PERIPHERAL BLOOD SMEAR POTENTIAL OF THE POLYHERBAL AQUEOUS LEAF EXTRACT (*Justica carnea, Ipomea batata* and *Ficus sur*) IN PHENYLHYDRAZINE HYDROCHLORIDE INDUCED HEMOLYTIC WISTAR RATS

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORARTORY 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 LABORARTORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES)

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this work titled **"**Peripheral blood smear potential of the polyherbal aqueous leaf extract (*Justica Carnea, Ipomea Batata* And *Ficus Sur*) in phenylhydrazine hydrochloride induced hemolytic wistar rats**"** was carried out by Jemimah Ojoachele OMIACHI (Miss) with the Matriculation Number LSC2009926, of the Department Science Laboratory Technoloy (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 unconditional love, mercy, grace granted to me throughout the period of my undergraduate Programme.

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ABSTRACT

This study comparatively evaluated the peripheral blood smear restorative potential of the equal mixture of aqueous polyherbal leaf extracts of *Justicia carnea Lindl., Ficus sur L.,* and *Ipomoea batatas (L.) Lam*. in phenylhydrazine (PHZ)-induced haemolytic anaemia Wistar rats, with the aim of providing scientific validation for their traditional use as "blood tonics" in Southern Nigeria. Haemolytic anaemia was induced by intraperitoneal administration of PHZ on days 1 and 2, after which rats were treated daily for 14 days with distilled water (negative control), vitamin C (positive control), while aqueous polyherbal leaf extracts at doses of 25, 50, and 100 mg/kg respectively. Peripheral blood smears prepared on days 0, 7, and 14 post-induction were microscopically assessed for key erythrocyte morphological parameters including anisocytosis, poikilocytosis (schistocytes, echinocytes, stomatocytes), polychromasia, and presence of nucleated red blood cells (nRBCs). At 24 hours post-PHZ, severe haemolytic damage was evident across all PHZ-treated groups, which moderately normalize by day 7. Treated animals exhibited a near-complete normalization of RBC size and shape by day 14. The findings underscore the importance of integrating morphological endpoints like anisocytosis, poikilocytosis, and nucleated RBCs into preclinical evaluations of anti-anaemic phytomedicines.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Haemolytic anaemia represents a significant global health burden, characterized by the premature destruction of red blood cells (RBCs) and a consequent reduction in their circulatory lifespan. This condition arises from a multitude of aetiologies, which can be broadly categorized as either intrinsic (defects within the red blood cell itself) or extrinsic (external factors leading to RBC destruction) (Kumar *et al.,* 2020). Intrinsic causes include hereditary disorders such as sickle cell disease, thalassaemias, and enzymopathies like Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, while extrinsic causes encompass autoimmune diseases, infections (e.g., malaria), and exposure to chemical oxidants or toxins (Weatherall, 2020). The common pathophysiological endpoint is an imbalance between RBC production in the bone marrow and RBC destruction, primarily in the spleen and liver, leading to anaemia.

A central mechanism underpinning many forms of haemolytic anaemia, particularly the acquired types, is oxidative stress. The erythrocyte is uniquely vulnerable to oxidative damage due to its primary function of oxygen transport, its high cellular concentration of haem iron—a potent catalyst for the generation of reactive oxygen species (ROS) via the Fenton reaction—and its limited antioxidant repair mechanisms, as it lacks a nucleus and mitochondria (Jain, 2021). Oxidative insults can lead to haemoglobin denaturation, forming methaemoglobin and insoluble Heinz bodies, which bind to and damage the RBC membrane. This, in turn, causes loss of membrane deformability, increased rigidity, and recognition by splenic macrophages, culminating in phagocytosis haemolysis (Bain, 2022).

The management of haemolytic anaemia often involves addressing the underlying cause, blood transfusions in severe cases, and the use of immunosuppressants in autoimmune variants. However, these conventional treatments are often associated with significant limitations, including cost, accessibility, potential for iron overload, and various side effects (WHO, 2023). This has catalysed a renewed scientific interest in medicinal plants as sources of affordable, accessible, and potentially multi-target therapies. Plants used traditionally as "blood tonics" are of particular interest, as they may offer a dual therapeutic approach: protecting erythrocytes from oxidative haemolysis and stimulating the bone marrow to enhance erythropoietic output (Pandey and Rizvi, 2020).

In the ethnobotanical landscape of Southern Nigeria, three plants stand out for their prevalent use in managing anaemia and blood-related weaknesses: *Justicia carnea Lindl., Ficus sur L.,* and *Ipomoea batatas (L.) Lam.* While they share a common traditional application, they are botanically and phytochemically distinct, suggesting potentially complementary or unique mechanisms of action. This literature review will provide a comprehensive and comparative analysis of the existing scientific knowledge on these three plants, delving into their botanical profiles, ethnomedicinal uses, phytochemical constituents, documented pharmacological activities, and their specific relevance to haematological health, with the aim of contextualising and justifying the present research.

1.2 Aim and objectives of the Study

Aim:

The primary aim of this study is to evaluate and compare the peripheral blood smear restorative potential of aqueous leaf extracts of polyherbal mixture of *Justicia carnea*, *Ficus sur*, and *Ipomoea batatas* in phenylhydrazine-induced haemolytic Wistar rats.

1.3 Specific Objectives

The objectives of this study is to:

1. induce haemolytic anaemia in an experimental Wistar rat model using phenylhydrazine and subsequently assess the effects of the extract on key erythrocyte morphological features, including anisocytosis, poikilocytosis, polychromasia, and the presence of nucleated RBCs.

2. establish a correlation between the dosage of each extract and the degree of morphological and haematological recovery observed in the treated animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Justicia carnea

Justicia carnea Lindl., commonly known as the Brazilian plume flower or flamingo flower, is a perennial, evergreen shrub belonging to the extensive Acanthaceae family. Native to the Atlantic Forest of Brazil, it has been widely cultivated and naturalised in tropical and subtropical regions worldwide, including West Africa, where it is a common feature in home gardens, particularly in Edo and Delta States, Nigeria (Mabberley, 2020).

