

**HEMATINIC PROPERTY OF THE POLYHERBAL AQUEOUS LEAF  
EXTRACT (*Ipomoea batatas*, *Justicia carnea*, and *Ficus sur*) IN  
PHENYLHYDRAZINE HYDROCHLORIDE INDUCED ANAEMIA IN  
WISTAR RATS**

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**NOVEMBER 2025**

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE  
LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY  
OF BENIN, BENIN CITY, NIGERIA IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN  
SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES)**

**NOVEMBER 2025**

## CERTIFICATION

This is to certify that this work titled “**Hematinic property of the polyherbal aqueous leaf extract (*Ipomoea batatas*, *Justicia carnea*, and *Ficus sur*) in phenylhydrazine hydrochloride induced anaemia in wistar rats**” was carried out by Oghenetega Christiana EMOGHENE (Miss) with the Matriculation Number LSC2009959, of the Department Science Laboratory Technology (Microbiology Techniques), Faculty of Life Sciences, University of Benin, Benin City, under the supervision of Prof. E. O. Oshomoh.

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## **DEDICATION**

This work is dedicated to God Almighty, the giver and sustainer of life, for His faithfulness, mercy, grace granted to me throughout the period of my undergraduate Programme.

## **ACKNOWLEDGEMENTS**

I would love to appreciate Almighty God, the giver and sustainer of life, for strength, love, greater grace and mercy throughout the period of my undergraduate programme. My heartfelt gratitude to my project supervisor and co-supervisor, Prof. E. O. Oshomoh and Dr. B. O. Gabriel for his guidance, moral support and correction during the cause of this project work.

I will also like to acknowledge my wonderful mum, MRS. SUCCESS OMOVERERE EMOGHENE and siblings (Elohour, Great and Favor) for their immeasurable support and also Dr. Nicholas Ebeigbe for his unwavering support.

I also appreciate my friends, Oluwabukola Adeseye, Mrs Peace Chioke, Gift Joseph, Elizabeth, Omiachi Jemima, Efechuku Efemena, Victor Igwe, Tega Joy, Emeruo Joy, Ahikojoe God's gift, Onyemечи Favor, THE ELECTS, neighbors (Joyce, Esosa, Anita, Blessing), my SUCF family, my LFCE family and others for their support towards the success of this work.

I want to also acknowledge the Head of Department and all the lecturers in the department of Science laboratory technology for the knowledge impacted in me during my duration of study.

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## ABSTRACT

Anaemia remains a significant global health challenge, with conventional iron supplementation frequently associated with gastrointestinal side effects and poor patient compliance. This has necessitated the exploration of herbal alternatives with improved safety profiles and better tolerability. This study evaluated the hematinic potential of a polyherbal aqueous extract combining leaves of *Justicia carnea*, *Ipomoea batatas*, and *Ficus sur* using an experimental Wistar rat model of hemolytic anaemia. Thirty-six Wistar rats were subjected to phenylhydrazine hydrochloride-induced hemolytic anaemia (40 mg/kg for seven consecutive days). Following confirmation of anaemia through significant reductions in red blood cell count, hemoglobin concentration, and packed cell volume on Day 1, the animals were randomly assigned to six groups: three treatment groups receiving the polyherbal extract at graded doses of 25, 50, and 100 mg/kg; a positive control group administered 5 mg/kg folic acid; a negative control receiving no treatment; and a normal control group without induction or treatment. Treatment interventions continued on daily basis for 14 days. Post-treatment assessment demonstrated dose-dependent hematological recovery across all measured parameters. The highest dose (100 mg/kg) exhibited remarkable efficacy, producing a 60.15% increase in Red Blood Cell (RBC) count, 38.50% elevation in hemoglobin levels, and 55.66% improvement in Pack Cell Volume (PCV), with the performance comparable to the standard folic acid control. Statistical analysis revealed significant inter-group differences in RBC count at Day 7 ( $p = 0.005$ ) and PCV at Day 14 ( $p = 0.05$ ). 100 mg/kg dose of the polyherbal extract was the most effective, producing hematinic results comparable to the standard folic acid treatment by Day 14. The 50 mg/kg dose is also a strongly effective concentration, demonstrating significant recovery and representing a viable therapeutic option. These findings establish that the polyherbal aqueous leaf extract possesses substantial hematinic properties, effectively reversing experimentally-induced anaemia in a dose-dependent fashion. The therapeutic efficacy is attributed to the synergistic effects of bioactive compounds including iron, vitamin C, and antioxidant phytochemicals that stimulate erythropoiesis and protect red blood cells from oxidative damage. This research validates the traditional medicinal use of these plants and highlights their potential as safe, effective natural alternatives for anaemia management.

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF STUDY

Anaemia stands as one of the most pressing health challenges worldwide, touching the lives of billions of people regardless of whether they live in resource-rich or resource-limited settings. According to the World Health Organization, anaemia develops when the body lacks adequate red blood cells or when these cells cannot effectively transport oxygen to meet the body's metabolic needs, criteria that differ based on factors like age, gender, and elevation above sea level (Gabriel *et al.*, 2021). Beyond being a medical diagnosis, this condition substantially diminishes people's well-being and their ability to contribute economically to society. At its core, anaemia involves a compromised ability of blood to deliver oxygen throughout the body, typically arising from insufficient red blood cells or hemoglobin molecules (Cotoraci *et al.*, 2021). The underlying causes are remarkably diverse: inadequate intake of essential nutrients, inherited blood disorders such as thalassemia and sickle cell disease, long-standing illnesses, or accelerated breakdown of red blood cells as observed in hemolytic anaemia (Cotoraci *et al.*, 2021). Standard medical practice typically addresses anaemia through hematinic medications, particularly iron-based supplements like ferrous sulphate. Unfortunately, these treatments often bring uncomfortable side effects such as upset stomach, constipation, and nausea that discourage patients from maintaining their treatment regimen and ultimately compromise therapeutic success (Gabriel *et al.*, 2021). These challenges have motivated researchers to investigate alternative treatment approaches.

The growing interest in plant-based medicines rests on solid foundations: centuries of traditional use, easy availability in many communities, and a generally favorable safety record. Roughly 80% of people worldwide depend on traditional plant remedies as their

primary source of healthcare (Beppe *et al.*, 2023). Many plants have demonstrated encouraging potential in addressing blood-related disorders. This research investigates three particular plants, each with a rich history of traditional medicinal use: *Justicia carnea*, popularly called the "Blood of Jesus plant," has long been employed in West African traditional healing systems to treat anaemia. Recent scientific investigations have started to confirm these traditional practices, revealing that extracts from its leaves can meaningfully enhance blood parameters in laboratory animals with induced anaemia (Onyeabo *et al.*, 2017; Cotoraci *et al.*, 2021). While *Ipomoea batatas*, or sweet potato, is primarily recognized as a nutritious food source, its leaves harbor substantial amounts of iron and vitamins A and C, along with powerful antioxidant capabilities stemming from abundant flavonoids and phenolic compounds (Gabriel *et al.*, 2021). Studies have shown that n-hexane extracts from its leaves can counteract phenylhydrazine-induced anaemia and stimulate bone marrow function in rats. *Ficus sur*, known as the Cape Fig, occupies an important place in traditional medicine for treating various conditions. Scientific evidence has shown that methanol-based extracts from its stem bark and fruit can promote blood cell formation in rats with experimentally induced hemolytic anaemia, pointing to its nutritional and therapeutic value (Adebayo *et al.*, 2017; Cotoraci *et al.*, 2021).

What makes this investigation particularly interesting is the polyherbal formulation approach. While each plant shows individual promise, combining them may create synergistic interactions where the therapeutic benefit of the mixture exceeds what each plant could achieve alone. This strategy could potentially boost treatment effectiveness while allowing for lower doses of each component.

## **1. 2 AIM AND OBJECTIVES OF THE STUDY**

### **Aims**

To evaluate the hematinic (blood-boosting) property of polyherbal aqueous leaf extract comprising of *Justicia carnea*, *Ipomoea batata*, and *Ficus sur* in phenylhydrazine hydrochloride induced anaemia in Wistar rats.

