing mothers consume bitter leaf soup, known as "Ofe Onugbu," to promote milk production and flow.
- Culinary uses**:** Beyond its medicinal properties, bitter leaf is notable for its culinary applications. The leaves are a staple vegetable in soups and stews across various cultures in equatorial Africa. They are typically washed thoroughly to reduce their inherent bitterness before being incorporated into dishes. The soup prepared from the leaf is consumed as both food and medicine.

V. amygdalina contains both major and trace elements that are responsible for some of the observed pharmacological properties. Stem and root bark are used as chewing sticks in some parts of Africa to serve as cleansing agents and antimicrobial agents in oral applications. Anthelmintic, antimalarial, and anti-tumorigenic properties have been properly reported for extracts from this plant. Other studies have demonstrated hypoglycaemic and hypolipidaemic effects of leaf extract

in experimental animals. Throughout history, medicinal plants like *V. amygdalina* have been extensively utilized in traditional medicine to prevent and treat various diseases, owing to their effectiveness, affordability, and widespread availability. (Atolani *et al*., 2024).

Pharmacological uses of *Vernonia amygdalina*

- Antidiabetic properties

V. amygdalina has been shown to lower blood glucose levels and improve insulin sensitivity. Studies suggest that its active compounds may stimulate insulin secretion and enhance glucose uptake in peripheral tissues (Adekemi *et al*., 2023).

- Anti-inflammatory effects

The plant exhibits significant anti-inflammatory activity, which may be attributed to its ability to inhibit pro-inflammatory cytokines and enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) (Nguyen *et al.,* 2021).

- Antimicrobial activity

Extracts from *V. amygdalina* have demonstrated antimicrobial activity against various pathogens, including bacteria and fungi. This property is particularly beneficial in treating infections (Imarenezor *et al*., 2022).

- Antioxidant activity

The antioxidant properties of *V. amygdalina* are attributed to its high content of flavonoids and phenolic compounds, which help in scavenging free radicals and reducing oxidative stress (Fawwaz, 2023).

- Hepatoprotective effects

Studies indicate that *V. amygdalina* may protect the liver from damage caused by toxins and promote liver health by enhancing detoxification processes (Uchendu, 2021).

- Cardiovascular benefits

Some research suggests that the plant may have cardioprotective effects by improving lipid profiles and reducing blood pressure (Ubah *et al.,* 2024).

- Anticancer properties

Preliminary studies indicate that extracts from *V. amygdalina* may possess anticancer properties by inducing apoptosis in cancer cells and inhibiting tumor growth (Joseph *et al*., 2023).

Figure 1.1: Picture of *Vernonia amygdalina* leaf (Asteraceae) gotten from the vicinity of University of Benin.

1.3.2 Spondias mombin

Spondias mombin, commonly known as yellow mombin or hog plum, is a tropical fruit tree belonging to the Anacardiaceae family. It is native to Central and South America but is also found in parts of Africa and the Caribbean. The tree is valued for its edible fruits, which are rich in vitamins and minerals, as well as for its traditional medicinal uses.

Local names:

English name: Hog plum, Yellow mombin, Golden apple, Ashanti plum, Jamaica plum.

Nigeria: Iyeye (Yoruba), Udara (Igbo), Yayantarwa (Hausa)

Geographical distribution and Taxonomy

S. mombin is a deciduous tree typically reaching 15-25 meters. It possesses smooth, gray bark and pinnately compound leaves with 7-19 leaflets (Pinto *et al*., 2022). The tree produces small, white flowers in panicles followed by oval-shaped, yellow fruits, 2-4 cm long, with a thin, waxy skin, juicy, acidic pulp, and a fibrous stone (Akinwumi *et al*., 2022). It thrives in tropical climates with well-drained soils, often in disturbed areas and secondary forests (Da Silva *et al.,* 2023).

Table 1.2: Taxonomical classification of *S. mombin*

Classification	Taxonomy
Kingdom	Plantae
Clade	Angiospermae
Order	Sapindales
Family	Anacardiaceae
Genus	*Spondias*
Species	*S. mombin* Linn

Phytochemical constituents

S. mombin is rich in vitamins, minerals, and phytochemicals such as tannins, saponins, and flavonoids. Research has demonstrated its antimicrobial and anti-inflammatory properties, which support its use in traditional medicine for treating various ailments (Adeyemi *et al*., 2023). The synergistic effects of these compounds may contribute to the overall therapeutic efficacy of *S. mombin* (Musa *et al*., 2024).

• Flavonoids are known for their antioxidant properties, which help protect cells from oxidative stress (Olufunmilayo *et al*., 2022).

• Tannins present in various parts of the plant exhibit antimicrobial and anti-inflammatory activities (Akinmoladun *et al*., 2023).

• Phenolic compounds contribute to the antioxidant capacity of the plant, which is beneficial for health (Cavalcante *et al*., 2024).

• The fruit is rich in vitamin C and other essential nutrients that support immune function (Nwankwo *et al*., 2021).

Ethnomedicinal uses of *Spondias mombin*

S. mombin has a rich history in traditional medicine (Ogunmefun *et al*., 2024):

• Leaves: Used for fever, inflammation, diarrhea, dysentery, and wounds. (Da Silva *et al*., 2023).

• Bark: Used as an astringent, anti-inflammatory, and antipyretic (Da Silva *et al*., 2023).

• Fruits: Eaten fresh or processed into juices, jams, providing vitamins and antioxidants (Akinwumi *et al*., 2022).

Traditional healers prepare decoctions, infusions, or poultices, with specific uses and methods varying regionally (Dos Santos *et al*., 2021).

Pharmacological uses of *Spondias mombin*

- Antioxidant activity

S. mombin exhibits significant antioxidant properties due to its high content of phenolic compounds and flavonoids. These compounds help scavenge free radicals and reduce oxidative stress, which is linked to various chronic diseases (Ojo *et al*., 2022).

- Anti-inflammatory properties

Extracts from *S. mombin* have demonstrated anti-inflammatory effects in various studies. These effects may be beneficial in treating conditions characterized by inflammation, such as arthritis (Agbaje *et al*., 2022).

- Antimicrobial activity

It has shown antimicrobial activity against a range of pathogens, including bacteria and fungi. This property suggests its potential use in treating infections. (Samuggam *et al*., 2021).

- Antidiabetic effects

Some studies have indicated that extracts of *S. mombin* may help lower blood glucose levels and improve glycemic control in diabetic models, suggesting its potential as a complementary treatment for diabetes (Bello *et al.,* 2022).

- Cardiovascular benefits

The plant may offer cardiovascular benefits by improving lipid profiles and exhibiting antihypertensive effects, which could help reduce the risk of heart disease (Ojo *et al*. 2022).

- Anticancer properties

Preliminary studies indicate that *S. mombin* extracts might possess anticancer properties by inhibiting the proliferation of cancer cells and inducing apoptosis (Metibemu *et al*., 2021).

- Digestive health

Traditionally, *S. mombin* has been used to treat digestive issues such as diarrhea and dysentery due to its astringent properties (Araruna *et al.* 2021).

Figure 2: Picture of *Spondias mombin* leaf (Anacardiaceae) gotten from the vicinity of University of Benin.

1.4 Justification for study

Vernonia amygdalina and *Spondias mombin* has gained significant attention in herbal medicine due to its diverse pharmacological properties. However, the need for comprehensive toxicity evaluation remains crucial to ensuring its safety for human consumption.

1.5 Aim of the study

This study aims to conduct a phytochemical analysis and evaluate the safety profile of the ethanol leaf extracts of *V. amygdalina* and *S. mombin* using Female Wistar rats.

1.6 Objectives

- To identify the phytochemical constituents of *V. amygdalina* and *S. mombin.*
- To assess the sub-acute toxicity profile using Female Wistar rat by determining
    - Liver function
    - Lipid profile
    - Kidney function
    - Haematological indices
- To assess the structural safety of the plant extract via histopathology of vital organs (Uterus, Lung, Kidney, Spleen, Liver and Heart).

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 Equipment and apparatus

This includes: milling machine, Soxhlet apparatus, Condenser, heating mantle, water-bath, microcentrifuge, semi-auto analyser/spectrophotometer (Mindray BA-88A Reagent system), micropipettes (50µL, 100µL, 1000µL), test tubes and test tube racks, refrigerator, freezer, automated hematology analyzer, porcelain dishes, stirrer, glass jars, spatula, cages, 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, Molisch's reagent, Benedict's reagent, Fehling's solution A and Fehling's solution B, Wagner’s reagent, Mayer's reagent, Hager’s reagent, Dragendorff's reagent, diethyl ether, ferric chloride solution, Sodium picrate paper, sodium potassium tartrate, 33 % acetic acid, glacial acetic acid and conc. sulfuric acid.

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

2.1.2 Consumables

This includes: latex hand gloves, hand sanitizer, detergent, facemask, cage beddings, and commercial pelleted feeds (Chikun feeds), surgical scissors, cotton wool, cage scrapers and syringes.

2.2 Methodology

2.2.1 Plant material collection and authentication

V. amygdalina and *S. mombin* leaves were collected in the school premises of University of Benin, Benin City, Nigeria. They were identified by a plant taxonomist Professor Akinnibosun as *Vernonia amygdalina* Delile and *Spondias mombin* Linn respectively. 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 voucher numbers, UBH-V342 and UBH-S345 for *V. amygdalina* and *S. mombin* respectively.

2.2.2 Preparation and extraction of plant material

962.10g and 464.50g of the leaves of *V. amygdalina* and *S. mombin* plants respectively were collected and dried at room temperature at the veranda of Pharmacognosy laboratory. The plants were pulverised using a milling machine. The pulverised powder was exhaustively extracted with 99.5% absolute ethanol, using Soxhlet extraction method at an operating temperature of 80°C. The extracts were reduced to dryness using a water bath at 60°C. The obtained plant extracts were weighed and their final weights recorded.

2.3 Qualitative phytochemical analysis

2.3.1 Phytochemical screening of *Vernonia amygdalina* and *Spondias mombin* Leaves extract.

The ethanol extract of *V. amygdalina* and *S. mombin* leaves underwent qualitative phytochemical screening to identify its constituent phytochemicals using the following methods and were referenced from Evans 2009:

Carbohydrate

- Molisch's Test: To 1 mL of the extract filtrate, 1 mL of Molisch's reagent (10% alcoholic solution of alpha naphthol) was added in a test tube, followed by the careful addition of 1 ml of concentrated sulfuric acid at angle 45° to form a distinct lower layer. The presence of carbohydrates was indicated by a deep violet colour at the interface between the two layers.
- Benedict's Test: To 1 mL of the extract filtrate, 1 mL of Benedict's reagent was added in a test tube and heated in a boiling water bath for about 2-5 minutes. A colour change from blue to green, yellow, or red indicates the presence of reducing sugars. The intensity of the colour change correlates with the amount of reducing sugar present.
- Fehling's Test: To 1 mL of the extract filtrate, 1 mL of Fehling's solution A (copper (II) sulfate) and Fehling's solution B (alkaline sodium tartrate) was added in a test tube and heated gently. A brick-red precipitate indicates the presence of reducing sugars.

Alkaloids

- Wagner's Test: A few drops of Wagner's reagent were added to a portion of the extract, leading to the formation of a brownish precipitate, which indicated the presence of alkaloids.
- Mayer's Test: A few drops of Mayer's reagent (mercuric chloride in potassium iodide) were added to the plant extract. A creamy precipitate indicates the presence of alkaloids.
- Hager's Test: A few drops of Hager's reagent were added to the plant extract. A yellow precipitate indicates the presence of alkaloids.
- Dragendorff's Test: A few drops of Dragendorff's reagent (bismuth nitrate in potassium iodide) were added to the extract. An orange or red precipitate indicates the presence of alkaloids.

Anthraquinones

- Borntrager's Test: 5 mL of chloroform were added to a portion of the extract in a dry test tube and shaken for at least 5 minutes. After filtration, the 5 mL of the filtrate was mixed with 1 mL of ammonia. The appearance of a bright pink colour in the aqueous upper layer indicated the presence of free anthraquinones.

Tannins (Phenolic compounds)

- Ferric Chloride Test: Three to five drops of ferric chloride solution were added to a portion of the extract. A greenish-black precipitate suggested the presence of condensed tannins, while hydrolyzable tannins produced a blue or brownish-blue precipitate.
- Iron complex test: 5 mL of plant extract was added to 5 mL 0.5 % ferric ammonium citrate and 0.5g sodium acetate in a test tube. It was boiled and cooled. A purple violet or blackish

bulky precipitate, which is insoluble in hot water or blue solution indicates the presence of gallic acid, pseudo tannins.

- Modified Iron complex test: 5 mL of plant extract was added to one drop 33 % acetic acid and 1g sodium potassium tartrate in a test tube. It was boiled, cooled and filtered. 0.25 % ferric ammonium citrate solution was added to the filtrate. A purple or blackish precipitate, which is insoluble in hot water, alcohol or dilute ammonia indicates the presence of pyrogallol tannins.

Saponins

- Frothing Test: Approximately 10 mL of distilled water was added to a portion of the extract, followed by vigorous shaking for 30 seconds. The tube was then allowed to stand upright for 30 minutes. The formation of a persistent honeycomb-like froth for 10-15 minutes signified the presence of saponins.

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. This mixture was then transferred to a dry test tube, and 1 ml of concentrated sulfuric acid was added along the side of the tube to create a lower layer. The presence of deoxy sugars was indicated by a purple-brown ring at the interface, while a pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides.
- Salkowski Test: 0.5g of the extract was dissolved in 2 mL chloroform. 2 drops of concentrated sulfuric acid were carefully added to form a layer. A reddish-brown colour at the interface indicates the presence of steroidal nucleus (aglycone of cardiac glycosides).

- Liebermann-Burchard Test: Equal volumes of acetic anhydride and chloroform were mixed with a portion of the extract in ice. One milliliter of concentrated sulfuric acid was carefully added along the side of the test tube to create a lower layer. An immediate colour change, followed by further changes, indicated the presence of steroids and triterpenes. A red, pink, or purple colour suggested triterpenes, while a blue or green hue indicated steroids.