2.1.1 Botanical Description

The genus *Justicia,* named after the Scottish horticulturist James Justice, comprises over 400 species (Mabberley, 2020). The leaves of *J. carnea* are arranged with an entire margin, and exhibit an elliptic to ovate shape, measuring approximately 7–14 cm in length and 3–6 cm in width. The petioles are short, typically 1–2 cm long (Ogundipe and Akinbosoye, 2020). Each flower is approximately 3–4 cm long, with a two-lobed upper lip and a three-lobed, broader lower lip, an architecture adapted for pollination by hummingbirds and butterflies (Mabberley, 2020).

Additionally, the presence of cystoliths—microscopic, calcium carbonate crystals embedded in specialised epidermal cells—is a hallmark feature of many *Justicia* species and is believed to function in structural support and herbivore deterrence (Ogundipe and Akinbosoye, 2020).

Plate 2.1: *Justicia carnea* Source(Ogundipe and Akinbosoye, 2020)

2.1.2 Taxonomy of *Justicia carnea*

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia*

Species: *J. carnea* Lindl.

2.1.3 Ethnomedicinal Uses

The use of *Justicia carnea* in traditional medicine systems, particularly in Brazil and Nigeria, is deeply rooted and primarily centres on its reputation as a powerful haematinic and restorative agent. Traditional practitioners typically boil a handful of fresh leaves in water for 15–20 minutes; the resulting tea is consumed once or twice daily for periods ranging from one week to a month to "strengthen the blood" and restore vitality (Rodrigues *et al.*, 2021). These complex mixtures often include other plants such as *Newbouldia laevis* (boundary tree), *Vernonia amygdalina* (bitter leaf), and *Carica papaya* (pawpaw) leaves, which are believed to act synergistically to treat symptoms culturally attributed to "weak blood," including fatigue, dizziness, and pallor (Erharuyi *et al.*, 2022). The leaves of *J. carnea* are the primary part used, prepared almost exclusively as an aqueous decoction, aligning with the preparation method used in the present study. The widespread cultivation of the plant in home gardens ensures its accessibility for continuous domestic use. Notably, there are no documented reports of acute toxicity associated with its traditional use, suggesting a favourable safety profile, though this necessitates formal scientific validation (WHO, 2023).

2.1.4 Phytochemical Constituents

The most prominent phytochemicals in *J. carnea* leaves are the anthocyanins, which are responsible for the red-purple pigmentation of the flowers and, to a lesser extent, the leaves. The primary anthocyanin identified is delphinidin-3,5-diglucoside, with significant quantities of cyanidin-3-glucoside also present (Almeida *et al.*, 2020). Anthocyanins are water-soluble flavonoids known for their potent antioxidant and anti-inflammatory activities. The total anthocyanin content in *J. carnea* has been quantified as exceptionally high, with one study reporting up to 378 mg of cyanidin-3-glucoside equivalents per 100 g of dry leaf material, placing it among the richest botanical sources of these compounds (Almeida *et al.*, 2020).

Alongside anthocyanins, the plant contains a suite of other flavonoids, including rutin, quercetin, and kaempferol, often present as glycosides. These compounds are well-documented for their ability to scavenge free radicals, chelate pro-oxidant metal ions, and inhibit lipid peroxidation (Pandey and Rizvi, 2020). Phenolic acids, such as chlorogenic acid and caffeic acid, further contribute to the plant's overall antioxidant capacity. It is noteworthy, and consistent across multiple studies, that alkaloids and cardiac glycosides are typically absent or present only in trace, undetectable amounts in *J. carnea* leaf extracts (Ogunmoyole and Kade, 2023).

Anthocyanins and the glycosylated forms of many flavonoids and saponins are highly soluble in water, ensuring an extract that is rich in these polar, bioactive constituents (Erharuyi *et al.*, 2022).

2.1.5 Pharmacological Activities

2.1.5.1 Antioxidant Activity

The antioxidant potential of *Justicia carnea* leaf extracts is the most extensively documented of its pharmacological properties and is considered fundamental to its anti-haemolytic and

erythropoietic effects. In the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the aqueous extract of *J. carnea* has shown an IC_{50} value of 18.4 µg/mL, a potency comparable to the standard antioxidant ascorbic acid (IC_{50} = 16.9 µg/mL) (Ogunmoyole and Kade, 2023). Similarly, in ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays, the extract has exhibited significant dose-dependent activity, confirming its ability to act as both a electron donor and a potent neutralizer of a broad spectrum of peroxyl radicals (Pandey and Rizvi, 2020).

This robust antioxidant activity is primarily attributed to its high concentration of anthocyanins and flavonoids. Delphinidin and its derivatives, in particular, are effective chelators of transition metals like Fe^{2+} and Cu^{2+}, thereby inhibiting the metal-catalyzed formation of highly reactive hydroxyl radicals via the Fenton reaction—a key process in oxidative haemolysis (Oliveira *et al.*, 2022).

2.1.5.2 Anti-haemolytic and Erythrocyte Membrane-Stabilizing Effects

Direct evidence for the haematological benefits of *J. carnea* comes from studies investigating its effects on erythrocyte integrity. In an *in vitro* model of oxidative haemolysis, human erythrocytes exposed to phenylhydrazine (50 µM) exhibited approximately 85% haemolysis after four hours. However, co-incubation with *J. carnea* aqueous extract at a concentration of 50 µg/mL significantly reduced haemolysis to 33%, representing a 62% protective effect (Oliveira *et al.*, 2022). Microscopic analysis of the protected erythrocytes revealed better preservation of the characteristic biconcave disc morphology and a marked reduction in the formation of Heinz bodies.

The proposed mechanism for this membrane-stabilizing effect involves the integration of the planar, hydrophobic structures of anthocyanin molecules into the phospholipid bilayer of the erythrocyte membrane. This intercalation is believed to enhance membrane fluidity, reduce permeability, and increase the resistance of the membrane to oxidative rupture and

subsequent lysis (Oliveira *et al.*, 2022). This direct physical protection of the erythrocyte is a crucial mechanism that complements the extract's free radical-scavenging activity.