### **Objectives**

The objectives of this study are to:

1. assess the hematinic and erythropoietic effects of the polyherbal aqueous leaf extract on RBC count, hemoglobin concentration, and packed cell volume in phenylhydrazine-induced anemic rats over a 14-day treatment period.
2. determine the dose-dependent hematinic efficacy of the polyherbal aqueous leaf extract (25, 50, and 100 mg/kg) by comparing its effects with the positive control (folic acid) and negative control groups.

## CHAPTER TWO

### LITERATURE REVIEW

#### **2. 1 *Ipomoea batatas* (L.)**

Widely recognized as sweet potato, this root crop holds considerable agricultural significance on a global scale, positioned as the world's seventh most important food source (Wahyuni *et al.*, 2022). What makes this crop particularly valuable isn't just its impressive nutritional profile, but also its remarkable adaptability to various tropical and subtropical growing conditions (Antonio *et al.*, 2021). While sweet potatoes have long been a staple food in many traditional diets, contemporary research has uncovered exciting new possibilities for their use in both nutraceutical and pharmaceutical applications. The entire plant proves useful, from storage roots to foliage and stems, each component offering economic value and potential health benefits that have captured the attention of researchers investigating disease prevention and health promotion (Nawaz *et al.*, 2022).

#### **2. 1. 2 Botanical Description**

Sweet potato is technically a perennial species, though farmers typically grow it as an annual crop specifically for harvesting the enlarged storage roots (Lebot, 2020). The plant develops long, slender vines that creep along the ground, producing roots at stem joints wherever they touch soil. Leaf shape varies considerably throughout the plant, generally resembling hearts but frequently showing deeply divided lobes in a hand-like pattern (International Potato Center, 2023). Although the plant can produce attractive funnel-shaped flowers typically white or soft pink with purple centers, many commercial varieties bloom unpredictably or not at all, and seed production is uncommon. Farmers therefore propagate sweet potatoes vegetatively, using either stem segments or slips (young shoots emerging from storage roots),

which guarantees offspring will match the parent variety exactly (Lebot, 2020). The storage roots themselves represent the plant's most economically important feature, displaying remarkable diversity in appearance. Both skin and internal flesh can range from pale white and yellow through brilliant orange and red to deep purple hues. These color differences reflect varying concentrations of specific phytochemicals, beta-carotenes produce the orange flesh while anthocyanins create purple pigmentation, and these same compounds contribute significantly to the crop's nutritional benefits (Truong *et al.*, 2018).



**Plate 2.1:** Leaves of *Ipomoea batatas*

**Source:** (Vecteezy,2023)

### **2. 1. 3 Taxonomy**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Convolvulaceae

Genus: Ipomoea

Species: *Ipomoea batatas* (L.)

### **2. 1. 4 Nutritional Content**

Sweet potato offers an impressive array of nutrients that differ between the storage roots and leafy portions. The foliage serves as an excellent iron source, supporting hemoglobin production and oxygen distribution throughout the body (Nawaz *et al.*, 2022). Both roots and leaves provide substantial amounts of ascorbic acid, a powerful antioxidant supporting immune defense and collagen formation, with leaves showing particularly high concentrations (Wahyuni *et al.*, 2022). The plant delivers abundant carbohydrates, dietary fiber, various vitamins (including provitamin A as beta-carotene in orange-fleshed types), and vital minerals like calcium, potassium, and magnesium (Antonio *et al.*, 2021).

Phytochemical analysis of sweet potato foliage reveals substantial quantities of biologically active compounds, including water-soluble vitamins, tannins, and saponins (Islam, 2019). Researchers assess standard physicochemical parameters to verify the quality and purity of leaf samples for scientific study or consumption. Critical measurements include moisture levels, total ash content, and acid-insoluble ash, which collectively confirm that plant material remains free from excessive inorganic impurities, soil particles, or siliceous materials (Mohd *et al.*, 2022).

### **2. 1. 5 Ethnomedicinal Benefits**

Sweet potato occupies an important position within traditional healing systems across diverse cultures, where practitioners utilize virtually every plant part: roots, leaves, and vines, to address numerous health concerns. The foliage particularly finds frequent medicinal use, typically prepared as either a boiled decoction or steeped infusion to reduce inflammation, lower fevers, and ease respiratory problems including asthma and bronchitis (Antonio *et al.*, 2021). Traditional applications extend beyond these conditions to encompass more serious ailments like tumors, stomach ulcers, chronic diarrhea, and diabetes (Nawaz *et al.*, 2022).

Modern phytochemical research increasingly validates the therapeutic value of sweet potato. The leaves contain abundant polyphenols, flavonoids, and caffeoylquinic acids and these are powerful antioxidants that combat oxidative stress and inflammation, scientifically explaining their traditional application for fevers and skin disorders (Wang *et al.*, 2016). For external treatment, crushed leaves are applied directly as poultices to calm skin irritation, facilitate burn recovery, and treat wounds, capitalizing on their antimicrobial and anti-inflammatory characteristics (Al-Amrousi *et al.*, 2023). The orange-fleshed storage roots serve not only as nutritious food but also help manage nausea and stimulate appetite, while purple-fleshed varieties, rich in anthocyanins, demonstrate antidiabetic and liver-protective properties

(Truong *et al.*, 2018). This alignment between traditional knowledge and scientific findings highlights sweet potato's dual role as both functional food and source of potentially valuable pharmaceutical compounds.

### **2. 1. 6 Pharmacological Activities**

Scientific research has substantiated many traditional applications of sweet potato through confirmation of its varied pharmacological properties, which encompass:

#### **Anti-cancer properties**

Sweet potato extracts, especially those containing anthocyanins from purple-fleshed varieties, have shown the ability to inhibit growth of multiple cancer cell types, including breast, colon, and stomach cancer cells (Antonio *et al.*, 2021; Wahyuni *et al.*, 2022).

#### **Antiulcer properties**

Both leaves and storage roots demonstrate stomach-protective qualities. Their antiulcer effect stems from their capacity to reinforce the stomach's protective mucosal layer and decrease acid production, thereby shielding the stomach lining from injury (Nawaz *et al.*, 2022).

#### **Antidiarrheal properties**

Extracts from foliage and roots have demonstrated effectiveness in reducing both excessive digestive tract movement and intestinal fluid secretion (Antonio *et al.*, 2021). This combined mechanism helps restore normal intestinal transit and prevent the fluid loss characteristic of diarrhea. The beneficial effect primarily results from high concentrations of tannins and flavonoids, which provide antispasmodic and astringent actions that calm the intestinal lining and suppress fluid and electrolyte secretion into the gut (Islam, 2019).

### **Antioxidant properties**

This represents one of the plant's most notable activities, driven predominantly by substantial concentrations of phenolics, flavonoids, and anthocyanins. These compounds neutralize damaging free radicals, diminishing oxidative stress associated with chronic diseases (Antonio *et al.*, 2021; Wahyuni *et al.*, 2022).

### **Anti-inflammatory and Antimicrobial properties**

The plant demonstrates considerable anti-inflammatory action by suppressing inflammatory signaling molecules. It also exhibits antimicrobial effectiveness against various bacterial and fungal species (Nawaz *et al.*, 2022).

### **Antidiabetic properties**

Leaf and root extracts have proven effective in lowering blood glucose levels and improving insulin sensitivity in experimental models. These effects appear mediated through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes and enhancement of glucose uptake by cells (Antonio *et al.*, 2021; Islam, 2019).

## **2. 1. 7 Phytoconstituents**

The medicinal properties of sweet potato directly correlate with its abundant and varied phytochemical composition. Key compounds include flavonoids such as quercetin and kaempferol, which function as potent antioxidants and contribute to the plant's anti-inflammatory, anticancer, and heart-protective effects (Wahyuni *et al.*, 2022). Phenolic acids, particularly caffeic and chlorogenic acid, occur in high concentrations in sweet potato and substantially contribute to its powerful free radical-neutralizing capacity (Antonio *et al.*, 2021). Saponins, which are glycoside compounds characterized by their membrane-active

properties, associate with antimicrobial, anti-inflammatory, and cholesterol-reducing activities (Nawaz *et al.*, 2022). Other important constituents include tannins, anthocyanins (especially abundant in purple varieties), carotenoids (like beta-carotene in orange varieties), and dietary fibers, all working synergistically to enhance the plant's health-promoting properties (Antonio *et al.*, 2021; Wahyuni *et al.*, 2022).