Cyanogenic glycosides

- Sodium picrate Test: Small amount of the plant extract was placed in 3 test tubes labelled A, B and C. The extract in A and B was mixed with water. Sodium picrate paper (yellow) was inserted in each 3 test tubes and stopper immediately. Tube B was placed in a boiling water bath for about 5 minutes. Tube A and C were kept at room temperature. At the end of about half an hour, the formation of a brick red precipitate (sodium isopurpurate) indicates the presence of cyanogenic glycosides.

2.4 Animal study

2.4.1 Source of laboratory animals

Female Wistar rats weighing between 120 – 159g 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 grouped into five (5) with each group (A, B, C, D and E) having five (5) rats each. A wire screen was placed on the cage 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.

2.4.2 Ethical clearance

Ethical approval for the use of laboratory animals was obtained from the Ethical committee, Faculty of Pharmacy, University of Benin (Ethical Clearance Number: EC/FP/025/08). The experimental protocols were carried out in accordance with the approval and recommendations provided by the University of Benin Ethical Committee, which followed worldwide norms on animal handling and corresponded to acceptable guidelines on the ethical use of animals in research.

2.4.3 Dosing of experimental animals

Doses of *V. amygdalina* and *S. mombin* extract used in this study as reported were selected based on the LD50 of the acute toxicity of plants leaf which is greater than 5000 mg/kg and 2000 mg/kg respectively. The obvious sign of toxicity like convulsion, bleeding, lethargy, vomiting and general distress were equally checked and none of which was recorded over the study period. (Olalekan *et al*., 2023, Yunusa *et al*., 2024). The Female Wistar rats were given doses via gavage. Throughout the experiment, animals were dosed once a day, with each dose volume determined by the animal's weekly recorded body weight. The oral route of administration was chosen because it is the commonly utilized route by humans.

2.5 Sub-acute toxicity

Female Wistar rats were divided into five groups comprising of 5 animals each. Group A received 0.5 mL distilled water (control). Groups B and C, and D and E were orally administered different

doses of *V. amygdalina* and *S. mombin* 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 12 hours 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, liver, spleen and uterus were obtained and stored in a universal bottle.

2.5.1 Biochemical analysis

Samples of blood were taken into plain bottles and left to stand at room temperature for 45 min before being centrifuged for 10 min at 3400 rpm. The collected serum stored at -25 °C was utilized to assess the lipids, renal and liver function tests. The parameters assayed included creatinine (Cr), urea (Ur), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), serum proteins (Tp), total bilirubin (Tb), triglycerides (TG), total cholesterol (T-CH), low-density lipoproteins (LDL), high-density lipoprotein (HDL) and serum electrolytes (Na+, K+, Cl−, HCO3 −). (Imade *et al*., 2024)

2.5.2 Haematological analysis

Additionally, hematological parameters were evaluated by a blood count utilizing an automated hematology analyzer on blood collected into EDTA bottles (Dymind 2000, China). The parameters

analyzed include white blood cell (WBC), red blood cells (RBC), red blood cell distribution width (CV), red blood cell distribution width (RDW), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), granulocytes (GRAN), platelets count (PCT) and hematocrit (HCT). (Imade *et al*., 2024)

2.5.3 Histological study

For histological analysis, the liver, heart, kidney, spleen, lung and uterus from the sacrificed animals were fixed in 10% neutral buffered formalin solution. These tissues were subsequently dehydrated in ascending grades of alcohol (70%, 90%, 96% and 100%), cleared in xylene, impregnated with molten paraffin wax and sectioned to slides. These sections (4–5 μm thick) were stained with hematoxylin after dewaxing with xylene and hydrating in descending grades of alcohol (100%, 96%, 70%) and water. Differentiation was done in 1% acid alcohol and the sections counter stained with eosin. Dehydration in ascending grades of alcohol was carried out again, and the sections were cleared in xylene and mounted with dibutylphthalate polystyrene using cover slips prior to microscopic examination. (Imade *et al*., 2024)

2.6 Statistical analysis

The results obtained from the various experiment above were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the extract treatment groups and control was carried out using ordinary one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison test. Analysis and data presentation were done using GraphPad Prism version 10.4.2. Results were considered significant where $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 Percentage yield

Weights of pulverized leaves *Vernonia amygdalina* and *Spondias mombin* were 962.10g and 464.50g respectively. Weights of absolute ethanol extracts of both plants were 98.06g and 71.27g respectively, therefore percentage yields of *V. amygdalina* and *S. mombin* were 10.19% and 15.34% respectively.

3.2 Phytochemical analysis

The phytochemical analysis of the ethanol extract of *V. amygdalina* and *S. mombin* is presented in Table 3.3. The presence (+) or absence (-) of key phytochemicals such as carbohydrates, reducing sugars, alkaloids, anthraquinones, phenolic compounds, steroids/triterpenes, saponins and glycosides was determined using standard qualitative tests.

Table 3.1: Phytochemical screening of ethanol extract of *V. amygdalina.*

S/N	TEST	OBSERVATION	INFERENCE
1	Carbohydrate		
	• Molisch's Test	A purple ring interface formed	Carbohydrate present
	• Benedict's Test	Blue colour change to green	Reducing sugar present
	• Fehling's Test	Brick-red ppt observed	Reducing sugar present
2	Alkaloids		
	• Wagner's Test	Brown ppt formed	Alkaloid present
	• Mayer's Test	Brown to creamy ppt observed	Alkaloid present
	• Hager's Test	Brown to yellow ppt observed	Alkaloid present
	• Dragendorff's Test	Brown to orange-red ppt formed	Alkaloid present
3	Anthraquinones		
	• Borntrager's Test	Pink colour at interface formed	Anthraquinones present
4	Phenolic compounds		
	• Ferric Chloride Test	Blackish ppt formed	Phenolic compounds present
	• Iron complex test	Purple-violet ppt observed	Pseudo tannin present
	• Modified Iron complex test	Brown to blackish ppt observed	Hydrolysable tannin present
5	Saponin		
	• Frothing Test	Frothing observed	Saponin present
6	Cardiac glycoside		
	• Keller-Killiani Test	Purple ring interface formed	Cardiac glycoside present
	• Salkowski's Test	Reddish-brown colour observed at the interface	Steroidal nucleus present
	• Liebermann-Burchard Test	Pink-red colour observed	Triterpenes present
7	Cyanogenic glycoside		
	• Sodium picrate Test	Sodium picrate paper remain yellow	Cyanogenic glycoside absent

Key: ppt = precipitate

Table 3.2: Phytochemical screening of ethanol extract of *S. mombin.*

S/N	TEST	OBSERVATION	INFERENCE
1	Carbohydrate		
	• Molisch's Test	A purple ring interface formed	Carbohydrate present
	• Benedict's Test	Blue colour change to green	Reducing sugar present
	• Fehling's Test	Brick-red ppt observed	Reducing sugar present
2	Alkaloids		
	• Wagner's Test	Brown ppt formed	Alkaloid present
	• Mayer's Test	Brown to creamy ppt observed	Alkaloid present
	• Hager's Test	Brown to yellow ppt observed	Alkaloid present
	• Dragendorff's Test	Brown to orange-red ppt formed	Alkaloid present
3	Anthraquinones		
	• Borntrager's Test	Pink colour at interface formed	Anthraquinones present
4	Phenolic compounds		
	• Ferric Chloride Test	Blackish ppt formed	Phenolic compounds present
	• Iron complex test	Purple-violet ppt observed	Pseudo tannin present
	• Modified Iron complex test	Brown to blackish ppt observed	Hydrolysable tannin present
5	Saponin		
	• Frothing Test	Frothing observed	Saponin present
6	Cardiac glycoside		
	• Keller-Killiani Test	Purple ring interface formed	Cardiac glycoside present
	• Salkowski's Test	Reddish-brown colour observed at the interface	Steroidal nucleus present
	• Liebermann-Burchard Test	Pink-red colour observed	Triterpenes present
7	Cyanogenic glycoside		
	• Sodium picrate Test	Sodium picrate paper remain yellow	Cyanogenic glycoside absent

Key: ppt = precipitate

Table 3.3: Phytochemical screening showing presence or absence of ethanol extract of *V. amygdalina* and *S. mombin*

S/N	Phytochemicals	*V. amygdalina*	*S. mombin*
1	Carbohydrate	+	+
2	Reducing sugar	+	+
3	Alkaloids	+	+
4	Anthraquinones	+	+
5	Phenolic compounds	+	+
6	Saponins	+	+
7	Steroids/triterpenes	+	+
8	Cardiac glycoside	+	+
9	Cyanogenic glycoside	-	-

3.3 Sub-acute toxicity results

3.3.1 Vernonia amygdalina

Effect of *Vernonia amygdalina* on hematological parameters

The results of the hematological parameters (Table 3.4) of the test groups at different doses were not significantly different ($p > 0.05$) when compared to the control.

Effects of *Vernonia amygdalina* on serum biochemical parameters

- **Effect on lipid profile tests**

  All the lipid profile parameters (Table 3.5) of the test groups obtained at different doses were not significantly different ($p > 0.05$) when compared to the control.

- **Effect on kidney function tests parameters**

  For the kidney function test (Table 3.6), extract administration at all doses did not significantly alter the concentration of sodium, potassium, chloride, urea and creatinine ($P > 0.05$) when compared to the control.

- **Effect on liver function tests parameters**

  The liver function test parameters (Table 3.7) of the test groups were also not significantly altered by administration of the extract ($P > 0.05$) when compared to the control.

Table 3.4: Results of hematological assay after 28 days administration of *V. amygdalina.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
WBC (10^3 /µL)	14.64 ± 1.50	12.54 ± 1.59	21.04 ± 2.31
LYM (%)	83.00 ± 1.66	23.26 ± 1.23	81.22 ± 3.03
MON (%)	2.06 ± 0.33	1.68 ± 0.29	2.66 ± 0.25
NEU (%)	10.56 ± 1.65	11.14 ± 0.73	11.76 ± 2.14
EOS (%)	0.84 ± 0.37	0.54 ± 0.17	0.56 ± 0.15
BAS (%)	3.50 ± 0.42	3.38 ± 0.73	3.80 ± 0.87
RBC (10^6 /µL)	6.38 ± 0.24	6.33 ± 0.21	6.63 ± 0.13
HGB (g/dL)	13.50 ± 0.51	13.68 ± 0.58	14.26 ± 0.27
HCT (%)	40.20 ± 1.56	40.80 ± 1.66	42.20 ± 0.86
MCV (µm^3)	50.28 ± 0.42	51.12 ± 1.05	51.44 ± 0.88
MCH (ρg)	21.16 ± 0.10	21.60 ± 0.43	21.52 ± 0.33
MCHC (g/dL)	42.12 ± 0.37	42.26 ± 0.15	41.80 ± 0.13
RDWC (%)	16.28 ± 0.88	14.88 ± 0.29	15.56 ± 0.39
RDWS (µm^3)	26.96 ± 1.65	24.78 ± 0.67	25.08 ± 0.81
PLT (10^3 /µL)	741.20 ± 98.38	707.80 ± 49.26	592.40 ± 21.90
MPV (µm^3)	6.54 ± 0.27	6.36 ± 0.09	6.36 ± 0.09
PCT (%)	0.49 ± 0.08	0.45 ± 0.03	0.45 ± 0.03
PDW (%)	24.98 ± 1.43	22.02 ±1.78	22.02 ±1.78
P-LCR (%)	5.96 ± 2.29	4.20 ± 0.45	4.20 ± 0.45

Data are expressed as mean ± SEM, n = 5. Values in the test groups were not significantly different compared to the control group according to Tukey-Kramer multiple comparison test. White blood cell (WBC), lymphocytes (LYM), monocyte (MON), neutrophil (NEU), eosinophil (EOS), basophil (BAS), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width SD (RDW-SD), red blood cell distribution width -coefficient of variation (RDW-CV), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelets count (PCT), platelet larger cell ratio (P-LCR).

Table 3.5: Lipid profile parameters after 28 days of administration of *V. amygdalina.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
T-CH (mg/dL)	82.20 ± 4.03	88.00 ± 5.54	91.60 ± 3.64
HDL (mg/dL)	25.40 ± 0.98	27.20 ± 1.28	27.60 ± 0.98
LDL (mg/dL)	36.60 ± 2.94	40.40 ± 4.29	44.60 ± 3.37
TG (mg/dL)	100.60 ± 2.89	101.4 ± 2.32	95.60 ± 2.44

Data are expressed as mean ± SEM, n=5. Values in the test groups were not significantly different compared to the control group according to Tukey-Kramer multiple comparison test. Total cholesterol (T-CH), high-density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG).

Table 3.6: Kidney function test parameters after 28 days of administration of *V. amygdalina.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
Urea (mg/dL)	45.40 ± 2.25	37.20 ± 3.31	44.80 ± 3.22
Creatinine (mg/dL)	0.68 ± 0.06	0.72 ± 0.04	0.80 ± 0.09
Na^+ (mmol/L)	140.60 ± 1.47	139.20 ± 1.32	140.40 ± 1.50
K^+ (mmol/L)	4.12 ± 0.10	4.34 ± 0.18	4.18 ± 0.14
HCO_3^- (mmol/L)	21.40 ± 0.28	21.20 ± 0.73	21.40 ± 1.08
Cl^- (mg/dL)	104.80 ± 0.73	104.8 ± 1.32	105.80 ± 0.80

Data are expressed as mean ± SEM, n=5. Values in the test groups were not significantly different compared to the control group according to Tukey-Kramer multiple comparison test.

Table 3.7: Liver function test parameters after 28 days of administration of *V. amygdalina.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
AST (µ/L)	77.40 ± 4.27	62.40 ± 4.20	71.60 ± 2.77
ALT (µ/L)	45.80 ± 2.42	37.00 ± 2.43	42.20 ± 1.66
ALP (µ/L)	59.60 ± 3.54	54.00 ± 5.41	59.80 ± 1.53
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	6.40 ± 0.25	6.50 ± 0.36
ALB (g/dL)	2.82 ± 0.02	2.98 ± 0.05	2.88 ± 0.06
GLo (g/dL)	3.52 ± 0.14	3.42 ± 0.25	3.62 ± 0.36

Data are expressed as mean ± SEM, n=5. Values in the test groups were not significantly different compared to the control group according to Tukey-Kramer multiple comparison test. Alkaline phosphatase (ALP), alanine aminotransferase (ALT) Aspartate aminotransferase (AST), total bilirubin (Tb), conjugated bilirubin (Cb), total protein (Tp), albumin (ALB), globulin (GLo).