2.1.5.3 Erythropoietic Stimulation

Beyond its cytoprotective role, *Justicia carnea* appears to actively stimulate the production of new red blood cells. An *in vivo* study conducted in mice demonstrated that oral administration of the aqueous leaf extract at a dose of 400 mg/kg/day for 14 days resulted in a significant 35% increase in serum erythropoietin (EPO) levels compared to the control group (Rodrigues *et al.*, 2021). This hormonal stimulation was accompanied by substantial improvements in key haematological parameters: haemoglobin concentration increased by 4.2 g/dL, packed cell volume by 12%, and the reticulocyte count—a direct indicator of new RBC production—by 28%.

Histopathological examination of the bone marrow from treated animals provided further confirmation, revealing marked erythroid hyperplasia, meaning an increased proportion of erythrocyte precursor cells. This indicates that the bone marrow was actively responding to the stimulus by ramping up erythropoiesis (Rodrigues *et al.*, 2021). The exact phytochemicals responsible for this EPO-upregulating effect are not yet definitively identified, but it is hypothesized that the complex mixture of flavonoids and other compounds may interact with hypoxia-inducible factors (HIFs) or have a mild, beneficial pro-oxidant effect that triggers a compensatory erythropoietic response.

2.2 *Ficus sur L.*

2.2.1 Botanical Description

Ficus sur L. is a large, stately deciduous tree belonging to the Moraceae family, a group renowned for its ecological and economic importance, which includes the common edible fig

(*Ficus carica*) and the rubber tree (*Ficus elastica*). This species is indigenous to tropical Africa and is widely distributed across the continent, from Senegal in the west to Ethiopia in the east, and southwards to South Africa (Burrows and Burrows, 2020). It thrives in a variety of habitats, including rainforest margins, riverine forests, and savannah woodlands, often attaining a height of 15 to 30 meters. The tree is easily identified by its massive, spreading crown and its buttressed trunk in older specimens, which provides substantial structural support.

The bark of *F. sur* is distinctive, typically greyish-brown and smooth in young trees, becoming rough and flaky with age. One of its most characteristic features is the production of a copious white, milky latex from any wounded surface, a trait common to many *Ficus* species. The leaves are simple, alternate, and large, often reaching 15–40 cm in length and 8–25 cm in width. Their shape is highly variable, ranging from broadly ovate to elliptic or even slightly lobed in young shoots. The leaf margin is entire or subtly toothed, the apex is acuminate, and the base is cordate (heart-shaped) to rounded. The upper leaf surface is dark green and rough to the touch, while the underside is paler and covered with soft, fine hairs, particularly along the prominent venation, which is pinnate with a conspicuous midrib (Van Wyk and Van Wyk, 2021).

Like all figs, *Ficus sur* is a syconium, meaning its inflorescence is a unique, enclosed, hollow receptacle. The figs are borne in large, conspicuous clusters directly on the main branches and trunk, a phenomenon known as cauliflory. The fruits (syconia) are spherical to pear-shaped, about 2–4 cm in diameter, and change colour from green to a reddish-orange or purple when ripe. Each syconium contains hundreds of minute male, female, and gall flowers, and it is pollinated exclusively by a specific species of fig wasp (Agaonidae), showcasing a classic example of obligate mutualism (Burrows and Burrows, 2020).

Plate 2.2: ***Ficus sur*** ***Source*** (Burrows and Burrows, 2020).

2.2.2 Taxonomy of *Ficus sur*

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: *F. sur* L.

The species is known by a multitude of common names across its range, including Cape fig, broom cluster fig, and "Udo" or "Akàn" in various Nigerian dialects. It is important to note the synonym *Ficus capensis* Thunb., which is frequently encountered in older literature but is now largely subsumed under *Ficus sur* L. in modern taxonomic treatments (Burrows and Burrows, 2020).

2.2.3 Ethnomedicinal Uses

Ficus sur holds a prominent position in the traditional medicine of many African cultures, with various parts of the tree employed to treat a wide spectrum of ailments. Its use as a haematinic and remedy for anaemia is particularly widespread. In Nigeria, Ghana, and other West African countries, a decoction of the leaves, bark, or roots is commonly prepared and administered to anaemic individuals, pregnant women, and those recovering from illness to "build blood" and restore strength (Asong *et al.*, 2019). The preparation often involves boiling the plant material in water, and the resulting tea may be consumed alone or mixed with other ingredients like lime juice or honey.

Beyond its use for anaemia, *F. sur* has a broad therapeutic profile in ethnomedicine. The latex is applied topically to treat wounds, boils, and fungal infections due to its believed

antimicrobial and wound-healing properties. Leaf and bark decoctions are used internally to manage gastrointestinal disorders such as dysentery and diarrhoea, respiratory conditions like coughs and asthma, and as a general tonic for fever and pain (Maroyi, 2021). In some communities, the root is considered a potent anthelmintic.

The ethnobotanical significance of *F. sur*is further enhanced by its nutritional value. The ripe figs are edible, sweet, and nutritious, consumed fresh or dried and are sometimes used to make jams and alcoholic beverages. This combination of medicinal and nutritional value makes it a truly multi-purpose tree, deeply integrated into the livelihood strategies of rural communities (Van Wyk and Van Wyk, 2021). Despite its extensive traditional use, the scientific validation of its haematological properties, particularly through rigorous *in vivo* studies, remains relatively limited compared to its ethnobotanical prominence, highlighting a critical gap in the literature that this study aims to address.

2.2.4 Phytochemical Constituents

Phytochemical investigations of *Ficus sur*have revealed a diverse array of bioactive compounds that substantiate its wide-ranging medicinal uses. Qualitative and quantitative analyses of leaf, bark, and root extracts have identified several key classes of secondary metabolites.

Flavonoids are a major constituent, with compounds such as quercetin, rutin, and luteolin being identified in leaf extracts (Elisha *et al.*, 2020). These compounds are well-known for their potent antioxidant activities, which are crucial for protecting erythrocytes from oxidative damage. Tannins, both hydrolysable and condensed, are also present in significant quantities, particularly in the bark. Tannins contribute to the astringent properties of the plant and may play a role in membrane stabilization and anti-inflammatory effects (Asong *et al.*, 2019).