## **2. 2 *Justicia carnea* Lindl.**

Popularly known as Brazilian plume flower or Jacobinia, this perennial evergreen shrub originates from the Atlantic Forest regions of South America, with Brazil serving as its primary native habitat (Afolabi *et al.*, 2022). Gardeners throughout tropical and subtropical zones cultivate it extensively as an ornamental species, valuing its striking pink or reddish flower clusters. Beyond decorative purposes, *J. carnea* holds substantial importance in traditional medicine, particularly throughout Nigeria, where practitioners commonly employ its leaves as a blood tonic to address anaemia, weakness, and fatigue (Odugbemi *et al.*, 2015). This established ethnomedicinal application has generated scientific interest in verifying its pharmacological activities and identifying its phytochemical constituents.

### **2. 2. 1 Botanical Description**

*Justicia carnea* presents soft, woody stems and typically reaches heights of approximately 1.5 m. The plant displays simple leaves arranged in opposite pairs, characterized by their dark green coloration and lanceolate to broadly lanceolate shape, with pointed tips and smooth edges (Afolabi *et al.*, 2022). The soft-textured leaves can achieve lengths of 15 - 20 cm. Its most distinctive characteristic involves dense, terminal flower clusters, comprising large, conspicuous, spike-like arrangements of tubular flowers colored pink to reddish, each

measuring roughly 5 - 6 cm in length (Odugbemi *et al.*, 2015). The plant flourishes in partially shaded, humid conditions and reproduces readily through stem cuttings.



**Plate 2. 2:** Leaves and flowers of *Justicia carnea*

**Source:** (Auckland Botanic Gardens, 2019)

### **2. 2. 2 Taxonomy**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia*

Species: *Justicia carnea* Lindl.

### **2. 2. 3 Nutritional Content**

The foliage of *Justicia carnea* provides abundant essential nutrients, supporting its recognized health benefits. The leaves contain notably high iron levels, a vital mineral for hemoglobin formation and blood oxygen transport, which directly correlates with its traditional application against anaemia (Odugbemi *et al.*, 2015). Additionally, the leaves supply considerable quantities of ascorbic acid, which enhances the absorption and bioavailability of plant-derived non-heme iron (Afolabi *et al.*, 2022). This beneficial combination of elevated iron and vitamin C content offers scientific rationale for its effectiveness in treating iron-deficiency anaemia. Proximate analysis of its leaves reveals significant physicochemical characteristics. The plant material exhibits substantial moisture

content, along with measurable total ash, acid-insoluble ash, and water-soluble ash values which are standard parameters for evaluating herbal material quality and purity (Afolabi *et al.*, 2022). Extractive values obtained using various solvents (including water and ethanol) demonstrate the presence of polar and semi-polar phytochemical constituents, suggesting the potential yield of bioactive compounds during preparation.

#### **2. 2. 4 Ethnomedicinal Benefits**

Within traditional medical systems of Southwestern Nigeria, *J. carnea* leaf represents a prominent and well-recognized herbal remedy. Practitioners most commonly prepare it as a blood tonic, consuming aqueous extracts or infusions to combat anaemia, fatigue, and general weakness (Odugbemi *et al.*, 2015). However, the plant's therapeutic uses extend considerably beyond this primary application. Traditional healers also utilize it for managing diverse health conditions, including hemorrhoids, fever, pain, and diabetes (Afolabi *et al.*, 2022). Furthermore, practitioners apply the leaves topically to wounds for accelerating healing. Scientific investigation increasingly examines the pharmacological foundation for these varied applications; research has confirmed its wound-healing and anti-inflammatory activities, attributed to bioactive flavonoids and tannins present in the plant (Adebayo *et al.*, 2019). This convergence of established traditional knowledge with emerging scientific evidence emphasizes the considerable medicinal value of *J. carnea* within West African therapeutic traditions (Elufioye *et al.*, 2017).

#### **2. 2. 5 Pharmacological Activities**

Scientific studies have confirmed several traditional applications of *J. carnea* through investigation of its pharmacological properties, including:

### **Anti-anemic Activity**

The substantial iron and vitamin C content directly supports its demonstrated effectiveness in elevating hemoglobin levels and red blood cell counts in animal studies, validating its anti-anemic properties (Odugbemi *et al.*, 2015).

### **Anticancer Activity**

Extracts of *J. carnea* have exhibited toxic effects against multiple cancer cell lines. This activity largely stems from bioactive compounds including flavonoids and phenolics, which can trigger programmed cell death and inhibit cancer cell multiplication (Afolabi *et al.*, 2022).

### **Antiulcer Activity**

The plant has demonstrated significant stomach-protective effects in experimental studies. This protective action likely results from its flavonoid content, which can strengthen the stomach's protective mucosal barrier and reduce acid production (Odugbemi *et al.*, 2015).

### **Antidiarrheal Activity**

Extracts have shown effectiveness in reducing digestive tract movement and secretion in animal studies, supporting its traditional application for managing diarrhea. Further research indicates that *J. carnea* possesses antioxidant, anti-inflammatory, and antimicrobial properties, which explain its use in treating wounds, fever, and infections (Afolabi *et al.*, 2022).

## **2. 2. 6 Phytoconstituents**

Phytochemical screening of *Justicia carnea* leaf extracts verifies the presence of numerous

bioactive compounds responsible for its pharmacological effects. The plant's therapeutic potential largely derives from its rich array of bioactive phenolic compounds, encompassing sub-classes including flavonoids, phenolic acids, and tannins (Afolabi *et al.*, 2022). These compounds closely associate with the plant's potent pharmacological activities, particularly its capacity to neutralize free radicals. By donating electrons to stabilize highly reactive oxygen and nitrogen species, these phenolics directly produce powerful antioxidant effects, representing a fundamental mechanism underlying the plant's documented anti-inflammatory, wound-healing, and general protective properties (Ezekwe *et al.*, 2023). Flavonoids significantly contribute to the plant's antioxidant, anti-inflammatory, antiulcer, and anticancer activities (Afolabi *et al.*, 2022). Saponins have been identified and correlate with various biological effects, including antimicrobial and anti-inflammatory properties (Odugbemi *et al.*, 2015).

### **2.3 *Ficus sur* Forssk.**

The genus *Ficus*, an important member of the Moraceae family, encompasses over 800 species of woody trees, shrubs, and vines predominantly distributed throughout tropical and subtropical ecosystems globally (Ojo *et al.*, 2022). A characteristic feature of this genus involves the production of milky latex when plant tissues are injured. *Ficus sur*, commonly called Cape fig or broom cluster fig, represents a notable species within this genus (Mbakam *et al.*, 2023).

#### **2.3.1 Botanical Description**

Botanically, *F. sur* develops as a large deciduous tree capable of reaching heights between 25 and 40 meters, with trunk diameters potentially exceeding one meter. The fruit represents a specialized, fleshy, hollow inflorescence termed a syconium, which contains numerous

minute flowers and develops into the edible structure commonly recognized as a fig (Ojo *et al.*, 2022).



**PLATE 2. 3:** Leaves of *Ficus sur*

**Source:** (iNaturalist, 2022)

### **2. 3. 2 Taxonomy**

Kingdom: Plantae

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *Ficus sur* Forssk.

Synonyms: *Ficus capensis* Thunb.

### **2. 3. 3 Nutritional Content**

Specific micronutrient data for *Ficus sur* fruits, particularly iron and vitamin C concentrations, represents a notable gap in current scientific literature (Ojo *et al.*, 2022). Nevertheless, research clearly establishes that fruits from edible Ficus species generally provide valuable macronutrients and essential minerals. They typically supply significant quantities of digestible carbohydrates for energy and serve as good sources of dietary fiber, supporting digestion and promoting intestinal health (Mwamatope *et al.*, 2020). While specific data for *F. sur* remains limited, proximate analyses of related species indicate these fruits also contribute beneficial minerals including potassium, calcium, and magnesium to the diet (Tolera & Tadese, 2021).