3.3.2 Spondias mombin.

Effect of *Spondias mombin* on hematological parameters

The results of the hematological parameters (Table 3.8) of the test groups revealed a decrease ($p < 0.01$) in red blood cell distribution width-coefficient of variation (RDW-CV) level at 200 mg/kg when compared to the control. The extract administration at all other doses did not significantly alter the concentration of other parameters ($P > 0.05$).

Effects of *Spondias mombin* on serum biochemical parameters

- **Effect on lipid profile tests**

  All the lipid profile parameters (Table 3.9) of the test groups obtained at different doses were not significantly different ($p > 0.05$) when compared to the control.

- **Effect on kidney function test parameters**

  The results of the kidney function test (Table 3.10) revealed a decrease ($p < 0.001$) in urea level at 200 mg/kg. The extract administration at all other doses did not significantly alter the concentration of sodium, potassium, chloride, urea and creatinine ($P > 0.05$) when compared to the control.

- **Effect on liver function test parameters**

  The results of the liver function test (Table 3.11) revealed a decrease ($p < 0.01$) in Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) level at 100 mg/kg. The extract administration at all other doses did not significantly alter the concentration of other parameters ($P > 0.05$) when compared to the control.

Table 3.8: Results of hematological assay after 28 days administration of *S. mombin.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
WBC (10^3 /µL)	14.64 ± 1.50	15.68 ± 3.05	18.90 ± 1.14
LYM (%)	83.00 ± 1.66	80.48 ± 3.53	80.73 ± 3.53
MON (%)	2.06 ± 0.33	1.98 ± 0.30	1.80 ± 0.19
NEU (%)	10.56 ± 1.65	12.56 ± 2.31	12.15 ± 2.80
EOS (%)	0.84 ± 0.37	0.42 ± 0.12	0.92 ± 0.46
BAS (%)	3.50 ± 0.42	4.56 ± 1.29	4.20 ± 0.73
RBC (10^6 /µL)	6.38 ± 0.24	6.69 ± 0.24	6.56 ± 0.06
HGB (g/dL)	13.50 ± 0.51	14.40 ± 0.35	14.00 ± 0.35
HCT (%)	40.20 ± 1.56	42.60 ± 0.68	41.00 ± 1.05
MCV (µm^3)	50.28 ± 0.42	50.42 ± 0.45	49.90 ± 1.05
MCH (ρg)	21.16 ± 0.10	21.56 ± 0.27	21.32 ± 0.39
MCHC (g/dL)	42.12 ± 0.37	42.80 ± 0.28	42.78 ± 0.37
RDW-CW (%)	16.28 ± 0.88	15.64 ± 0.42	13.83 ± 0.36[b]
RDWS (µm^3)	26.96 ± 1.65	25.86 ± 0.72	24.32 ± 0.37
PLT (10^3 /µL)	741.20 ± 98.38	618.80 ± 18.56	658.00 ± 38.70
MPV (µm^3)	6.54 ± 0.27	6.58 ± 0.09	6.50 ± 0.11
PCT (%)	0.49 ± 0.08	0.41 ± 0.01	0.43 ± 0.02
PDW (%)	24.98 ± 1.43	26.28 ± 1.91	26.02 ± 2.84
P-LCR (%)	5.96 ± 2.29	5.40 ± 0.61	4.68 ± 0.54

Data are expressed as mean ± SEM, n = 5. Values in the test groups not carrying any letter when compared to the control group are not significantly different according to Tukey-Kramer multiple comparison test. Letter b was considered statistically significant with $p<0.01$ when compared to the control. White blood cell (WBC), lymphocytes (LYM), monocyte (MON), neutrophil (NEU), eosinophil (EOS), basophil (BAS), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width SD (RDW-SD), red blood cell distribution width-coefficient of variation (RDW-CV), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelets count (PCT), platelet larger cell ratio (P-LCR).

Table 3.9: Lipid profile parameters after 28 days of administration of *S. mombin.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
T-CH (mg/dL)	82.20 ± 4.03	89.60 ± 4.48	89.00 ± 4.14
HDL (mg/dL)	25.40 ± 0.98	26.40 ± 0.98	25.60 ± 1.12
LDL (mg/dL)	36.60 ± 2.94	44.40 ± 3.31	45.80 ± 3.18
TG (mg/dL)	100.60 ± 2.89	94.00 ± 8.59	87.80 ± 3.68

Data are expressed as mean ± SEM, n=5. Values in the test groups were not significantly different compared to the control group according to Tukey-Kramer multiple comparison test. Total cholesterol (T-CH), high-density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG).

Table 3.1.0: Kidney function test parameters after 28 days of administration of *S. mombin.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
Urea (mg/dL)	45.40 ± 2.25	49.00 ± 2.61	29.00 ± 1.00[c]
Creatinine (mg/dL)	0.68 ± 0.06	0.70 ± 0.03	0.72 ± 0.05
Na^+ (mmol/L)	140.60 ± 1.47	139.20 ± 1.39	141.5 ± 0.92
K^+ (mmol/L)	4.12 ± 0.10	4.30 ± 0.22	4.40 ± 0.17
HCO_3^- (mmol/L)	21.40 ± 0.28	21.60 ± 0.81	21.00 ± 0.84
Cl^- (mg/dL)	104.80 ± 0.73	105.00 ± 1.05	105.60 ± 0.75

Data are expressed as mean ± SEM, n=5. Values in the test groups not carrying any letter when compared to the control group are not significantly different according to Tukey-Kramer multiple comparison test. Letter c was considered statistically significant with $p<0.001$ when compared to the control.

Table 3.11: Liver function test parameters after 28 days of administration of *Spondias mombin.*

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
AST (µ/L)	77.40 ± 4.27	57.00 ± 4.53[b]	75.00 ± 3.78
ALT (µ/L)	45.80 ± 2.42	33.40 ± 2.62[b]	44.60 ± 2.29
ALP (µ/L)	59.60 ± 3.54	49.80 ± 4.13	51.80 ± 3.50
Tb (mg/dL)	0.28 ± 0.02	0.28 ± 0.02	0.26 ± 0.02
Cb (mg/dL)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Tp (g/dL)	6.34 ± 0.12	6.92 ± 0.11	6.26 ± 0.18
ALB (g/dL)	2.82 ± 0.02	3.00 ± 0.05	2.82 ± 0.02
GLo (g/dL)	3.52 ± 0.14	3.92 ± 0.10	3.42 ± 0.17

Data are expressed as mean ± SEM, n=5. Values in the test groups not carrying any letter when compared to the control group are not significantly different according to Tukey-Kramer multiple comparison test. Letter b was considered statistically significant with $p<0.01$ when compared to the control. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin (Tb), conjugated bilirubin (Cb), total protein (Tp), albumin (ALB), globulin (GLo).

3.4 Histological Evaluation

Sections of the uterus taken from the control group rats given baseline feed and water freely show normal histological architecture with well-defined uterine cavity, surrounded by the endometrial lining (membrane), and endometrium containing the glands embedded in the stroma. Sections taken from the uterus of rats treated with the doses of *V. amygdalina* and *S. mombin* show normal histological architecture. (Figure 3.1-3.5).

Sections of the lungs taken from the control group 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. Sections taken from rats given the doses of *V. amygdalina* and *S. mombin* show normal alveolar sacs. However, sections of the lungs taken from rats treated with graded doses of *S. mombin* show bronchiolar ulceration (Figure 3.6-3.10).

Sections of the liver taken from the control group 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). The same was also observed in sections taken from rats treated with 100mg of *V. amygdalina* and *S. mombin.* Sections taken from the liver of rats treated with 200mg *V. amygdalina* show mild inflammation in the portal region of the liver, also known as portal hepatitis (Figure 3.11-3.15).

Sections of the spleen taken from the control group 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. Sections treated with the doses of *V. amygdalina* and *S. mombin* showed varying degrees of activation (boosting) of the lymphoid follicles, however, 200mg/kg body weight *V. amygdalina* had the most marked boosting of the local immune system of the spleen (Figure 3.16-3.20).

Sections of the heart taken from the control group rats given baseline feed and water freely, and sections taken from rats given the doses of *V. amygdalina* show normal histological architecture, with well-defined bundles of myocardial fibres, interstitial space, coronary arteries and cardiac veins. It also shows the beneficial haemodynamic and vasogenic changes of increased blood circulation (active vascular congestion) and vasodilatation. However, the doses of *S. mombin* causes coronary artery ulceration (Figure 3.21-3.25).

Sections of the kidney taken from rats given baseline feed and water freely, as well as sections taken from rats treated with the doses of *V. amygdalina* and *S. mombin* show normal histological architecture, with well-defined tubules, glomeruli, interstitial space and arcuate blood vessels (Figure 3.26-3.25).

UTERUS

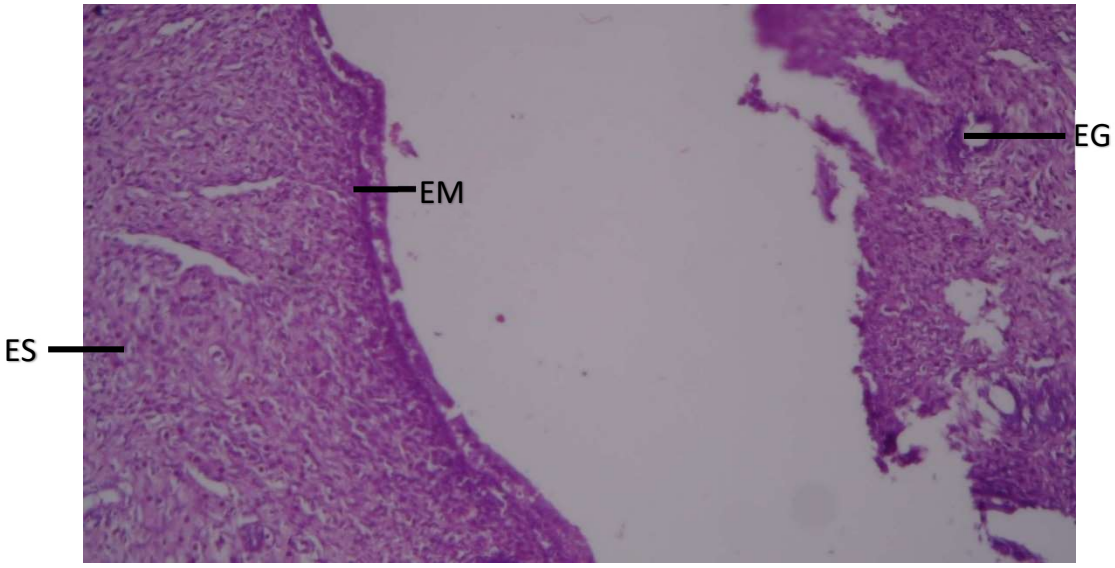

Figure 3.1: Rat uterus, control, showing: normal architecture: endometrial membrane (EM), stroma (ES) and glands (EG): H&E 400 X

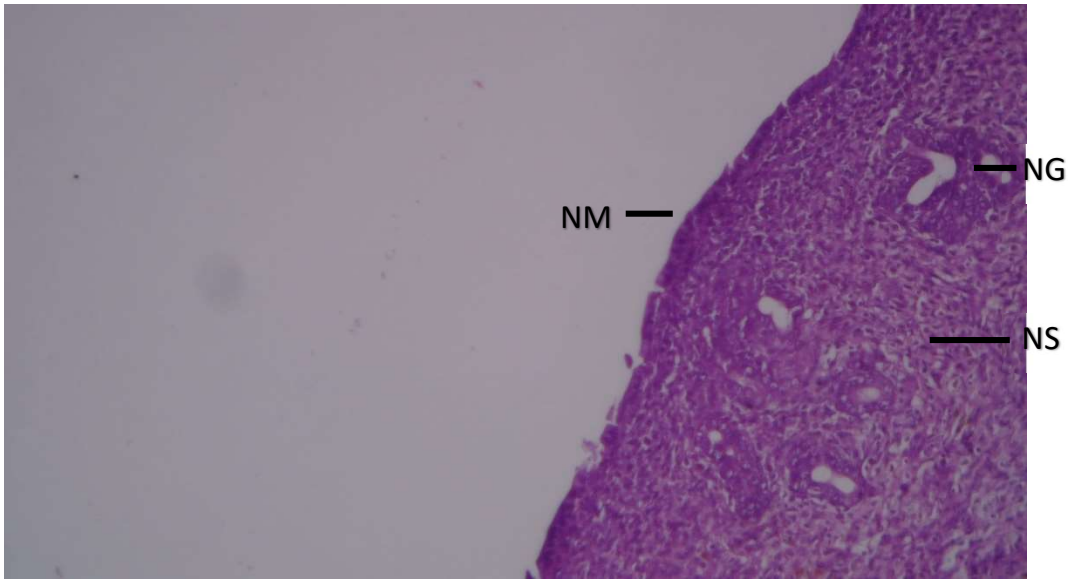

Figure 3.2: Rat uterus given 100mg/kg *V. amygdalina* showing: normal endometrial glands (NG), stroma (NS) and membrane (NM): H&E 400 X