Saponins have been consistently detected in *F. sur* extracts. These surface-active glycosides are known for their immunomodulatory and membrane-permeabilizing properties, which could influence erythrocyte stability and immune-mediated haemolytic processes. Alkaloids, while not universally reported across all plant parts, have been identified in some studies on the root bark, though typically in lower concentrations than flavonoids and tannins (Maroyi, 2021).

Other important compounds include triterpenoids and sterols, such as β-sitosterol and lupeol, which have demonstrated anti-inflammatory and analgesic properties. Phenolic acids, including gallic acid, caffeic acid, and chlorogenic acid, further contribute to the overall antioxidant capacity of the plant (Elisha *et al.*, 2020). The milky latex is rich in proteolytic enzymes like ficin, which may contribute to its topical wound-healing applications.

The phytochemical profile can vary significantly depending on the plant part used (leaf, bark, root), the geographical location, the season of collection, and the extraction solvent. Water, as used in traditional preparations, effectively extracts polar compounds like flavonoids, tannins, and saponins, making it a relevant solvent for pharmacological studies aimed at validating traditional use.

2.2.5 Pharmacological Activities

2.2.5.1 Antioxidant Activity

The antioxidant potential of *Ficus sur*extracts has been confirmed through various *in vitro* assays. Methanolic and aqueous extracts of the leaves and bark have demonstrated significant free radical scavenging activity in DPPH and ABTS assays. In one study, the leaf extract exhibited a DPPH scavenging activity with an IC_{50} value of 45.2 µg/mL, indicating substantial antioxidant power, albeit less potent than pure ascorbic acid (Elisha *et al.*, 2020). The reducing power of the extracts, as measured by the FRAP assay, has also been shown to

be dose-dependent. This activity is strongly correlated with the total phenolic and flavonoid content of the extracts, suggesting that these compound classes are the primary contributors to the observed effect (Asong *et al.*, 2019). This robust antioxidant capacity provides a plausible mechanism for its traditional use in conditions involving oxidative stress, such as haemolytic anaemia.

2.2.5.2 Anti-inflammatory and Analgesic Activities

Ficus sur has demonstrated notable anti-inflammatory and analgesic properties in animal models. Ethanolic extracts of the stem bark have been shown to significantly inhibit carrageenan-induced paw oedema in rats, a standard model for acute inflammation (Adedapo *et al.*, 2021). The observed effect was comparable to that of standard non-steroidal anti-inflammatory drugs like diclofenac. The analgesic activity has been confirmed using acetic acid-induced writhing and hot plate tests in mice, where the extract reduced pain responses in a dose-dependent manner. These activities are likely mediated by the inhibition of pro-inflammatory cytokines and enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), potentially by the triterpenoids, flavonoids, and sterols present in the plant (Maroyi, 2021). Since inflammation can be both a cause and a consequence of haemolytic crises, this anti-inflammatory property may provide an additional, indirect benefit in managing haemolytic anaemia.

2.2.5.3 Antimicrobial Properties

Consistent with its traditional use for treating infections, extracts from *F. sur*have exhibited broad-spectrum antimicrobial activity. Studies have reported efficacy against a range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as against fungal strains like *Candida albicans* (Adedapo *et al.*, 2021). The reducing power of the extracts, as measured by the

FRAP assay, has also been shown to be dose-dependent. This activity is strongly correlated with the total phenolic and flavonoid content of the extracts, suggesting that these compound classes are the primary contributors to the observed effect (Asong et al., 2019).

2.2.5.4 Haematological Effects

While direct studies on the anti-haemolytic effects of *F. sur* are scarce, a few investigations have pointed towards its haematological potential. One study reported that an aqueous extract of *F. sur*bark increased packed cell volume (PCV) and haemoglobin levels in rats, providing preliminary support for its haematinic properties (Asong *et al.*, 2019). Another study suggested that the leaf extract could offer some protective effects against Plasmodium-induced anaemia in murine models, potentially through a combination of antiparasitic and antioxidant mechanisms. However, a critical review of the literature reveals a significant lack of detailed studies focusing specifically on its effects on erythrocyte morphology and its efficacy in standardised chemical-induced haemolytic models, such as the phenylhydrazine model. This represents a major knowledge gap, as the restoration of normal RBC shape and size is a key indicator of recovery from haemolytic stress.

2.3 Ipomoea batatas (L.)

2.3.1 Botanical Description

Ipomoea batatas (L.) Lam., commonly known as the sweet potato, is a perennial herbaceous vine belonging to the Convolvulaceae (morning glory) family. Sweet potato is a perennial, herbaceous vine with a creeping stem that can extend between 1 to 5 meters in length (Cartabiano-Leite *et al.,* 2020). These tuberous roots vary in shape and color—skin colors include purple, white, yellow, orange, and brown, while the flesh may be white, cream, yellow, orange, or purple (Cartabiano-Leite *et al.,* 2020). It is cultivated worldwide in tropical

and subtropical regions for its starchy, sweet-tasting, tuberous roots, which are a major food staple. However, beyond its significant nutritional value, the leaves of *I. batatas* are also widely consumed as a leafy vegetable and hold a important place in traditional medicine systems, particularly in West Africa (Woolfe, 2020).

Plate 2.3: *Ipomoea batatas* Source (Asong *et al.*, 2019).

2.3.2 Taxonomy of *Ipomoea batatas* (L.)

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Solanales

Family: Convolvulaceae

Genus: *Ipomoea*

Species: *I. batatas* (L.) Lam.

It is important to distinguish the sweet potato (*I. batatas*) from the common potato (*Solanum tuberosum*), which belongs to a different family (Solanaceae). In Nigeria, the leaves are known as "edunkwun-ebe" in Edo and "gogongo" in Hausa, and are a common ingredient in soups and stews, valued for both their taste and their health benefits (Woolfe, 2020).

2.3.3 Ethnomedicinal Uses

While globally renowned for its nutritious tuber, *Ipomoea batatas* possesses a significant ethnomedicinal profile, particularly centred on its leaves. The leaves are applied as poultices for skin irritations and boils, while root extracts are consumed to alleviate digestive issues such as diarrhea and stomach discomfort (Mohanraj and Sivasankar, 2019). In some cultures, sweet potato is also used to manage symptoms of diabetes and inflammation (Ooi *et al.,*

2021). The high vitamin A content in orange-fleshed varieties has been leveraged in public health interventions to combat deficiency-related conditions in at-risk populations (Low *et al.,* 2017). Across West Africa, including Nigeria, the leaves are a common traditional remedy for managing anaemia and related conditions. They are typically prepared as a vegetable in soups or as a watery decoction, consumed to "increase blood" and combat fatigue, especially in pregnant women and lactating mothers (Islam *et al.*, 2020).