### **2. 3. 4 Ethnomedicinal Benefits**

The ethnomedicinal applications of *F. sur* are thoroughly documented across numerous African cultures, with virtually all plant parts including leaves, bark, roots, and sap is being employed for therapeutic purposes (Abdallah *et al.*, 2022). Boiled preparations from leaves and roots commonly treat respiratory infections, tuberculosis, and diarrhea, utilizing the plant's documented antimicrobial properties to fight bacterial and fungal pathogens (Nguele *et al.*, 2023). Within certain Nigerian and Sudanese communities, practitioners administer *F. sur* preparations to manage central nervous system disorders, including epilepsy and convulsions, suggesting potential neuropharmacological activity (Abdallah *et al.*, 2022). A significant traditional application involves its use as a powerful diuretic. Fresh leaf preparations are consumed to stimulate kidney function and enhance urine output, a practice specifically employed for managing kidney disorders and hypertension (Nguele *et al.*, 2023). The plant finds both topical and oral use for relieving pain, reducing fevers, and treating inflammatory conditions like arthritis and wounds, supported by scientific studies confirming its anti-inflammatory and pain-relieving effects (Mwamatope *et al.*, 2020). The latex and crushed leaves are applied directly to wounds, burns, and skin infections to disinfect affected areas, reduce inflammation, and facilitate healing (Tshikalange *et al.*, 2016).

### **2. 3. 5 Pharmacological Activities**

Research has shown that a methanol extract from the stem bark is a powerful antioxidant. It effectively neutralizes harmful free radicals like 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), an activity that is directly linked to its high concentration of phenolic compounds (Ojo *et al.*, 2022). The plant also

shows promise in treating specific diseases by targeting key enzymes. The methanolic stem bark extract can inhibit the activity of  $\alpha$ -amylase, suggesting it may help lower blood sugar levels, and acetylcholinesterase, indicating a potential use for neurodegenerative conditions like Alzheimer's (Abdallah *et al.*, 2022). Furthermore, *F. sur* exhibits anticancer, or antineoplastic, properties. Both infusions and methanolic extracts from the stem bark have been toxic to human cervical and colon cancer cells in lab studies, marking it as a potential source for new anticancer drugs (Mbakam *et al.*, 2023). Its traditional use for treating epilepsy is also backed by science, as an ethanol extract has been shown to have a significant anticonvulsant effect (Nguele *et al.*, 2023). Finally, the plant's use for stomach problems is well-supported. The tannins present have astringent properties that can help reduce diarrhea, while flavonoid-rich extracts have shown gastroprotective effects that could help prevent ulcers (Abdallah *et al.*, 2022).

### **2.3.6 Phytoconstituents**

The medicinal effects of *Ficus sur* come from its wide array of bioactive compounds, known as phytoconstituents. Analyses have revealed that the plant is rich in several important classes of these compounds. Leaf extracts, especially those made with methanol, contain high levels of phenolic acids like quinic and citric acid, along with various flavonoid glycosides (Ojo *et al.*, 2022). Other notable compounds found include methyl gallate and hydroxycoumarin. When leaves are prepared as an aqueous infusion (like a tea), they contain compounds such as hydroxycaffeoylquinic acid, glucogallic acid, and ferulic acid derivatives (Abdallah *et al.*, 2022). The stem bark is a major source of tannins, catechins, and procyanidins in methanolic extracts. Its water-based infusion contains flavonoids like apigenin, luteolin, and kaempferol, along with their glycosides (Mbakam *et al.*, 2023). A detailed analytical study using LC-ESI-QTOF-MS/MS confirmed the presence of over 118 distinct compounds in the plant, with a

generally greater diversity of compounds found in the leaves compared to the stem bark (Ojo *et al.*, 2022).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3. 1 Plant Collection and Identification

On July 8th, 2025, fresh leaf specimens of *Ipomoea batatas*, *Justicia carnea*, and *Ficus sur* were harvested from locations within Benin City, Edo State, Nigeria. To verify the botanical identity of these specimens, authentication was carried out by Prof. Akinnibosun Henry Adewale from the Department of Plant Biology and Biotechnology at the University of Benin. Following standard herbarium practice, voucher specimens bearing the reference codes UBH-I493 (*Ipomoea batatas*), UBH-J386 (*Justicia carnea*), and UBH-F331 (*Ficus sur*) were archived in the University's herbarium to serve as permanent reference materials (Akinnibosun, 2025).

#### 3. 2 Plant Preparation and Polyherbal Extraction

The harvested leaves underwent thorough washing with water to eliminate dirt and surface contaminants, after which they were air-dried at ambient temperature in a shaded environment over a two to three-week period. This drying approach was adopted to maintain the integrity of thermolabile bioactive constituents. Once fully desiccated, each plant material was independently ground into fine powder using an industrial-grade mechanical blender. A polyherbal formulation was prepared by mixing 93 grams of each powdered specimen, yielding a combined mass of 279 grams in equal proportions (1:1:1 ratio). The extraction procedure utilized cold maceration; a technique selected to optimize the recovery of

hydrophilic phytochemicals while avoiding heat-induced degradation. The powdered composite was placed in a glass extraction vessel and soaked in 1900 ml of cold distilled water, which was introduced gradually in successive aliquots of 1000, 500, and 400 ml to achieve uniform distribution. The sealed container was maintained at room temperature for 72 hours, during which the mixture was agitated at four-hour intervals to facilitate efficient solute transfer. Subsequently, the macerate underwent dual filtration; initially through a mesh strainer and then by vacuum filtration to yield a clarified liquid extract. This filtrate was then evaporated to a semi-solid consistency using a water bath. From this concentrated extract, a working stock solution was formulated and its concentration was determined to enable precise dosing calculations.

### **3.3 Experimental Animals and Anaemia Induction**

Thirty-six adult Wistar rats comprising both males and females were utilized in this investigation. The inclusion of both sexes was intentional, aimed at eliminating sex-related bias while acknowledging that females exhibit greater vulnerability to hemolytic anaemia, potentially linked to physiological factors such as menstrual bleeding. The animals were randomly allocated into six experimental groups of six rats each ( $n = 6$ ). Hemolytic anaemia was experimentally induced in Groups 1, 2, 3, 4, and 6 through administration of freshly prepared Phenylhydrazine hydrochloride (PHZ-HCl) solution. To optimize drug absorption, the animals underwent a 24-hour fasting period before induction commenced. A daily dose of 40 mg/kg body weight of PHZ-HCl was delivered via orogastric gavage for seven consecutive days. Successful anaemia induction was verified by the appearance of classical anemic manifestations, including pallor of the eyes, ears, tail, and paws, respiratory distress evidenced by labored breathing and gasping, marked lethargy with extended periods of inactivity, and diminished food intake, which became apparent by the sixth day of induction.

### **3. 4 Experimental Design and Dosage Administration**

The experimental groups were organized according to the following scheme:

Group 1: Induced with anaemia + treated with 25 mg/kg of the polyherbal extract.

Group 2: Induced with anaemia + treated with 50 mg/kg of the polyherbal extract.

Group 3: Induced with anaemia + treated with 100 mg/kg of the polyherbal extract.

Group 4: Induced with anaemia + Positive Control, treated with 5 mg/kg of Folic Acid.

Group 5: Normal Control, neither induced with anaemia nor treated with any substance.

Group 6: Induced with anaemia + Negative Control, receiving no treatment.

Therapeutic interventions began immediately upon confirmation of anaemia and extended over a 14-day period. Daily dosing of the polyherbal extract for individual animals in the treatment groups was determined based on their current body weight using the equation:  $\text{Dose (mg/kg)} \times \text{Body Weight (g)} / 1000$ . This computation yielded the exact volume of stock solution to be administered orally once daily via orogastric intubation. The positive control group similarly received Folic acid at 5 mg/kg body weight. Animals in both the negative and normal control groups received no pharmacological treatment. At the study's conclusion, each rat was anesthetized in a glass desiccator chamber containing chloroform-soaked cotton wool until complete unconsciousness and abolition of reflexes were observed, after which they were humanely sacrificed. Biological samples were subsequently harvested for laboratory analysis.