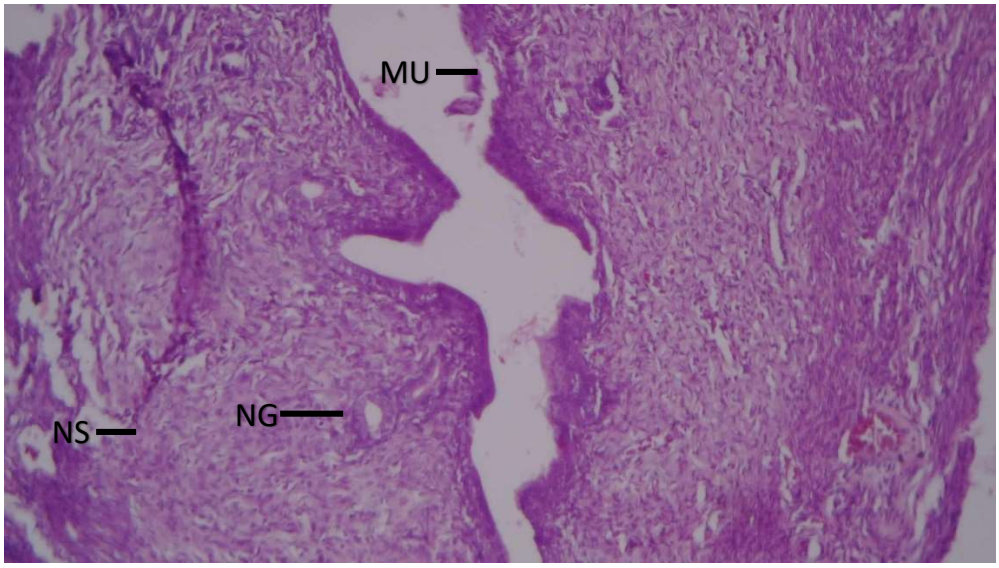

Figure 3.3: Rat uterus given 200mg/kg *V. amygdalina* showing: normal endometrial layer (MU), normal glands (NG) and stroma (NS): H&E 400 X

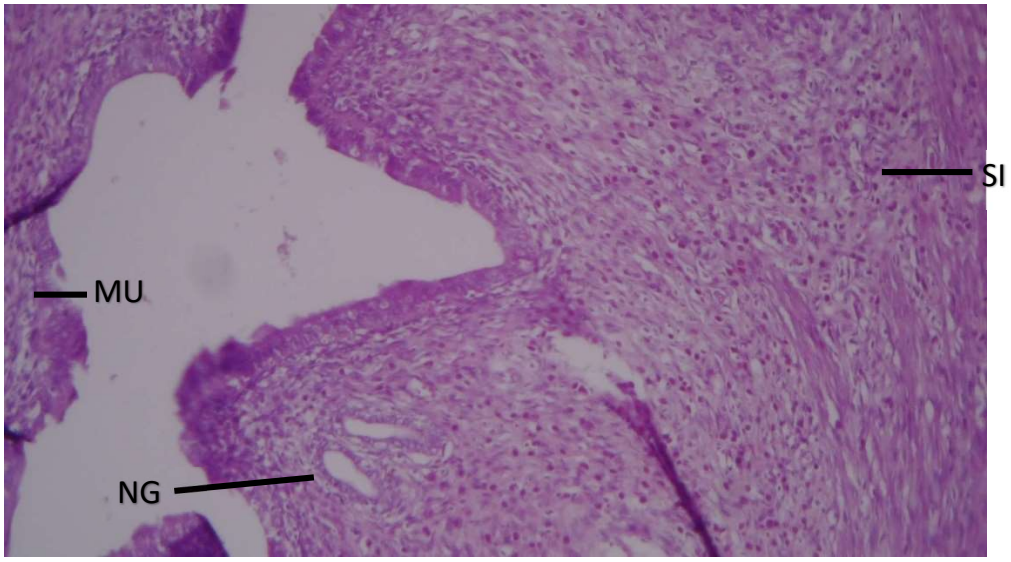

Figure 3.4: Rat uterus given 100mg/kg *S. mombin* showing: normal endometrial layer (MU), stromal infiltrates of inflammatory cells (SI) and normal endometrial glands (NG): H&E 400 X

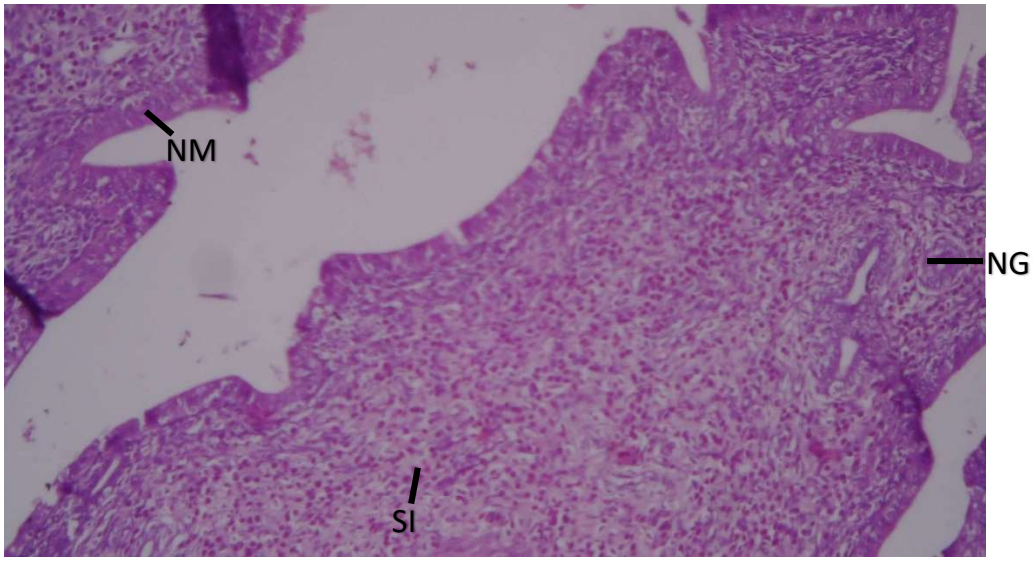

Figure 3.5: Rat uterus given 200mg/kg *S. mombin* showing: normal endometrial membrane (NM), stromal infiltrates of inflammatory cells (SI) and normal glands (NG): H&E 400 X

LUNG

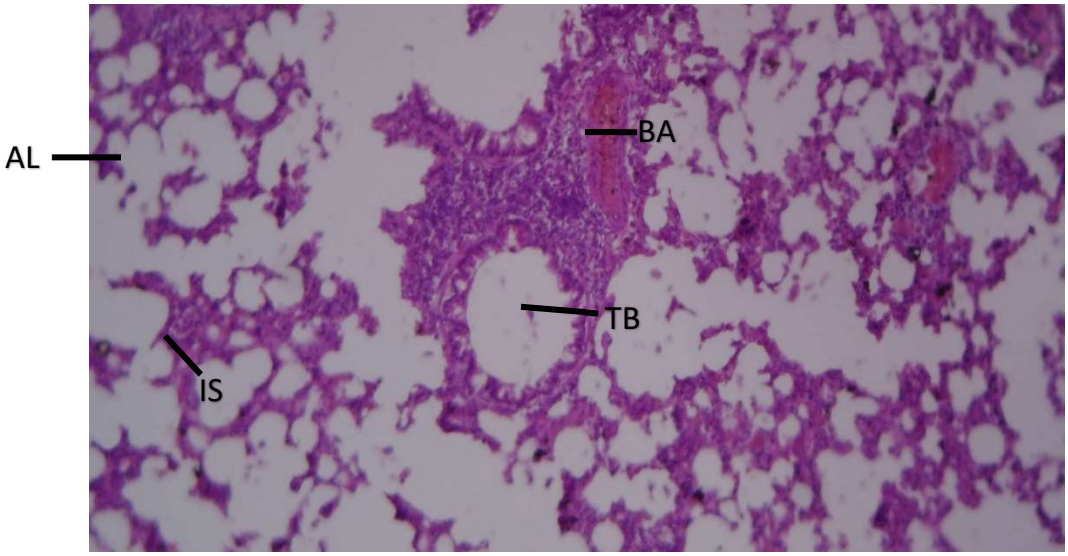

Figure 3.6: Rat lungs, control, showing normal architecture: alveoli (AL), interstitial space (IS), terminal bronchiole (TB) and bronchial artery (BA): H&E 400 X

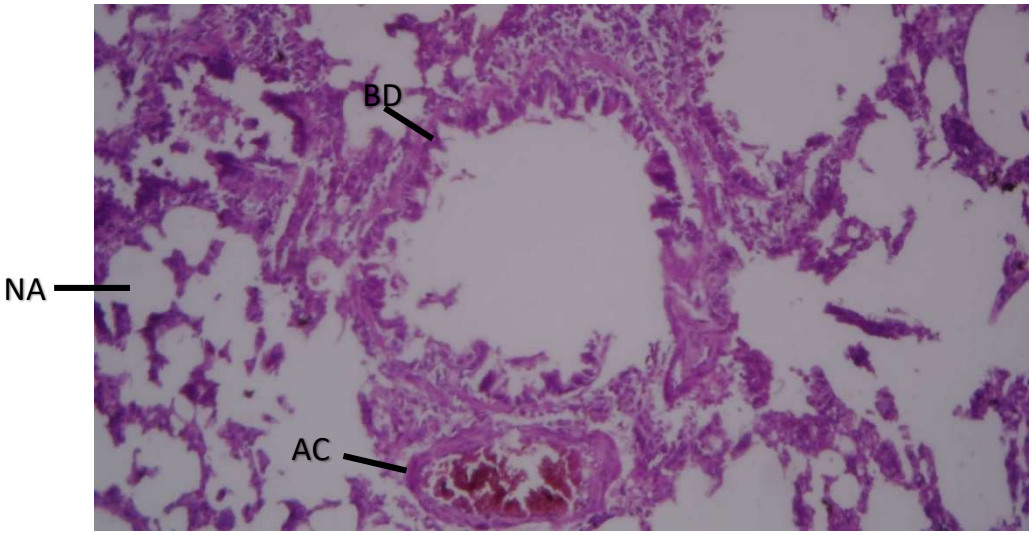

Figure 3.7: Rat lungs given 100mg/kg *V. amygdalina* showing normal alveoli (NA), bronchiolar dilation (BD) and active vascular congestion (AC): H&E 400 X

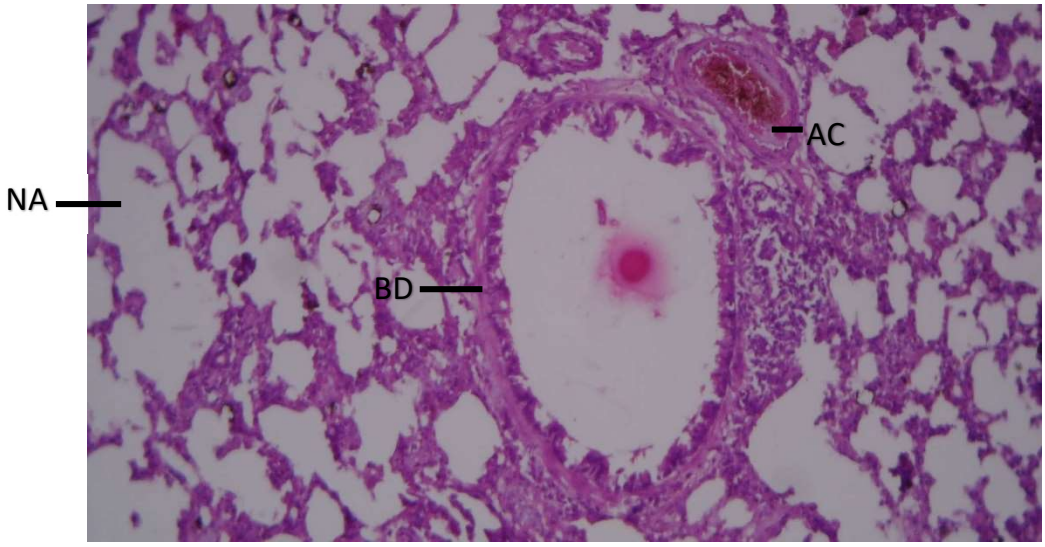

Figure 3.8: Rat lungs given 200mg/kg *V. amygdalina* showing normal alveoli (NA), bronchiolar dilation (BD) and active vascular congestion (AC):

H&E 400 X

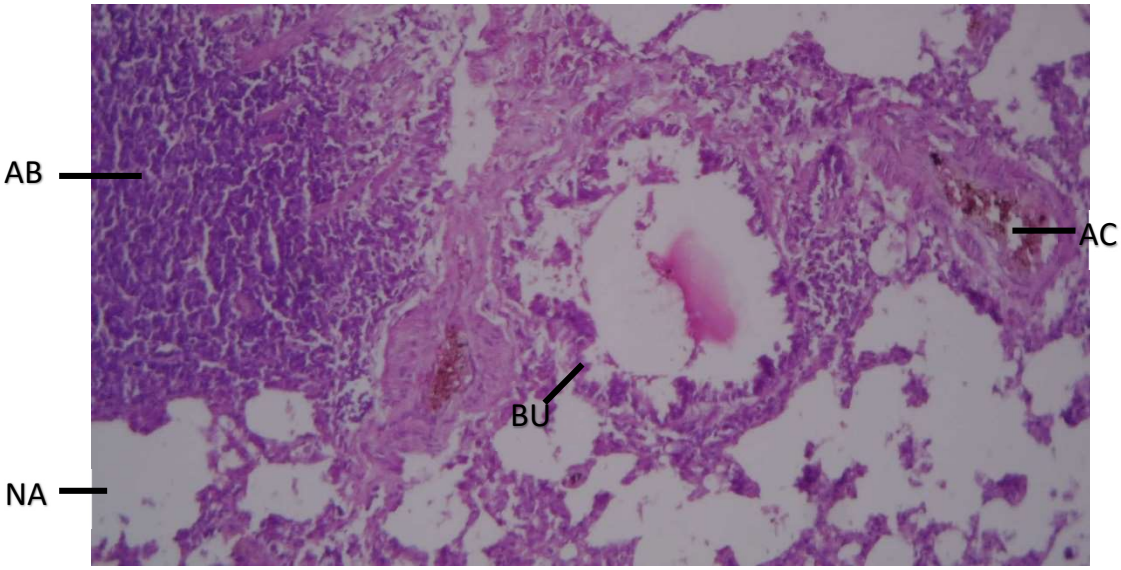

Figure 3.9: Rat lungs given 100mg/kg *S. mombin* showing normal alveoli (NA), bronchiolar ulceration (BU), active vascular congestion (AC) and activation of bronchiolo-alveolar lymphoid aggregate (AB): H&E 400 X