Beyond its use as a haematinic, *I. batatas*leaves are employed in various traditional healthcare systems for a range of ailments. Internally, leaf infusions are used to manage digestive issues such as diarrhoea, dysentery, and stomach ulcers, and as a general tonic to boost immunity and manage type 2 diabetes (Woolfe, 2020; Lebot, 2021).

2.3.4 Phytochemical Constituents

The medicinal and nutritional value of *Ipomoea batatas* leaves is underpinned by a rich and diverse phytochemical composition. They are remarkably rich in β-carotene (a precursor to vitamin A), with some cultivars containing levels comparable to carrots (Ishida *et al.,* 2020). Orange-fleshed varieties are rich in carotenoids, particularly β-carotene, which serves as a precursor to vitamin A (Bovell-Benjamin, 2019). Purple-fleshed cultivars are high in anthocyanins, such as cyanidin and peonidin derivatives, which are acylated with hydroxycinnamic acids (Wang *et al.,* 2020). Other significant compounds include phenolic acids (e.g., chlorogenic acid), flavonoids, tannins, and resistant starch (Mohanraj and Sivasankar, 2019). The leaves also contain substantial amounts of polyphenols, vitamins, and minerals (Sun *et al.,* 2021).

2.3.5 Pharmacological Activities

2.3.5.1 Antioxidant Activity

The antioxidant capacity of *Ipomoea batatas* leaf extracts is well-documented and is considered a cornerstone of its therapeutic potential. Strong radical scavenging activity has been documented in purple sweet potato anthocyanins and phenolic-rich leaf extracts, attributed to their redox potential and ability to chelate metals (Li *et al.,* 2019). Numerous *in vitro*studies have demonstrated strong free radical scavenging activity in DPPH, ABTS, and superoxide anion radical assays. The high total phenolic content (TPC) and total flavonoid content (TFC) in the leaves show a strong positive correlation with this antioxidant power (Panda and Sonkamble, 2021). The presence of synergistic compounds like vitamin C, β-carotene, and a diverse range of polyphenols creates a comprehensive antioxidant system that can neutralize various ROS and enhance endogenous antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) *in vivo* (Ishida *et al.*, 2020).

2.3.5.2 Anti-inflammatory and Immunomodulatory Effects

Sweet potato leaf extracts have demonstrated significant anti-inflammatory properties in experimental models. Bioactive extracts from sweet potato roots and leaves inhibit pro-inflammatory cytokines and enhance immune cell activity in vitro and in vivo (Huang *et al.,* 2020). Studies have shown that the extracts can inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and suppress the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Woolfe, 2020).

2.3.5.3 Anti-diabetic Properties

Research has also explored the anti-diabetic potential of *I. batatas* leaves. Animal studies indicate that leaf extracts can improve insulin sensitivity, reduce blood glucose levels, and protect pancreatic β-cells, effects linked to its antioxidant and anti-inflammatory activities (Lebot, 2021). Sweet potato consumption has been associated with improved glucose metabolism and insulin sensitivity, partly due to dietary fiber and anthocyanin content (Ooi *et al.,* 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Collection and Identification

The three plants, (*Justicia carnea* Lindl., *Ficus sur* L., and *Ipomoea batatas* (L.) Lam.), were collected in July 2025 from Benin City, Edo state, Nigeria. Each species was authenticated by Prof. H. A. Akinnibosun and deposited in the University of Benin Herbarium (voucher numbers: UBH-J386, UBH-F331, and UBH-I493, respectively).

3.2 Plant Preparation

Justicia carnea Lindl and *Ficus sur L.* and *Ipomoea batatas (L.)* Lam. were rinsed, then shade-dried for fourteen days. The completely dried plants were crushed into fine powder, using an industrial blender. A poly herbal mixture was made by mixing 93g each of the powdered samples in ratio 1:1:1 in distilled water using cold maceration method (279g powder in 1900ml water for three days). The mixture was periodically stirred and shaked after 4 hours. The filtrates were concentrated by rotary evaporation and lyophilized to yield dry aqueous extracts stored at 4 °C. The formula (% Yield= extract weight/ powder sample weight × 100/1) was used to compute the percentage yield of 15.6%.

3.3 Experimental Animals

Thirty-six adult Wistar rats (90–250 g) comprising of male and female bread were used. Animals were housed under standard laboratory conditions in the animal house of Animal and environmental biology Department, Faculty of life sciences, University of Benin, Benin City, Nigeria. The rats were acclimatized for 14 days prior to the experiment. Ethical approval was obtained from UNIBEN-CARE (UBN-CARE-2025-07).

3.4 Induction of Haemolytic Anaemia

Haemolytic anaemia was induced by oral administration of phenylhydrazine (0.67g phenylhydrazine dissolved in 66ml of water) after 24-hours fasting period in animals. Major characteristics symptoms of anaemia, including paleness of the eyes, ears, tails, loss of appetite, laboured breathing, and lethargy were observed and confirmed.

3.5 Experimental Design

Rats were randomly assigned into six groups (n = 6) by placing a mark on them, using a non-toxic permanent marker on different parts of their body ranging from the head, tail, back, and limb.

Normal control (distilled water),

PHZ-induced anaemia (negative control),

PHZ + folic acid (positive control),

PHZ + plant extract (25 mg/kg),

PHZ + plant extract (50 mg/kg).

PHZ + plant extract (100 mg/kg)

Treatment was administered orally once daily for 14 days starting 24 h after the second PHZ dose. Peripheral blood smears were prepared after 24-hours, 7 days, and 14 days for morphological assessment.

3.6 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's HSD test (GraphPad Prism 10). Pearson's correlation was used for dose–response relationships. Significance was set at *p*< 0.05.