### **3. 5 Statistical Analysis**

All hematological and biochemical data obtained were presented as Mean  $\pm$  Standard Error of the Mean (SEM). Statistical evaluation was performed using the Statistical Package for the Social Sciences (SPSS), version 16.0 software. One-way analysis of variance (ANOVA) was

employed to detect significant variations across experimental groups. In instances where ANOVA indicated significant differences, Duncan's Multiple Range Test was implemented as a post-hoc procedure to identify specific group differences. Results were deemed statistically significant when the probability value was equal to or less than 0.05 ( $p \leq 0.05$ ).

## CHAPTER FOUR

### RESULTS

This chapter presents the results of the study on the hematinic property of the polyherbal aqueous leaf extract of *Justicia carnea*, *Ipomoea batatas*, and *Ficus sur* in phenylhydrazine-induced anaemic Wistar rats. The hematological parameters evaluated include red blood cell (RBC) count, hemoglobin (HGB) concentration, and packed cell volume (HCT) at three different intervals: Day 1, Day 7, and Day 14 following the administration of the extract at varying doses (25, 50, and 100 mg/kg), a positive control (folic acid), a normal control, and a negative (untreated) control.

Data were expressed as mean  $\pm$  standard deviation (SD) for each group, and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc tests to determine the level of significance between groups at  $p < 0.05$ .

The effect of polyherbal aqueous leaf extract on red blood cell (RBC) count was shown in Table 4.1. At Day 1, the mean RBC count ranged from  $3.60 \pm 1.05 \times 10^6/\mu\text{L}$  in the negative control to  $7.22 \pm 0.57 \times 10^6/\mu\text{L}$  in the normal control. By Day 7, a noticeable increase in RBC count was recorded across all groups, with the extract-treated rats showing dose-related improvement. The ANOVA revealed a statistically significant difference among groups on Day 7 ( $p = 0.005$ ). On Day 14, RBC counts remained higher in the treated groups, with the

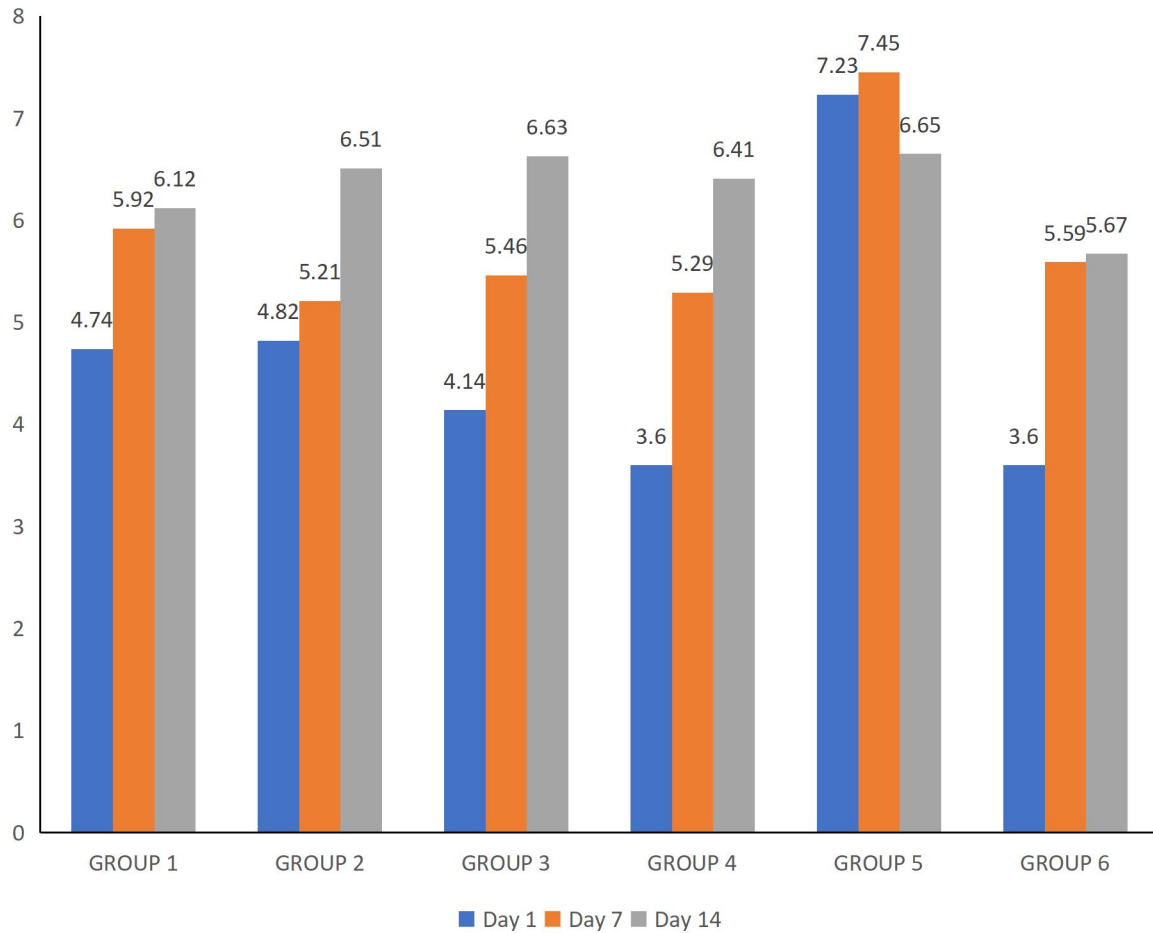
100 mg/kg extract and folic acid groups showing values approaching those of the normal control, although the difference was not statistically significant ( $p > 0.05$ ).

**Figure 4.1 showed the effect of polyherbal aqueous leaf extract on red blood cell (RBC) count in phenylhydrazine-induced anaemic wistar rats.** RBC levels increased progressively in all extract-treated groups (Groups 1–3) and in the positive control (Group 4) from Day 1 to Day 14. The **highest improvement (78.06%)** occurred in **Group 4 (Folic Acid-treated)**, followed by **Group 3 (100 mg/kg extract)** with **60.15%** and **Group 2 (50 mg/kg)** with **35.06%** recovery. The **Normal Control (Group 5)** showed a slight decline (–8.02%) over time, likely due to natural variation rather than treatment effect. The **Negative Control (Group 6)** showed moderate recovery (57.50%) from Day 1 to Day 14, suggesting partial physiological compensation. The polyherbal extract exhibited **dose-dependent erythropoietic activity**, with the 100 mg/kg dose showing the closest RBC recovery to the standard folic acid group. This indicates that the extract promotes red blood cell regeneration, possibly through antioxidant phytochemicals that protect erythrocyte membranes and enhance hematopoiesis.

**Table 4.1:** Effect of Polyherbal Aqueous Leaf Extract on Red Blood Cell (RBC) Count ( $\times 10^6/\mu\text{L}$ ) in Phenylhydrazine-Induced Anaemic Wistar Rats

Groups	Dose (mg/kg)	Day 1 (Mean $\pm$ SD)	Day 7 (Mean $\pm$ SD)	Day 14 (Mean $\pm$ SD)	<i>p</i> -value
Normal Control	—	7.22 $\pm$ 0.57 <sup>a</sup>	7.45 $\pm$ 0.58 <sup>a</sup>	6.65 $\pm$ 0.21 <sup>a</sup>	—
Negative Control (PHZ only)	—	3.60 $\pm$ 1.05 <sup>b</sup>	5.59 $\pm$ 0.08 <sup>b</sup>	5.67 $\pm$ 0.30 <sup>b</sup>	—
Positive Control (Folic Acid)	5	3.60 $\pm$ 1.94 <sup>b</sup>	5.29 $\pm$ 0.08 <sup>b</sup>	6.41 $\pm$ 0.27 <sup>ab</sup>	—
Extract (25 mg/kg)	25	4.74 $\pm$ 1.62 <sup>b</sup>	5.92 $\pm$ 0.06 <sup>b</sup>	6.11 $\pm$ 0.63 <sup>ab</sup>	—
Extract (50 mg/kg)	50	4.72 $\pm$ 0.87 <sup>b</sup>	5.21 $\pm$ 0.57 <sup>b</sup>	6.51 $\pm$ 1.02 <sup>a</sup>	—
Extract (100 mg/kg)	100	4.14 $\pm$ 1.04 <sup>b</sup>	5.46 $\pm$ 0.23 <sup>b</sup>	6.63 $\pm$ 0.17 <sup>a</sup>	—
<b><i>p</i>-value (ANOVA)</b>		0.175	<b>0.005</b>	0.475	

Values are expressed as mean  $\pm$  SD (n = 2). Means in the same column with different superscript letters (a–b) are significantly different at  $p < 0.05$ .