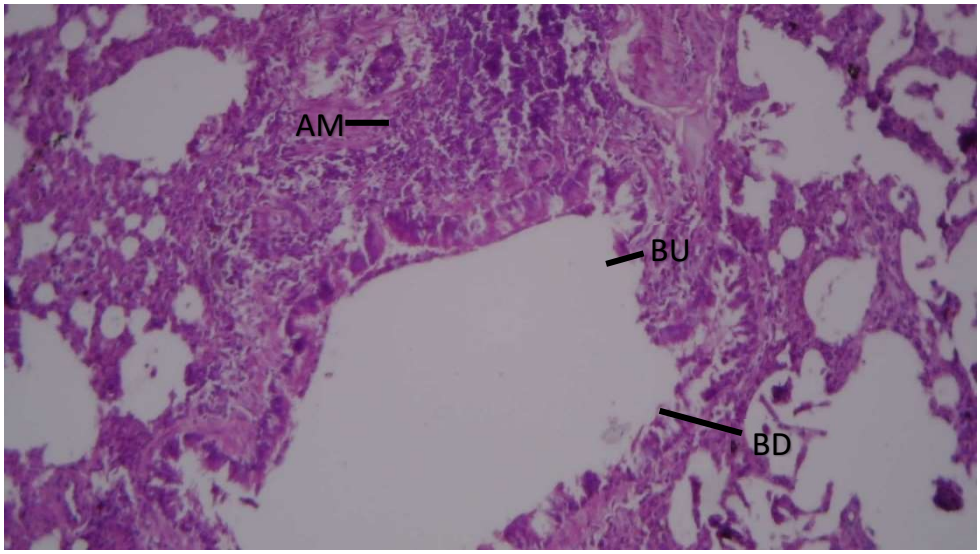

Figure 3.10: Rat lungs given 200mg/kg *S. mombin* showing: activation of cells of the mononuclear phagocyte system (AM), bronchiolar dilation (BD) and ulceration (BU): H&E 400 X

LIVER

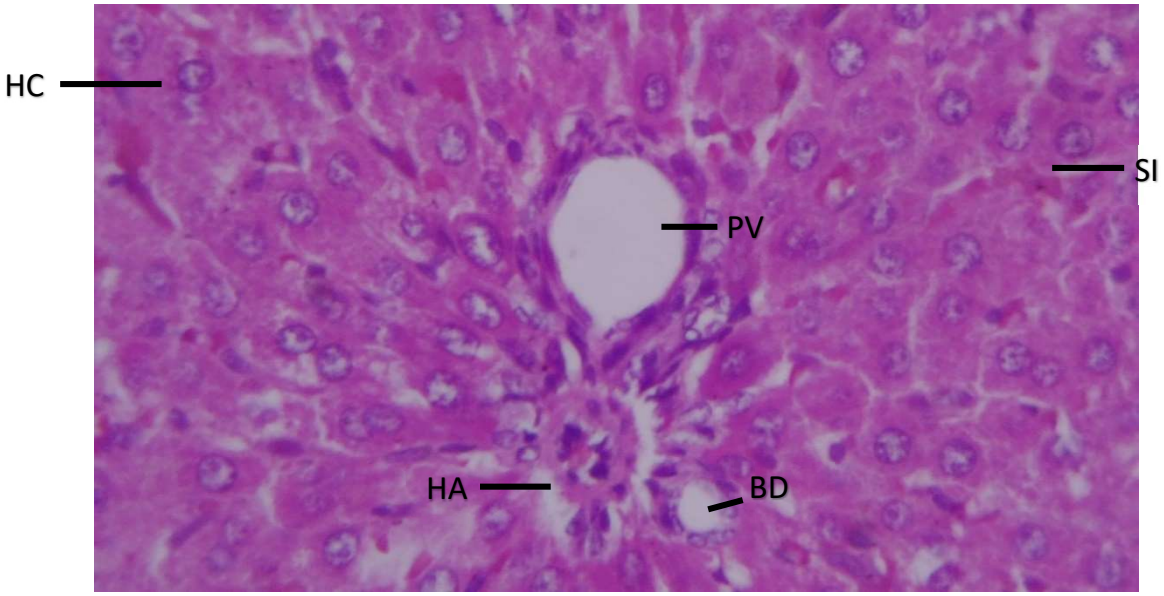

Figure 3.11: Rat liver, control, showing: normal architecture: hepatocytes (HC), sinusoids (SI), portal vein (PV), hepatic artery (HA) and bile duct (BD):

H&E 400 X

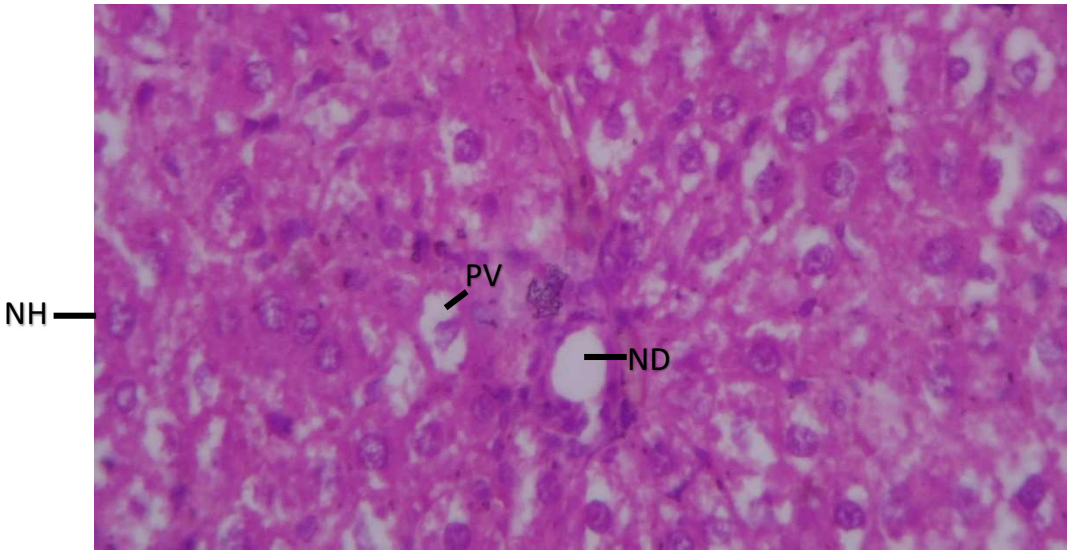

Figure 3.12: Rat liver given 100mg/kg *V. amygdalina* showing: normal hepatocytes (NH), portal vein (PV) and bile ducts (ND): H&E 400 X

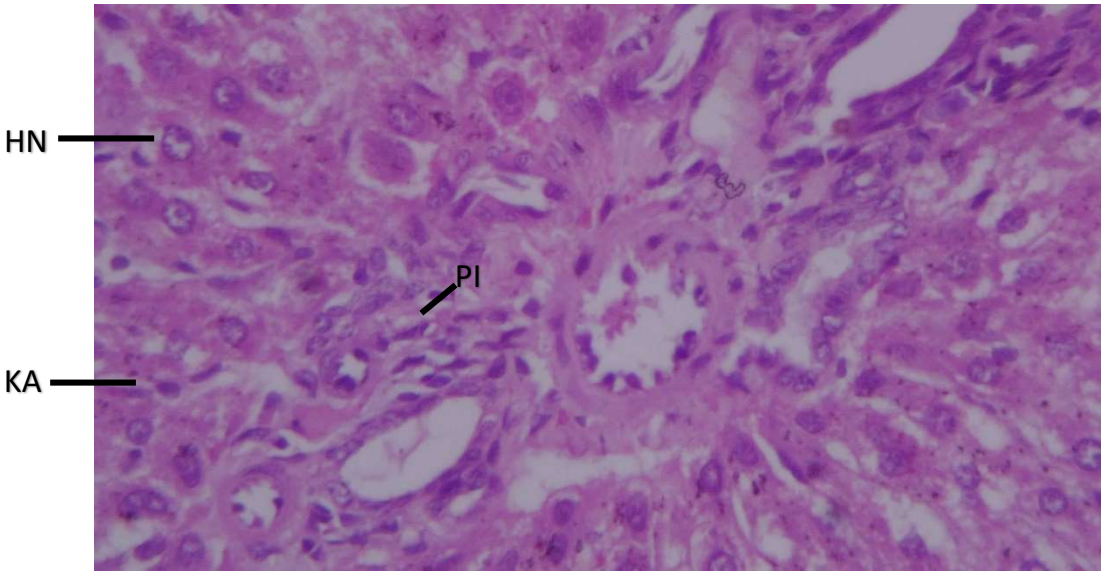

Figure 3.13: Rat liver given 200mg/kg *V. amygdalina* showing: normal hepatocytes with conspicuous nucleoli (HN), Kupffer cell activation (KA) and periportal infiltrates of inflammatory cells (PI): H&E 400 X

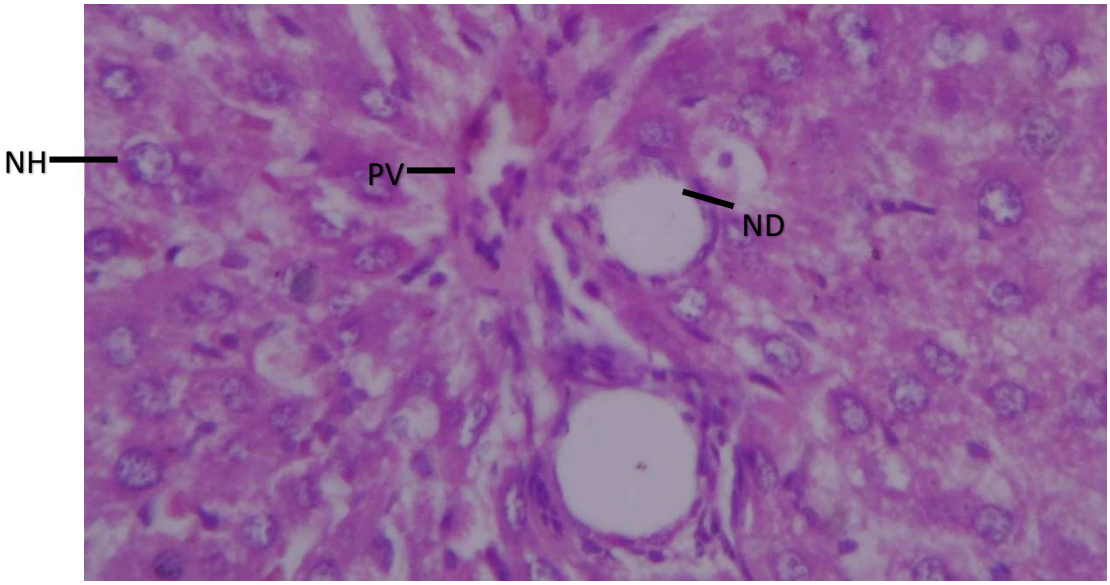

Figure 3.14: Rat liver given 100mg/kg *S. mombin* showing: normal hepatocytes (NH), portal vein (PV) and bile ducts (ND): H&E 400 X

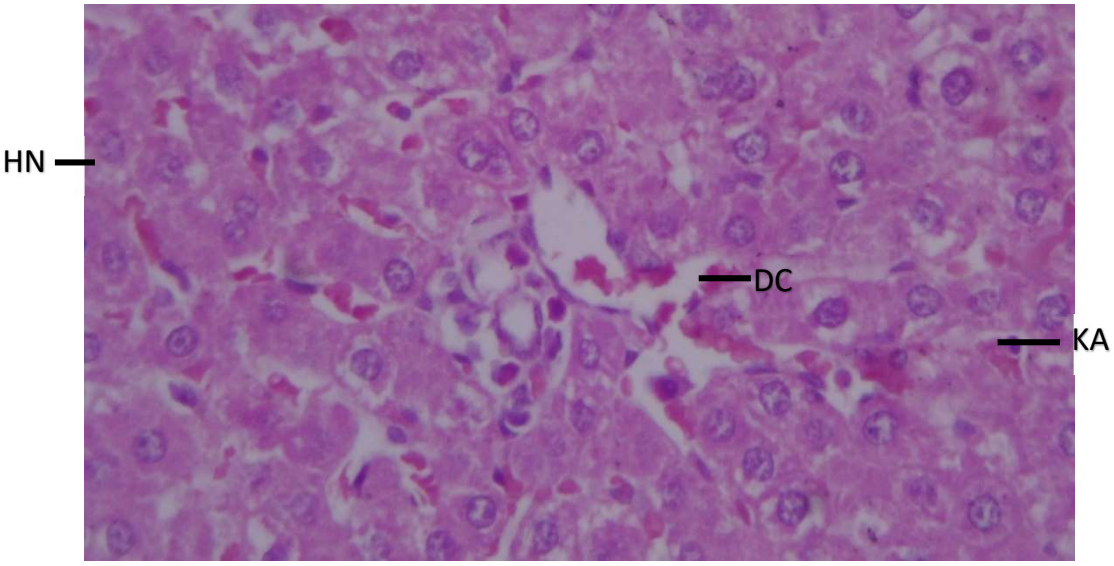

Figure 3.15: Rat liver given 200mg/kg *S. mombin* showing: normal hepatocytes with conspicous nucleoli (HN), vasodilatation and active congestion (DC) and Kupffer cell mobilization (KA): H&E 400 X