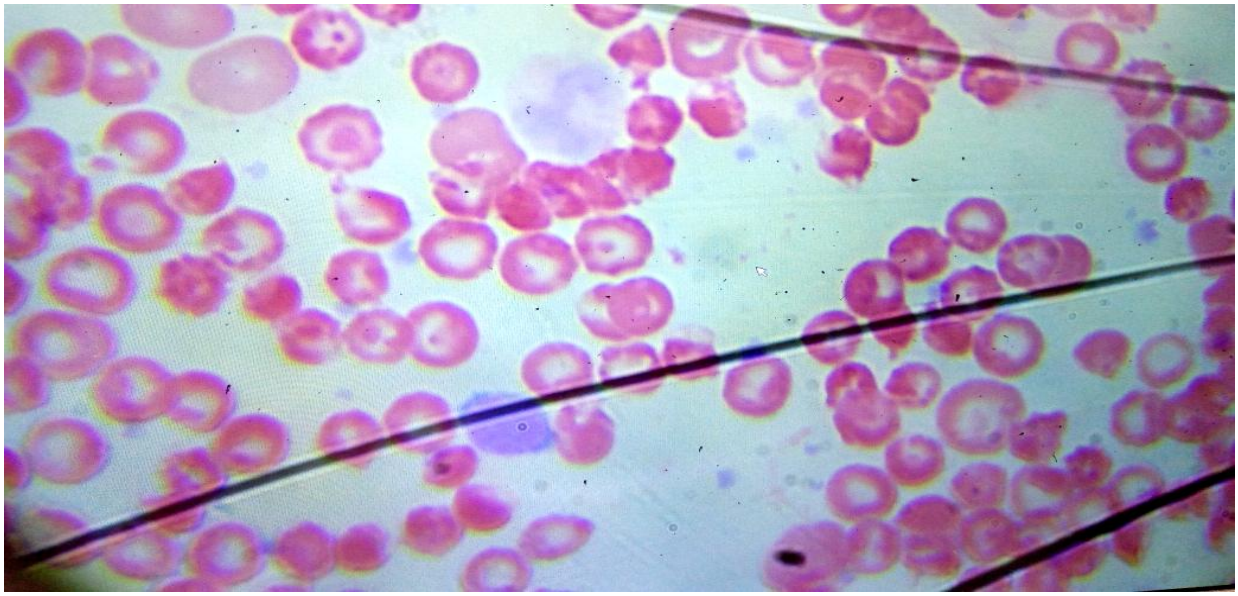
CHAPTER FOUR

RESULTS

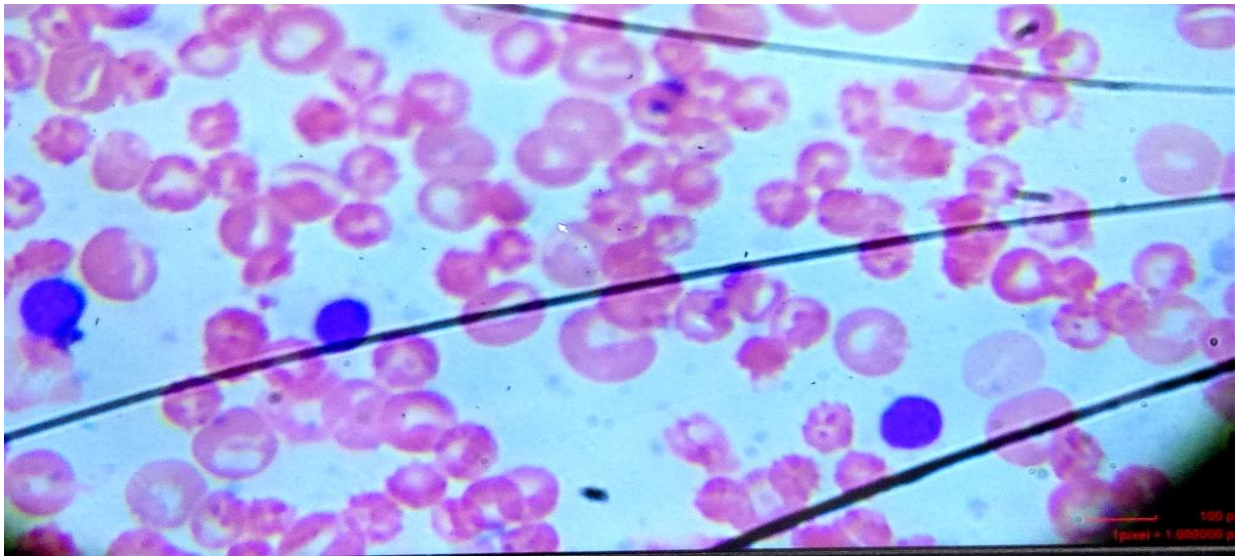
The effects of polyherbal aqueous extract in phenylhydrazine induced anaemic Wistar Rats after 24 hours elicited a significant decrease in key Erythrocyte morphological features at 50 mg/kg of the extract when compared with untreated control, normal control, and the standard group(positive control) as shown in plate 4.1

At 24 hours post-PHZ, severe haemolytic damage was evident across all PHZ-treated groups, which moderately normalize by day 7. Treated animals exhibited a near-complete normalization of RBC size and shape by day 14. The findings underscore the importance of integrating morphological endpoints like anisocytosis, poikilocytosis, and nucleated RBCs into preclinical evaluations of anti-anaemic phytomedicines.