**Figure 4.1: Effect of Polyherbal Extract on RBC Count Over 14 Days**

**KEY:** Group 1: 25 mg/kg of the polyherbal extract.

Group 2: 50 mg/kg of the polyherbal extract.

Group 3: 100 mg/kg of the polyherbal extract.

Group 4: 5 mg/kg of Folic Acid.

Group 5: Not induced

Group 6: phenylhydrazine-induced only, Untreated

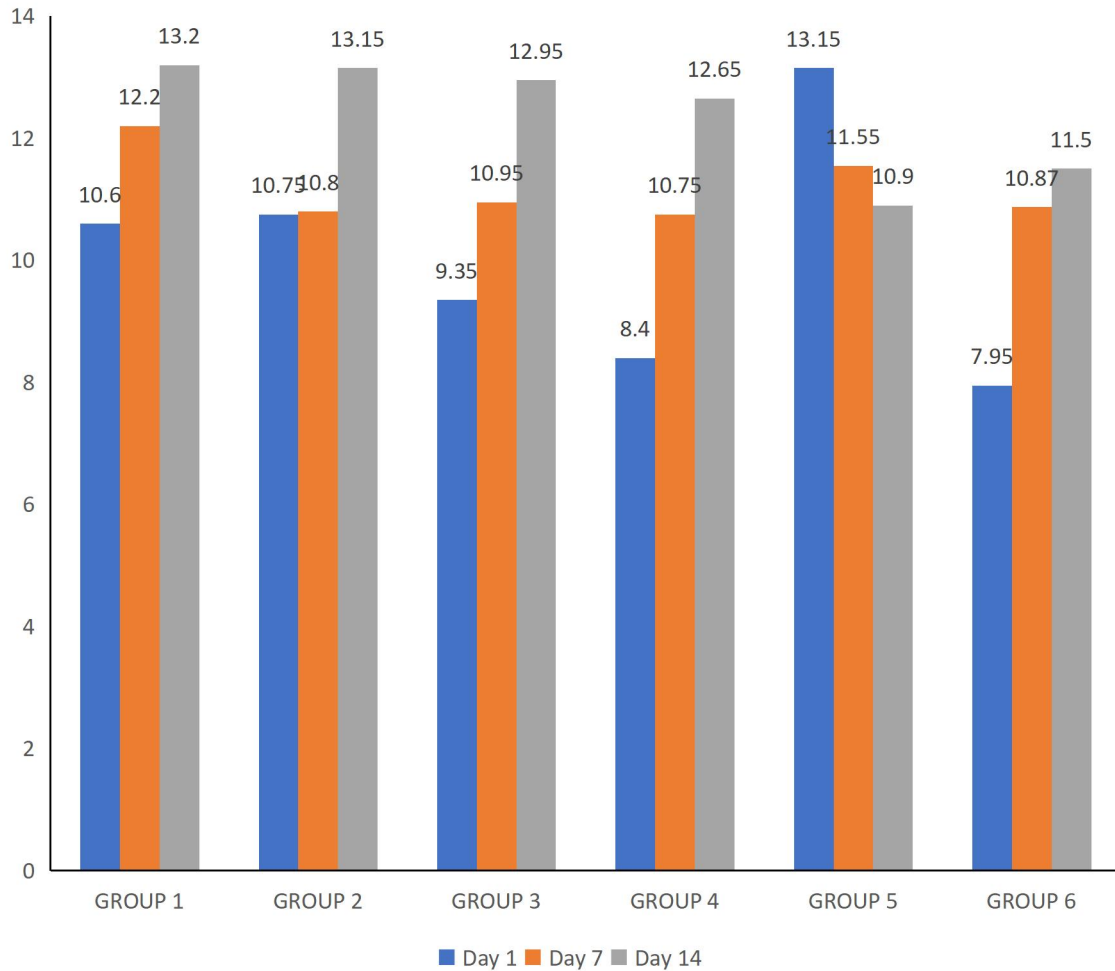
The effect of polyherbal aqueous leaf extract on hemoglobin (HGB) concentration was shown in table 4.2. Hemoglobin concentrations followed a general increasing trend across all groups throughout the treatment period. On Day 1, the anaemic control and positive control groups exhibited lower hemoglobin levels compared to the normal control. A steady rise was observed from Day 7 to Day 14 in extract-treated rats, with the 25 and 50 mg/kg doses recording the highest mean values on Day 14. No statistically significant difference was observed among groups across all days ( $p > 0.05$ ).

**Figure 4.2 showed the effect of polyherbal aqueous leaf extract on hemoglobin (hb) concentration (g/dl) in phenylhydrazine-induced anaemic wistar rats.** Hemoglobin concentration increased steadily across treatment groups (Groups 1–4) between Day 1 and Day 14. The **highest increase (50.60%)** was observed in the **Positive Control (Group 4)**, followed closely by **Group 6 (Negative Control)** with **44.65%**, **Group 3 (100 mg/kg)** with **38.50%**, and **Group 1 (25 mg/kg)** with **24.53%**. The **Normal Control (Group 5)** displayed a decline ( $-17.11%$ ), consistent with the absence of induced anaemia and treatment. All extract-treated groups showed marked Hb recovery, indicating restoration of oxygen-carrying capacity. The observed hemoglobin recovery suggests that the polyherbal extract enhances **erythropoiesis and hemoglobin synthesis**, comparable to folic acid. The improvement in Hb levels across doses demonstrates the extract's potential as a **natural hematinic agent**.

**Table 4.2:** Effect of Polyherbal Aqueous Leaf Extract on Hemoglobin (HGB) Concentration (g/dL) in Phenylhydrazine-Induced Anaemic Wistar Rats

Groups	Dose (mg/kg)	Day 1 (Mean $\pm$ SD)	Day 7 (Mean $\pm$ SD)	Day 14 (Mean $\pm$ SD)	<i>p</i> -value
Normal Control	—	13.15 $\pm$ 0.92 <sup>a</sup>	11.55 $\pm$ 0.49 <sup>a</sup>	10.90 $\pm$ 0.57 <sup>a</sup>	—
Negative Control (PHZ only)	—	7.95 $\pm$ 2.62 <sup>b</sup>	10.87 $\pm$ 0.38 <sup>ab</sup>	11.50 $\pm$ 0.57 <sup>a</sup>	—
Positive Control (Folic Acid)	5	8.40 $\pm$ 4.10 <sup>b</sup>	10.75 $\pm$ 0.64 <sup>ab</sup>	12.65 $\pm$ 1.06 <sup>a</sup>	—
Extract (25 mg/kg)	25	10.60 $\pm$ 2.83 <sup>b</sup>	12.20 $\pm$ 0.14 <sup>a</sup>	13.20 $\pm$ 0.71 <sup>a</sup>	—
Extract (50 mg/kg)	50	10.75 $\pm$ 2.05 <sup>b</sup>	10.80 $\pm$ 1.56 <sup>ab</sup>	13.15 $\pm$ 1.34 <sup>a</sup>	—
Extract (100 mg/kg)	100	9.35 $\pm$ 2.19 <sup>b</sup>	10.95 $\pm$ 0.07 <sup>ab</sup>	12.95 $\pm$ 1.63 <sup>a</sup>	—
<b><i>p</i>-value (ANOVA)</b>		0.471	0.400	0.278	

Values are expressed as mean  $\pm$  SD (n = 2). Means with different superscripts differ significantly at  $p < 0.05$ .