SPLEEN

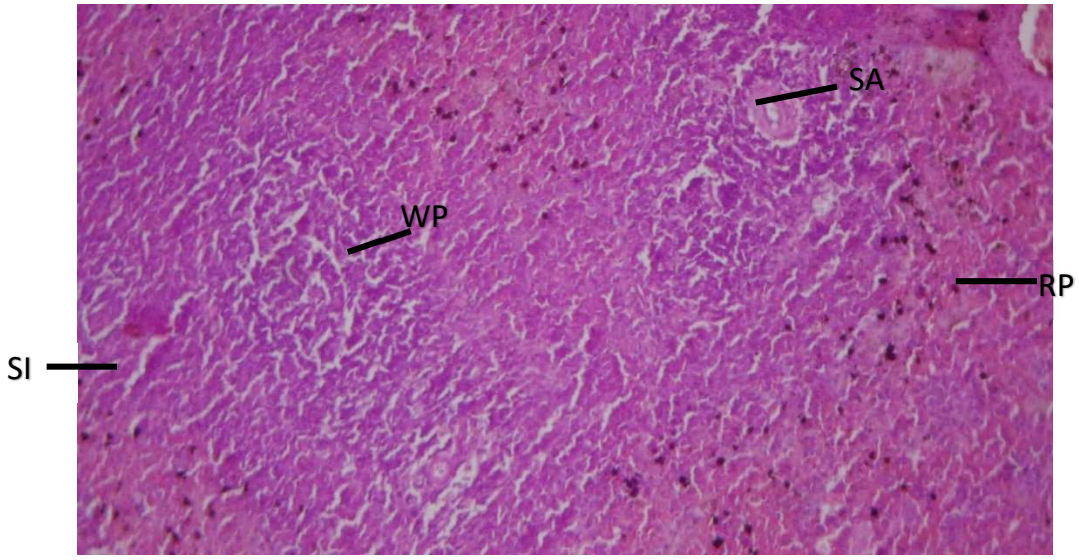

Figure 3.16: Rat spleen control, showing: normal architecture: white pulp (WP), sinuses (SI), splenic arterioles (SA) and red pulp (RP): H&E 400 X

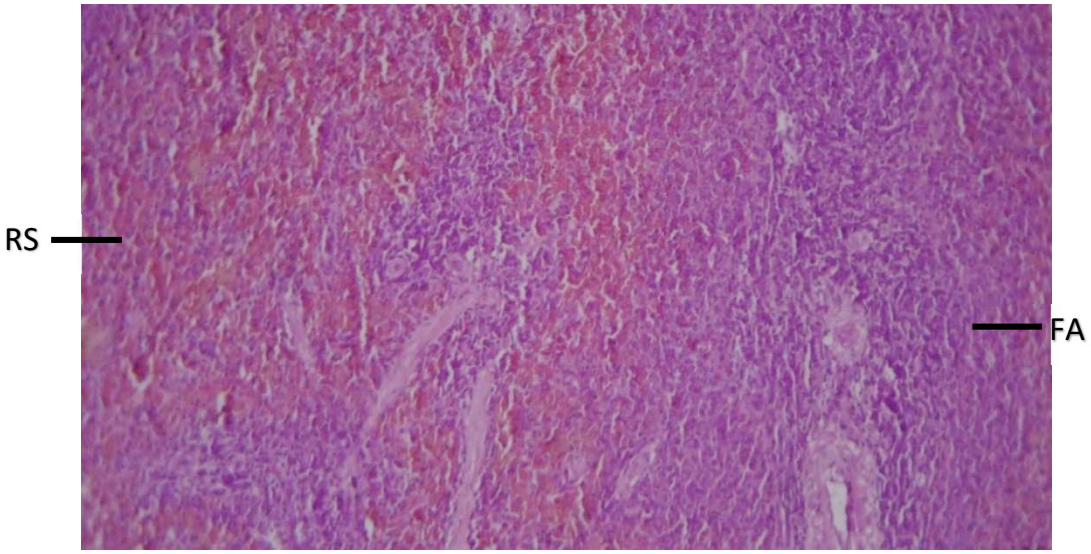

Figure 3.17: Rat spleen given 100mg/kg *V. amygdalina* showing: increased red cell sequestration (RS) and follicular activation (FA): H&E 400 X

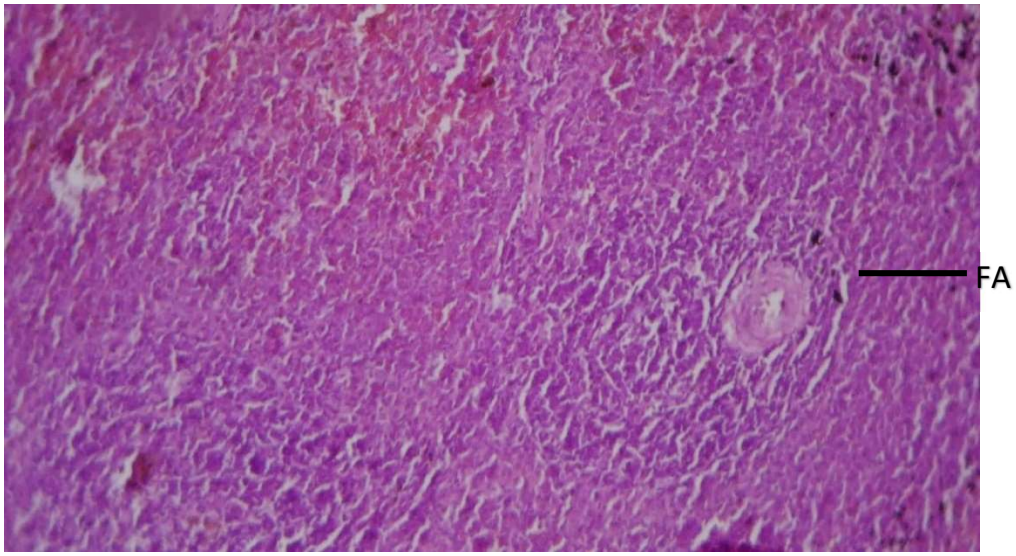

Figure 3.18: Rat spleen given 200mg/kg *V. amygdalina* showing: marked follicular activation (FA): H&E 400 X

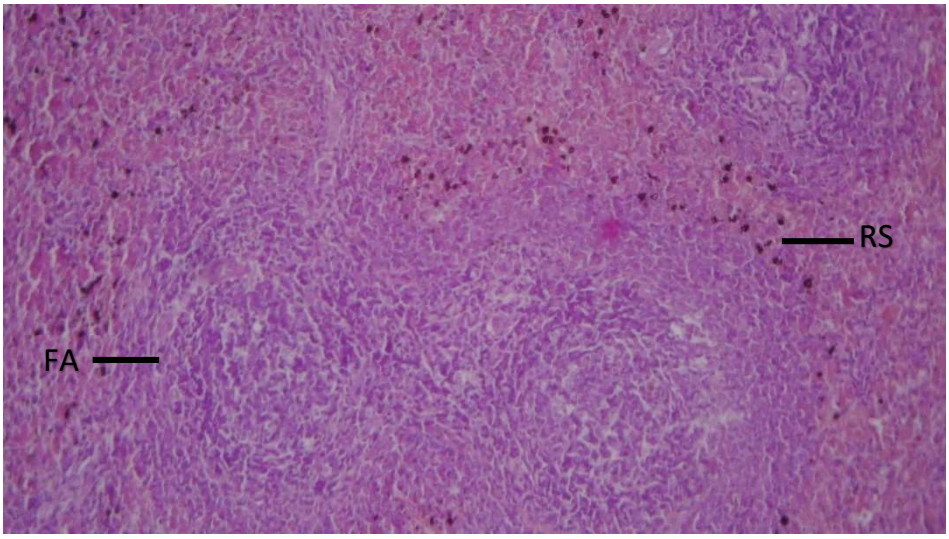

Figure 3.19: Rat spleen given 100mg/kg *S. mombin* showing: increased red cell sequestration (RS) and follicular activation (FA): H&E 400 X

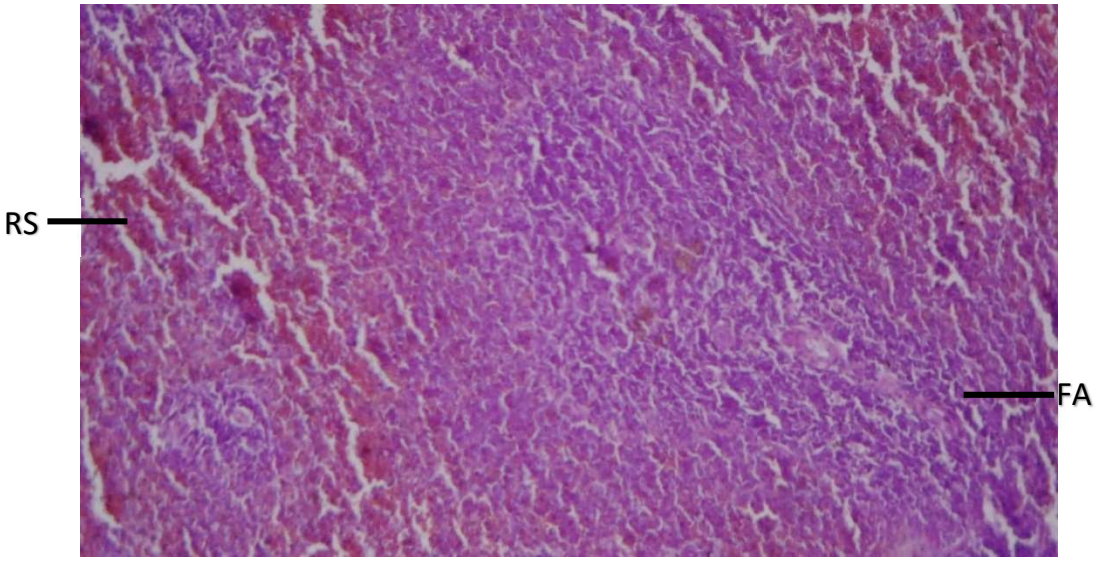

Figure 3.20: Rat spleen given 200mg/kg *S. mombin* showing: follicular activation (FA) and increased red cell sequestration (RS): H&E 400

HEART

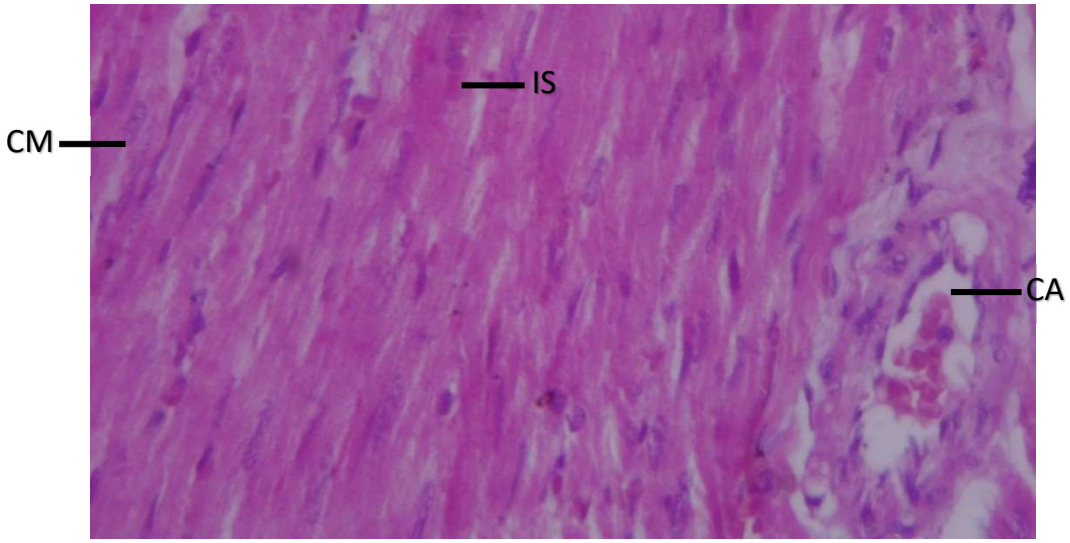

Figure 3.21: Rat heart, control, showing normal architecture: bundles of cardiomyocytes (CM), interstitial space (IS) and coronary artery (CA): H&E 400 X

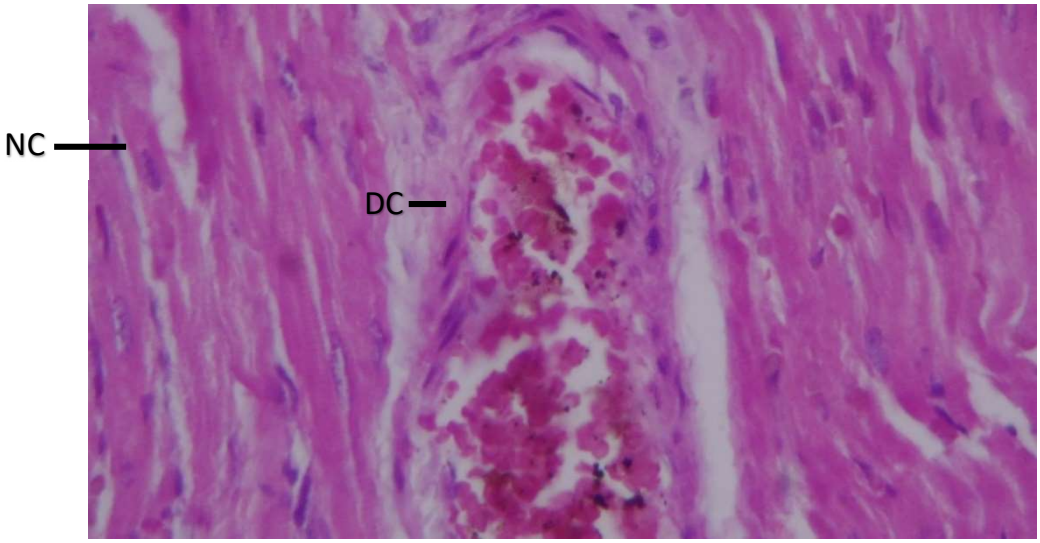

Figure 3.22: Rat heart given 100mg/kg *V. amygdalina* showing: normal bundles of cardiomyocytes (NC), vasodilatation and active vascular congestion (DC): H&E 400 X