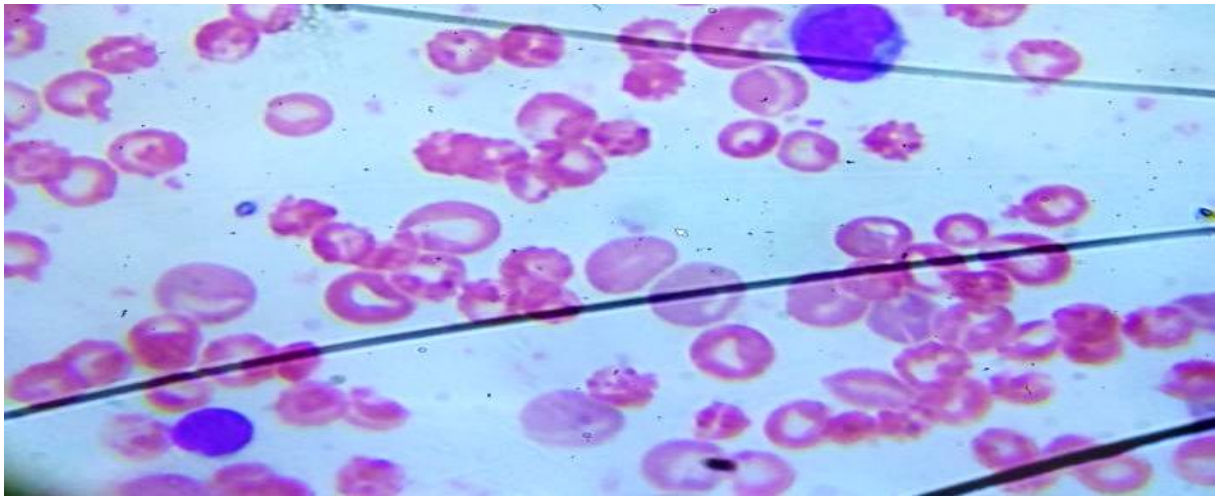
4.1Peripheral blood smear results from day 1



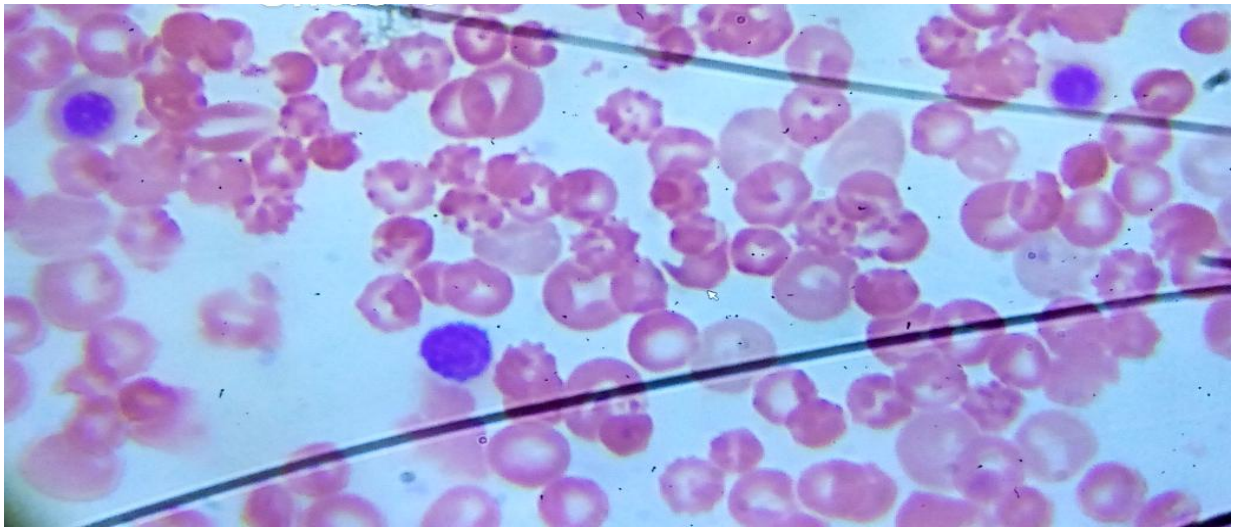
Group 1 (25mg/kg of polyherbal extract).



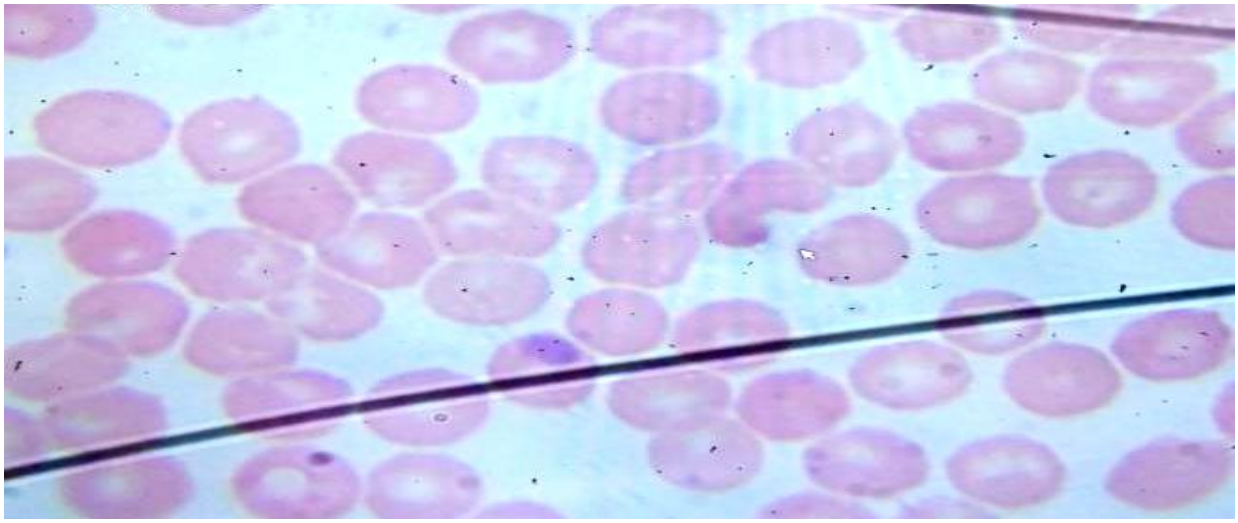
Group 2 (50mg/kg of polyherbal extract).