**Figure 4.2: Effect of Polyherbal Extract on Hemoglobin Concentration Over 14 Days**

**KEY:** Group 1: 25 mg/kg of the polyherbal extract.

Group 2: 50 mg/kg of the polyherbal extract.

Group 3: 100 mg/kg of the polyherbal extract.

Group 4: 5 mg/kg of Folic Acid.

Group 5: Not induced

Group 6: phenylhydrazine-induced only, Untreated

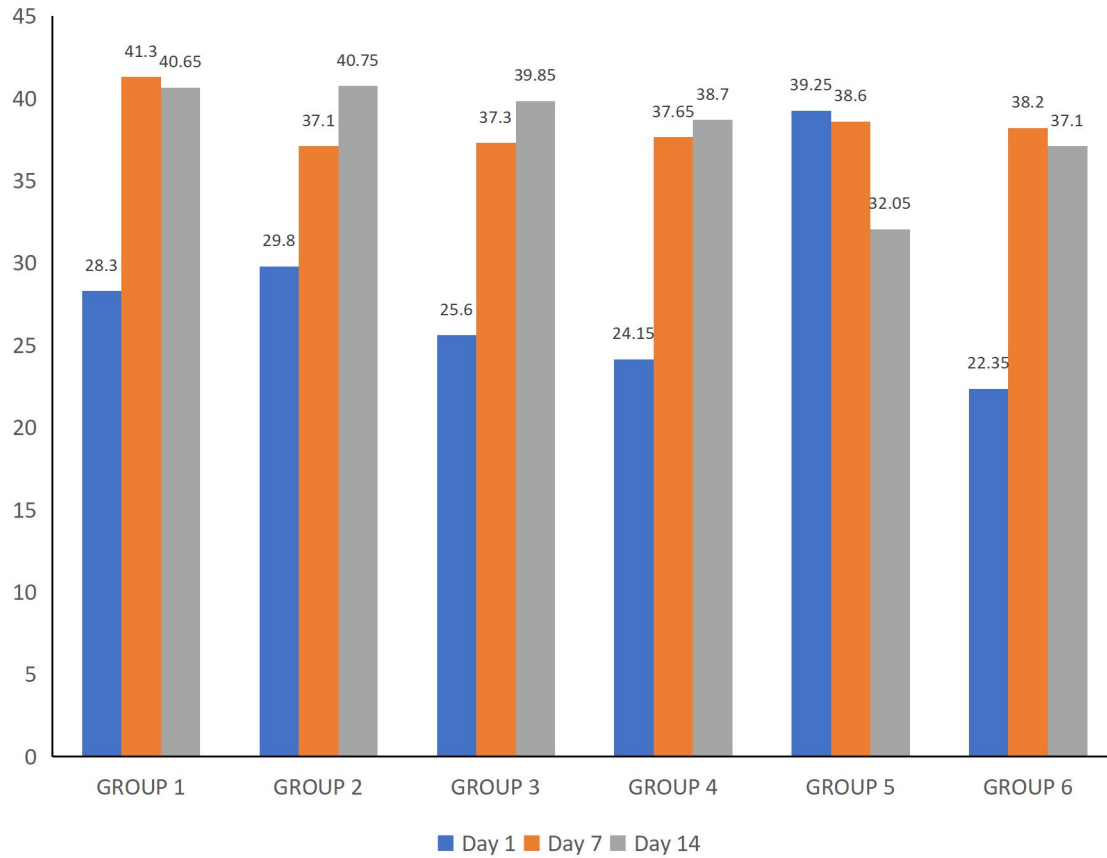
Table 4.3 showed the effect of polyherbal aqueous leaf extract on packed cell volume (HCT, %) in phenylhydrazine-induced anemic wistar rats. At Day 1, packed cell volume was notably reduced in the phenylhydrazine-treated rats compared to the normal control group. A progressive rise in HCT values was recorded from Day 7 to Day 14 across all treated groups. Statistical analysis revealed no significant differences among groups on Days 1 and 7 ( $p > 0.05$ ); however, a significant difference was observed on Day 14 ( $p = 0.05$ ), indicating improvement in erythrocyte mass after 14 days of treatment.

Figure 4.3 showed the effect of polyherbal aqueous leaf extract on packed cell volume (PCV) in phenylhydrazine-induced anemic wistar rats. PCV increased across treatment and control groups from Day 1 to Day 14, with varying degrees of recovery. The **Negative Control (Group 6)** recorded the highest increase (**65.99%**), followed by **Positive Control (Group 4)** with **60.25%** and **High-Dose Extract (Group 3)** with **55.66%**. The **25** and **50 mg/kg** extract groups improved by **43.63%** and **36.75%**, respectively. The **Normal Control (Group 5)** showed a decrease (**-18.34%**), reflecting natural fluctuation rather than pathological change. The rise in PCV among extract-treated groups indicates **effective restoration of red cell mass**, correlating with increases in RBC and Hb. The comparable recovery in the 100 mg/kg extract and folic acid groups reinforces the **dose-dependent hematinic effect** of the polyherbal formulation.

Across all hematological indices (RBC, HGB, and HCT), the polyherbal aqueous extract exhibited a time-dependent increase in values from Day 1 to Day 14, particularly at higher doses (50 and 100 mg/kg). ANOVA indicated significant differences among groups primarily on **Day 7 for RBC count** ( $p = 0.005$ ) and **Day 14 for HCT** ( $p = 0.05$ ), suggesting measurable variation in the response patterns among treatment groups.

**Table 4.3.** Effect of Polyherbal Aqueous Leaf Extract on Packed Cell Volume (HCT, %) in Phenylhydrazine-Induced Anaemic Wistar Rats

<b>Groups</b>	<b>Dose (mg/kg)</b>	<b>Day 1 (Mean ± SD)</b>	<b>Day 7 (Mean ± SD)</b>	<b>Day 14 (Mean ± SD)</b>	<b><i>p</i>-value</b>
Normal Control	—	39.25 ± 1.20 <sup>a</sup>	38.60 ± 3.82 <sup>a</sup>	32.05 ± 0.78 <sup>b</sup>	—
Negative Control (PHZ only)	—	22.35 ± 6.58 <sup>b</sup>	38.20 ± 3.25 <sup>a</sup>	37.10 ± 1.70 <sup>ab</sup>	—
Positive Control (Folic Acid)	5	24.15 ± 9.40 <sup>b</sup>	37.65 ± 0.49 <sup>a</sup>	38.70 ± 1.41 <sup>ab</sup>	—
Extract (25 mg/kg)	25	28.30 ± 6.65 <sup>b</sup>	41.30 ± 0.85 <sup>a</sup>	40.65 ± 0.49 <sup>a</sup>	—
Extract (50 mg/kg)	50	29.80 ± 4.38 <sup>b</sup>	37.10 ± 1.56 <sup>a</sup>	40.75 ± 3.75 <sup>a</sup>	—
Extract (100 mg/kg)	100	25.60 ± 6.36 <sup>b</sup>	37.30 ± 1.84 <sup>a</sup>	39.85 ± 3.18 <sup>a</sup>	—
<b><i>p</i>-value (ANOVA)</b>		0.239	0.537	<b>0.050</b>	



**Figure 4.3: Effect of Polyherbal Extract on Packed Cell Volume Over 14 Days**

**KEY:** Group 1: 25 mg/kg of the polyherbal extract.

Group 2: 50 mg/kg of the polyherbal extract.

Group 3: 100 mg/kg of the polyherbal extract.

Group 4: 5 mg/kg of Folic Acid.