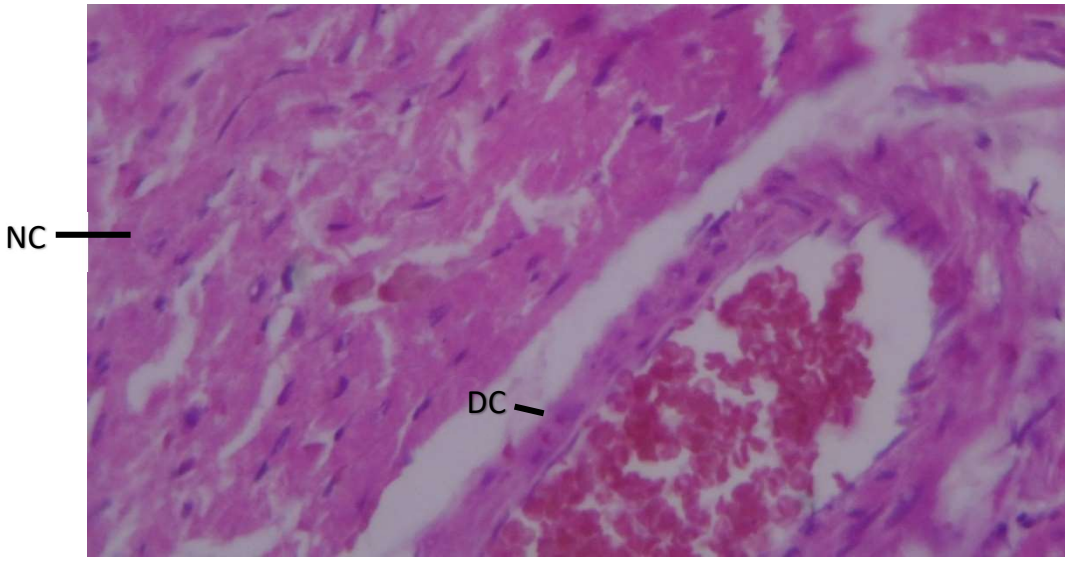

Figure 3.23: Rat heart given 200mg/kg *V. amygdalina* showing: normal bundles of cardiomyocytes (NC), vasodilatation and active congestion (DC):

H&E 400 X

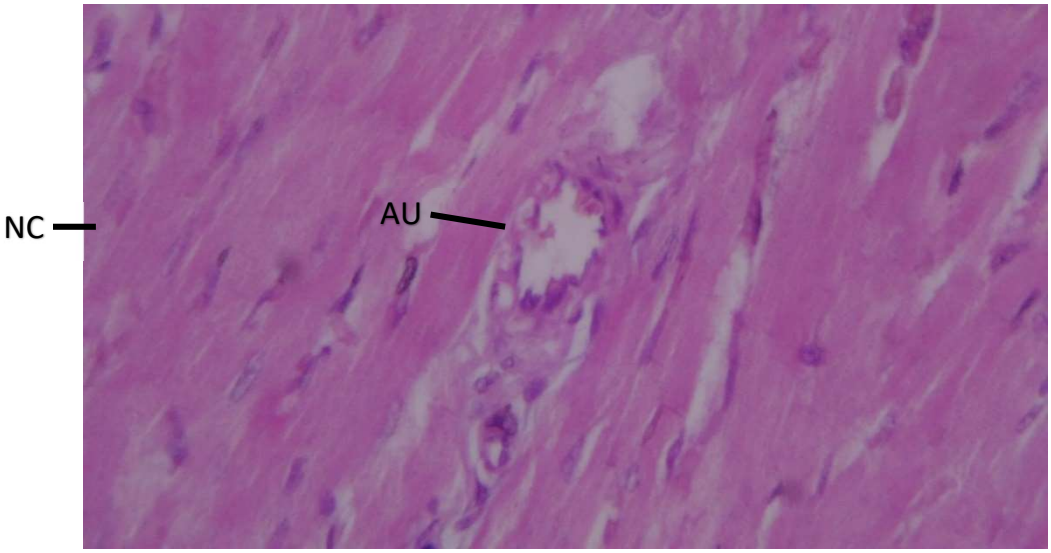

Figure 3.24: Rat heart given 100mg/kg *S. mombin* showing: normal bundles of cardiomyocytes (NC) and coronary artery ulceration (AU): H&E 400 X

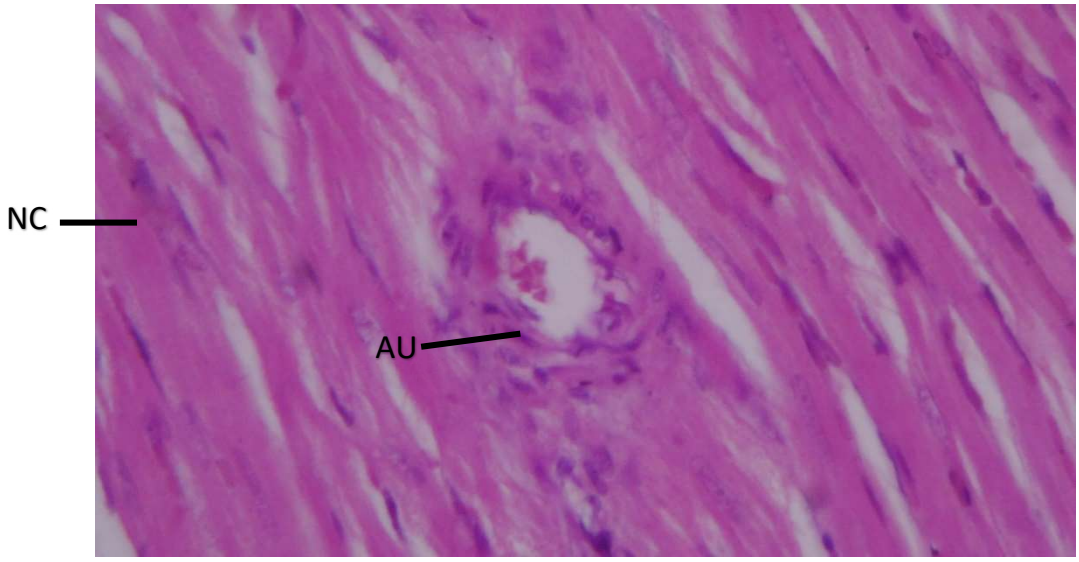

Figure 3.25: Rat heart given 200mg/kg *S. mombin* showing: normal bundles of cardiomyocytes (NC) and coronary artery ulceration (AU): H&E 400 X

KIDNEY

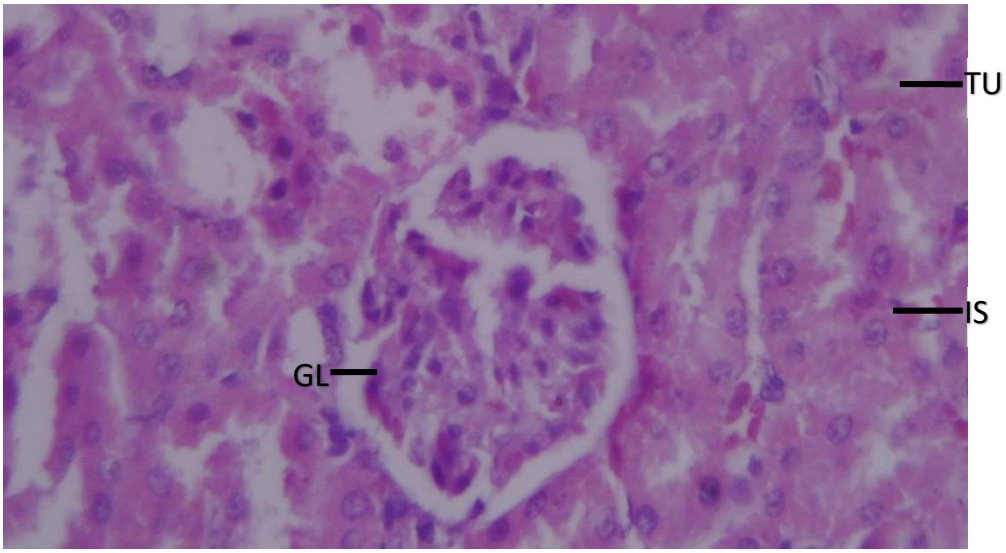

Figure 3.26: Rat kidney, control, showing normal architecture: tubules (TU), glomerulus (GL) and interstitial space (IS): H&E 400 X

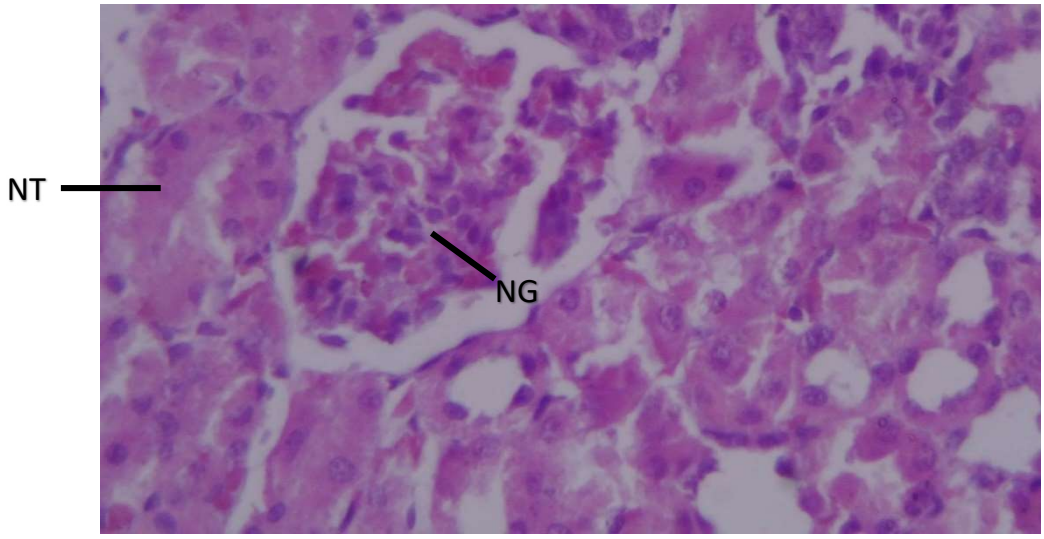

Figure 3.27: Rat kidney given 100mg/kg *V. amygdalina* showing: normal glomerulus (NG) and tubules (NT): H&E 400 X

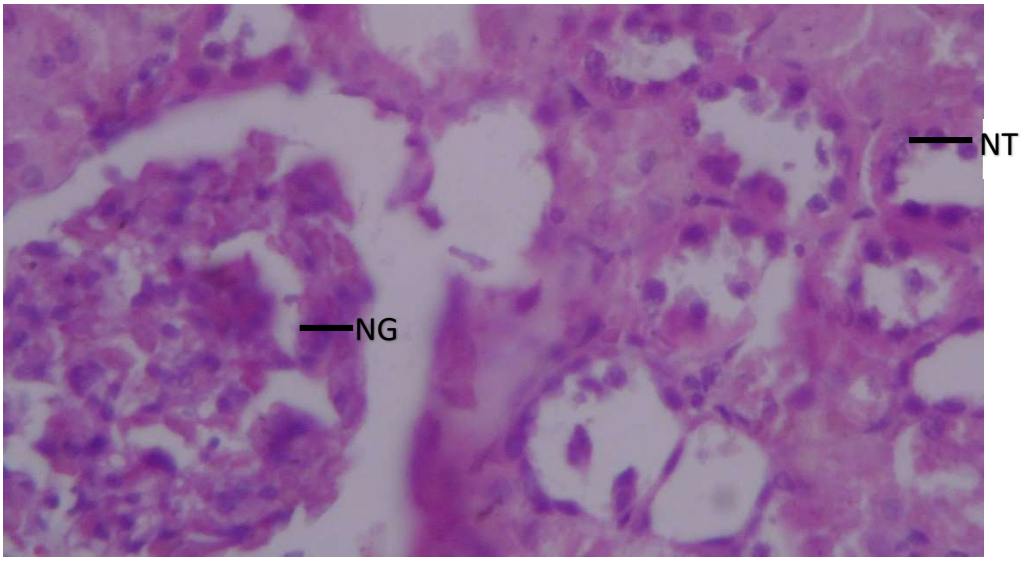

Figure 3.28: Rat kidney given 200mg/kg *V. amygdalina* showing: normal glomerulus (NG) and tubules (NT): H&E 400 X

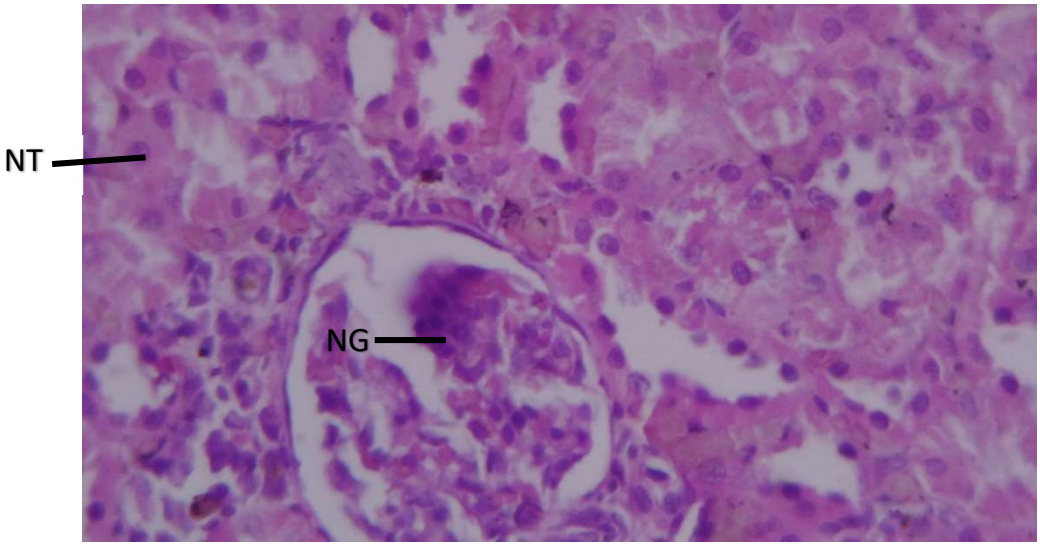

Figure 3.29: Rat kidney given 100mg/kg *S. mombin* showing normal glomerulus (NG) and tubules (NT): H&E 400 X

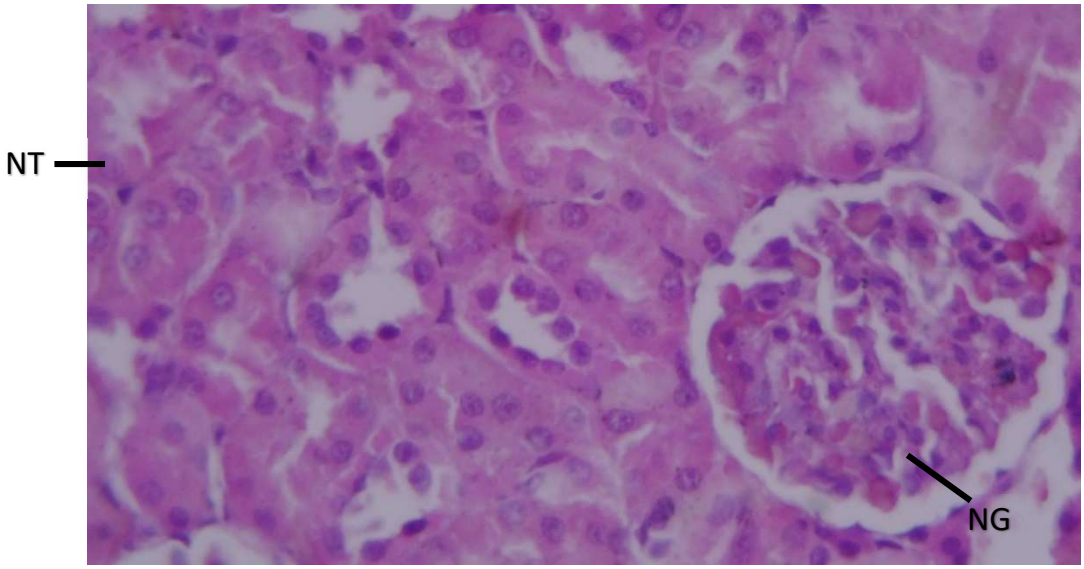

Figure 3.30: Rat kidney given 200mg/kg *S. mombin* showing: normal tubules (NT) and glomerulus (NG): H&E 400 X

CHAPTER FOUR

DISCUSSION

4.1 Discussion

The yield of both plant extracts in absolute ethanol shows that ethanol is a suitable solvent for extracting both plants. It also indicates that the continuous solvent extraction method using Soxhlet apparatus is suitable for extracting leaves of *Vernonia amygdalina* and *Spondias mombin*. The solubility of the extracts in ethanol, a semi polar solvent, indicates the plant consists of both polar and non-polar constituents.