Group 5: Not induced

Group 6: phenylhydrazine-induced only, Untreated

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

Phenylhydrazine (PHZ) administration resulted in pronounced hematological alterations at baseline (Day 1). The normal control group showed significantly higher red blood cell (RBC), hemoglobin (Hb), and hematocrit (HCT) values compared to the anemic control group. Specifically, RBC decreased by approximately 50.21%, Hb by 39.54%, and HCT by 43.06% following PHZ induction. These marked reductions confirm that PHZ effectively induced hemolytic anaemia in Wistar rats.

This finding is consistent with the well-documented mechanism of PHZ toxicity, where the compound induces oxidative damage to erythrocyte membranes, leading to red cell lysis and shortened erythrocyte lifespan (Onyeabo *et al.*, 2017; Ibe *et al.*, 2022). Similar decreases in hematological parameters following PHZ exposure have been reported in related studies, validating the reliability of this model for evaluating hematinic agents (Lalhriatpuii, Chhetri, Sohaila and Rachana, 2023; Okolo, 2021).

The observed decreases in RBC, Hb, and HCT reflect a state of oxidative hemolysis, which provides a suitable baseline for evaluating the erythropoietic potential of the polyherbal aqueous extract. This aligns with findings by Igwe, Ikpeazu, and Otuokere (2020), who also observed significant declines in erythrocyte indices in PHZ-induced rats prior to treatment. Therefore, the established anaemia model in this study effectively sets the foundation for assessing the hematinic efficacy of the plant extract combination. After treatment, RBC count increased progressively across all extract-treated groups and in the standard hematinic (folic acid) group. The highest recovery was recorded in the folic acid-treated group (78.06%), followed by the 100 mg/kg extract group (60.15%) and the 50 mg/kg extract group (35.06%). The 25 mg/kg group exhibited a moderate improvement of 29.11%, whereas the negative

control group (PHZ only) showed partial spontaneous recovery (57.50%) during the 14-day period.

The dose-dependent increases in RBC count suggest that the polyherbal aqueous extract exerts an erythropoietic effect. *Justicia carnea* and *Ficus* species have been previously reported to enhance red blood cell regeneration through antioxidant mechanisms and stimulation of erythropoiesis (Onyeabo *et al.*, 2017; Ibe *et al.*, 2022). Similarly, Sheth, Pawar, Mote, and More (2021) demonstrated that polyherbal formulations possess anti-anemic activity by promoting erythrocyte production and protecting cells from oxidative stress.

The remarkable increase in RBC at 100 mg/kg, which approached the effect of folic acid, indicates that the combined extract contains phytochemicals capable of supporting hematopoietic recovery. Anthonia, Ikechukwu, Uzoma, and Sunday (2019) attributed such hematinic effects of *Justicia carnea* to its high content of iron, vitamins, and antioxidant compounds that restore erythrocyte membrane stability and enhance hemoglobin synthesis.

Although the anemic control group showed some spontaneous improvement, the higher recovery in extract-treated groups indicates that the polyherbal preparation accelerated hematopoietic restoration. This observation aligns with findings by Obazelu and Odionyenma (2024), who reported that multi-herbal mixtures improve red cell parameters more effectively than single plant extracts in anemic rats.

Hemoglobin concentration followed a pattern similar to that of RBC count, with progressive increases observed across treatment groups over 14 days. The folic acid group exhibited the highest Hb increase (50.60%), followed by the 100 mg/kg extract group (38.50%), and lower doses (25 and 50 mg/kg) showing moderate recovery of 24.53 and 22.33%, respectively. These results demonstrate that the polyherbal extract enhances hemoglobin synthesis in a dose-dependent manner. *Justicia carnea* and *Ficus* species are rich in bioactive compounds such as flavonoids, phenolic acids, and iron complexes that support hemoglobin regeneration

and protect against oxidative degradation (Onyeabo *et al.*, 2017; Ibe *et al.*, 2022; Okafor and Atsu, 2022). Similar observations were made by Igwe *et al.* (2020), who found significant hemoglobin restoration following treatment with *Justicia carnea* in PHZ-induced anaemia. Furthermore, the improved Hb levels in treated rats suggest that the extract components may enhance erythropoietin activity or iron bioavailability, both of which are critical in erythropoiesis. Olukanni, Minari and Okpuzor (2023) demonstrated that plant-derived antioxidants, such as those found in *Moringa oleifera*, improve Hb synthesis by reducing oxidative stress in the bone marrow. The pattern observed here mirrors those findings, confirming that the combination of *Justicia*, *Ipomoea*, and *Ficus* leaves exerts similar hematinic effects.

The anemic control also showed some degree of hemoglobin recovery (44.65%), likely due to natural compensatory mechanisms following cessation of PHZ exposure (Lalhriatpuii *et al.*, 2023). However, the consistently higher Hb levels in the 100 mg/kg extract group relative to the lower doses reflect the efficacy of the extract as a hematinic and antioxidant remedy.

Packed cell volume (PCV) increased significantly in all treatment groups between Day 1 and Day 14, indicating restoration of blood volume and erythrocyte mass. The 100 mg/kg extract group and folic acid group showed comparable improvement, with 55.66 and 60.25% recovery, respectively. The 25 and 50 mg/kg groups demonstrated moderate recovery (43.63 and 36.75%), while the anemic control group showed the highest increase (65.99%), suggesting partial spontaneous recovery of erythropoiesis.

The extract's impact on PCV aligns with the RBC and Hb findings, confirming that the polyherbal formulation promotes complete hematinic recovery. Similar PCV improvements were reported by Idu, Alugeh and Gabriel (2022), who evaluated a polyherbal hematinic tonic (Mojeaga) and observed significant increases in red cell indices due to the combined antioxidant and nutritional properties of the constituent plants.

The results also correspond with findings by Ibe *et al.* (2022), who demonstrated that *Ficus capensis* extract restored PCV levels by protecting red cells against oxidative damage and supporting bone marrow regeneration. In the present study, the combined effects of *Justicia carnea*, *Ipomoea batatas*, and *Ficus sur* likely provided complementary bioactive compounds like iron, ascorbic acid, phenolics, and flavonoids that improved red cell survival and formation.

While the anaemic control group showed unexpectedly high PCV recovery, this may be attributed to physiological rebound erythropoiesis following PHZ-induced destruction, a phenomenon previously observed in similar studies (Lalhriatpuii *et al.*, 2023; Okolo, 2021). Nevertheless, the dose-dependent pattern among extract-treated groups suggests a genuine pharmacological effect rather than random fluctuation. The combined results from RBC, Hb, and PCV analyses demonstrate that the polyherbal aqueous leaf extract exhibits dose-dependent hematinic activity in phenylhydrazine-induced anemic rats. The extract's restorative effects are comparable to those of folic acid at higher doses, underscoring its therapeutic potential.

These findings corroborate earlier reports on the hematoprotective and antioxidant properties of *Justicia carnea*, *Ficus* species, and other medicinal plants (Onyeabo *et al.*, 2017; Ibe *et al.*, 2022; Okafor and Atsu, 2022; Igile *et al.*, 2018). Polyherbal formulations such as Raktavardhak Kadha and Mojeaga have also demonstrated potent anti-anemic activity, supporting the concept that herbal combinations yield synergistic effects (Sheth *et al.*, 2021; Idu *et al.*, 2022). The likely mechanism involves the combined antioxidant and nutrient-rich profile of the constituent plants. *Justicia carnea* is known to contain iron, folate, vitamins A and C, while *Ficus* and *Ipomoea* species are rich in polyphenols that protect erythrocytes from oxidative lysis (Anthonia *et al.*, 2019; Obazelu & Odionyenma, 2024). Together, these

compounds enhance erythropoiesis, restore hemoglobin synthesis, and improve red cell membrane stability.

## **5.2 Conclusion**

The results confirm that phenylhydrazine hydrochloride successfully induced hemolytic anaemia in Wistar rats, as evidenced by significant decreases in RBC, Hb, and HCT. Treatment with the polyherbal aqueous leaf extract of *Justicia carnea*, *Ipomoea batatas*, and *Ficus sur* significantly improved hematological parameters in a dose-dependent manner. The medium of the extract produced hematinic effects comparable to folic acid, demonstrating that the formulation possesses potent erythropoietic and antioxidant properties.

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