This study adopted a multi-faceted approach to evaluate the potential toxicity of *V. amygdalina* and *S. mombin*, incorporating qualitative phytochemical screening, biochemical analysis of key organ function markers, and histopathological examination to understand the range and severity of effects following sub-acute exposure in Wistar rats.

The qualitative phytochemical screening revealed a remarkably consistent phytochemical profile between the ethanol extracts of *V. amygdalina* and *S. mombin*. Both plants were positive for the major classes of secondary metabolites essential for their ethnobotanical relevance: carbohydrates/reducing sugars, alkaloids, anthraquinones, phenolic compounds (including hydrolysable and pseudo tannins), saponins, steroids/triterpenes, and cardiac glycosides. Notably, cyanogenic glycosides, known for high acute toxicity, were absent in both extracts, supporting the non-lethal findings of the acute toxicity assessments as seen in Table 3.3. These findings are consistent with previous phytochemical analysis of these plant extracts (Nowak, *et al*., 2022; Ogunlana *et al*., 2023).

The presence of reducing sugars (e.g., glucose, fructose) in both plants suggests that the plant extracts contain simple carbohydrates that can be readily metabolized for energy (Edo *et al*., 2022). However, studies have shown that *S. mombin* has antihyperglycemic effect (reducing high blood sugar). This is of relevance to those with diabetes (Gobinath *et al*., 2022).

Alkaloid are cyclic organic compound containing nitrogen in a negative oxidation state. Alkaloids have a wide range of physiological effects, including antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and anticancer activities (Jan *et al*., 2021). Alkaloids are useful in dietary supplements and medications. They are also a crucial component of organic synthesis (Dey *et al*., 2020). The qualitative screening indicates the presence of alkaloid in both plant extracts. According to Tura, *et al*., (2024), about 20 alkaloids were found in *V. amygdalina*. Similarly, According to Eze *et al*., (2024), significant number of alkaloids was screened and quantified in *S. mombin* leaf.

Anthraquinones are known for their laxative properties and are often found in plants used to treat constipation. Some anthraquinones also exhibit Hypoglycemic, antilipidemic, and tissue regeneration activities (Ez e *et al*., 2024).

Phenolic compounds are a large group of compounds characterized by the presence of one or more aromatic rings with hydroxyl groups. This class includes flavonoids, tannins, phenolic acids, and lignans. The qualitative screening indicates the presence of phenolic compounds in both plant extracts. Flavonoids are also significant for human health owing to their powerful pharmacological effects (Tura *et al*., 2024). They have a variety of beneficial biochemical and antioxidant properties against a number of illnesses, including cancer, Alzheimer's disease (AD), atherosclerosis, e.t.c (Ampem *et al*., 2024)

Saponins are glycosides with a characteristic soap-like foaming ability. The qualitative screening indicates the presence of saponin in both plant extracts. As versatile glycosidic compounds possessing several biological properties (anti-hyperglycemic, anti-inflammatory, hypocholesterolemic, anti-oxidant and anti-tumor properties), saponins have been employed for different applications in the pharmaceutical sector (one such application is the use of saponins by this industry as an initiative precursor for the semi-synthesis of steroidal drugs) (Imaga *et al*., 2023).

Steroids and triterpenes are a large group of compounds with diverse biological activities, including hormonal, anti-inflammatory, and anticancer effects. Steroids can affect hormone levels, potentially disrupting endocrine function. Triterpenes can exhibit a range of activities, including anti-inflammatory, antioxidant, and anticancer effects. Recent studies showed that the bark of *S. mombin* have significant level of steroids (Odoh *et al*., 2020).

Cardiac glycosides are compound that have potent effect on the heart. They are used to treat heart failure and arrhythmias. However, they have a narrow therapeutic window, and overdose can lead to serious cardiotoxicity, including arrhythmias, heart block, and even death. Owing to the richness of the leaf, root, and stem bark of *S. mombin* in cardiac glycosides, the valuable medicinal efficacy of the plant has been highlighted and recommended for drug formulation in pharmaceutical industries (Eze *et al*., 2024).

Cyanogenic glycosides are compounds that release hydrogen cyanide (HCN) upon hydrolysis. HCN is a potent respiratory inhibitor that can be lethal even in small amounts. The presence of cyanogenic glycosides is a significant safety concern and requires careful assessment of the potential for cyanide poisoning. The qualitative screening indicates absence of this compound in both plant extracts.

The sub-acute exposure of *V. amygdalina* and *S. mombin* leaf extracts resulted in significant alterations in key biochemical markers, providing insights into potential organ-specific toxicities. Significant changes in blood indices (red blood cells (RBC), white blood cells (WBC), platelets and their differentials) suggest that the chemical being administered is either toxic or protective to the hemopoietic tissue. The blood indices are used to monitor the physiological and pathological state of the body (Imade *et al*., 2024). Findings from this study (Table 3.4) report no significant effects on most of the important blood indices by the ethanol extract of *V. amygdalina* ($p>0.05$) across the 19 assayed indices (WBC, RBC, HGB, PLT, etc.). However, the results of the hematological parameters (Table 3.8) from the serum of the control and test groups of *S. mombin* revealed a significant effect ($p<0.05$) in red blood cell distribution width-coefficient of variation (RDW-CV) level at 200 mg/kg. The extract's non-significant effect on the RBC could mean that there was no change in the balance between blood corpuscle destruction to erythropoiesis and the rate of blood production. HGB, PCT and MCH levels did not significantly decrease ($p>0.05$) in female rats treated with the various doses; this could indicate that hemoglobin incorporation into red blood cells and red blood cell morphology were unaffected (Imade *et al*., 2024). Although, recent studies on *V. amygdalina* extract showed a moderate increase in WBC suggesting immunomodulation (Ikugbiyiyi et al., 2024).

LDL, total cholesterol and triglycerides are three primary components of the lipid profile that are linked to cardiovascular disease. A dysregulated lipid metabolism is indicated by changes in LDL and HDL levels, which may be caused by interference with lipolysis and the release of free fatty acids from peripheral depots (Imade *et al*., 2024). Although the levels of triglycerides, LDL, HDL and total cholesterol were not significantly different ($p>0.05$) from the control group, other studies

have shown that *V. amygdalina* leaf extract has the ability to decrease cholesterol, triglycerides, and LDL in broilers (Elemo 2023).

The primary function of the kidneys is to remove the harmful waste produced by the normal functioning of the body and transported by the blood. In fact, additional research amply supports the capacity of plant extracts to function as potent free radical scavengers in the kidney, avoiding their harmful effects on lipid peroxidation, which raises biochemical markers like creatinine and urea by rupturing membranes (Fawwaz, 2023). Conversely, the results of the kidney function test (Table 3.10) revealed a significant decrease ($p<0.05$) in urea level at 200 mg/kg of *S. mombin* extract suggesting a protection to the kidney. Meanwhile, this is in contrast with the study done by Ademola *et al*., (2020) revealing a significant increase in the level of urea in the blood at a dose of 500mg/kg.

The liver is essential for the detoxification and excretion of several endogenous and exogenous substances, and any harm or impairment to it can have a wide range of health effects on both humans and animals. Cellular necrosis, elevated tissue lipid peroxidation and glutathione depletion are linked to liver injury. Furthermore, liver illness is associated with increased serum levels of many biochemical indicators, including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) (Imade *et al*., 2024). All of these parameters, at all tested doses, revealed no significant difference ($p>0.05$) compared to the control. However, the results of the liver function test (Table 3.11) revealed a significant decrease ($p < 0.01$) in Alanine aminotransferase and Aspartate aminotransferase level at 100 mg/kg of *S. mombin* extract compared to the control suggesting a stabilizing effect on hepatocyte membranes or an enhanced hepatic antioxidant status and proffering a protective ability on the liver. Similarly,

recent findings suggest that *V. amygdalina* extract normalized ALT, AST, ALP, urea, creatinine in high-fat diet rats, restoring hepatic and renal health (Elemo 2023).

Histopathological examination refers to the microscopic study of tissues to detect disease. It is a gold standard diagnostic tool in medicine and research for assessing structural changes in tissues due to injury, disease, or treatment. This provides direct evidence of tissue damage, complementing the biochemical findings (Oshotse *et al*., 2021).

The plant extracts in the doses deployed in this study maintained a normal histological architecture, including well-defined endometrial layers, glands, and stroma, suggesting the extract is non-toxic to the female reproductive tract at the tested doses. This finding is consistent with the study reported by Johnson *et al*., (2025).

There were some added beneficial changes of bronchiolar dilation in the rats treated with all the different doses of the plant extract. The 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. However, sections of the lungs taken from rats treated with doses of *S. mombin* show bronchiolar ulceration. This appears to be the sole toxic effect observed causing pulmonary destruction.

In the *V. amygdalina* groups, the liver architecture was normal at 100 mg/kg, aligning with the stable serum liver function test. However, the 200 mg/kg dose induced mild inflammation localized to the portal region, characterized by periportal infiltrates of inflammatory cells (portal hepatitis). This finding is consistent with the study reported by Cecilia, *et al*., (2023) that the oral administration of *V. amygdalina* on albino rats 3mls and 4ml respectively showed marked alteration in the microscopic architectural changes in the liver. This hepatotoxicity occurred probably because the therapeutic range bitter leaf tonic was exceeded. Sections taken from rats

treated with 200mg dose of *S. mombin*, show additional beneficial haemodynamic and vasoactive effects of increased blood circulation and vasodilatation. They also boosted the local immune system of the liver, by activating the sinusoidal Kupffer cells.

The ethanol leaf extract of *V. amygdalina* protected the heart and the blood vessels from getting lesions. This agrees with study done by EHI-Omosun *et al*., (2023). Therefore, this study revealed a clear protective effect of *V. amygdalina*. The heart histology was compromised, showing coronary artery ulceration in both doses of *S. mombin*. These are grave findings, indicating severe damage to the delicate vascular endothelium lining the coronary arteries.

Sections treated with the doses of *V. amygdalina* and *S. mombin* showed varying degrees of activation (boosting) of the lymphoid follicles suggesting that the plant extracts boosted the activity of the local immune system of the spleen as also seen in the lungs where mononuclear phagocyte system are being mobilized. Moreover, at 100mg of *V. amygdalina* and graded doses of *S. mombin* show increase in the sequestration of red blood cells in the splenic red pulp suggesting enhancement in the physiological or immunological functions of the spleen. This agrees with study done by Oku *et al*., (2024) here was a significant increase in the number of white blood cells which help to strengthen the body's immune response.

Therefore, the sub-acute toxicological evaluation revealed two distinct and highly polarized safety profiles. *V. amygdalina* exhibited favorable systemic tolerance and clear evidence of immunomodulatory activity, confirming its suitability for internal use within the tested dose range. Conversely, *S. mombin*, despite demonstrating systemic protective effects in the liver and kidney, carries severe, dose-independent risks of tissue destruction in the lungs and heart.

4.2 Conclusion

The phytochemical analysis confirms that both plants possess a rich and diverse array of bioactive compounds that substantiate their traditional medicinal uses. The presence of alkaloids, flavonoids, phenolic compounds, saponins, steroids, triterpenes, and cardiac glycosides in both extracts provides a chemical basis for their documented pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic activities. Particularly noteworthy is the absence of cyanogenic glycosides in both plants, which eliminates a significant potential toxicity concern associated with some medicinal plants.

The study confirms that the ethanol leaf extract of *V. amygdalina* is systemically safe for internal administration up to 200 mg/kg in female Wistar rats. The stability of hepatic, renal, and hematological markers, coupled with histological evidence of active splenic follicular activation, supports its role as a safe and effective immunomodulator within its traditional therapeutic dose range. However, the findings for *Spondias mombin* are a critical health concern. Despite displaying measurable systemic benefits in biochemical markers (reduced AST/ALT and urea), the extract is unequivocally demonstrated to be unsafe in its crude form for internal use. The discovery of coronary artery ulceration and bronchiolar ulceration represents a severe and previously unrecognized toxicological hazard, necessitating urgent intervention.

This finding emphasizes that regulatory bodies and researchers must integrate detailed histopathology alongside standard biochemical evaluations to fully establish the safety margins of traditional plant medicines before their continued integration into modern therapeutics.

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