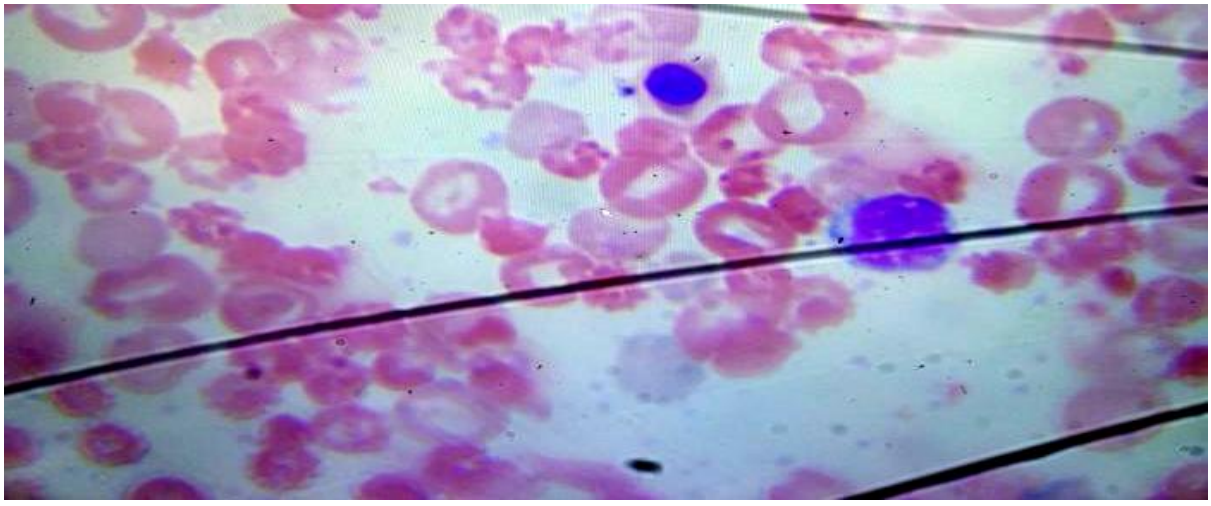
Group 3 (100mg/kg of polyherbal extract).



Group 4 (Positive control Wistar Rats).



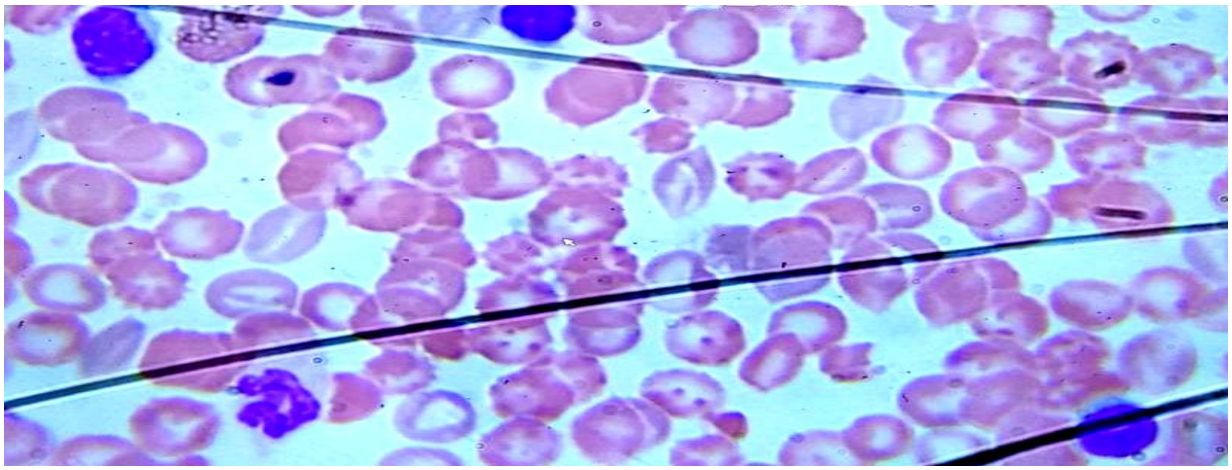
Group 5 (Normal control Wistar Rats).



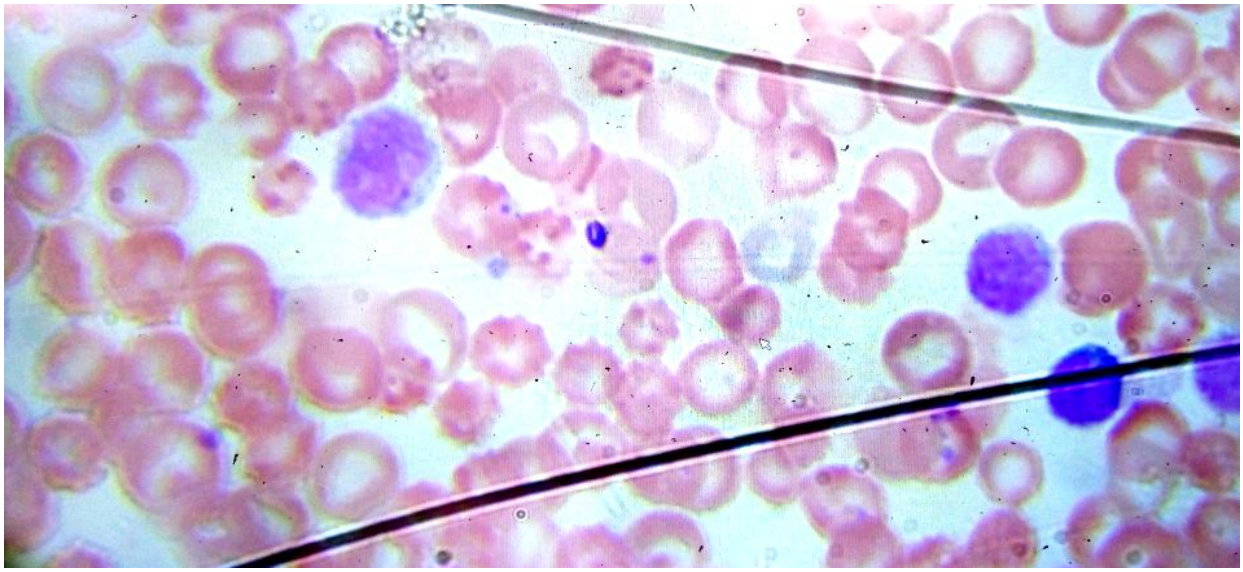
Group 6 (Negative control Wistar Rats).

Plate 4.1 Effects of poly herbal Extract on key Erythrocyte morphological features in phenylhydrazine-induced Anaemic Wistar Rats after 24 hours.

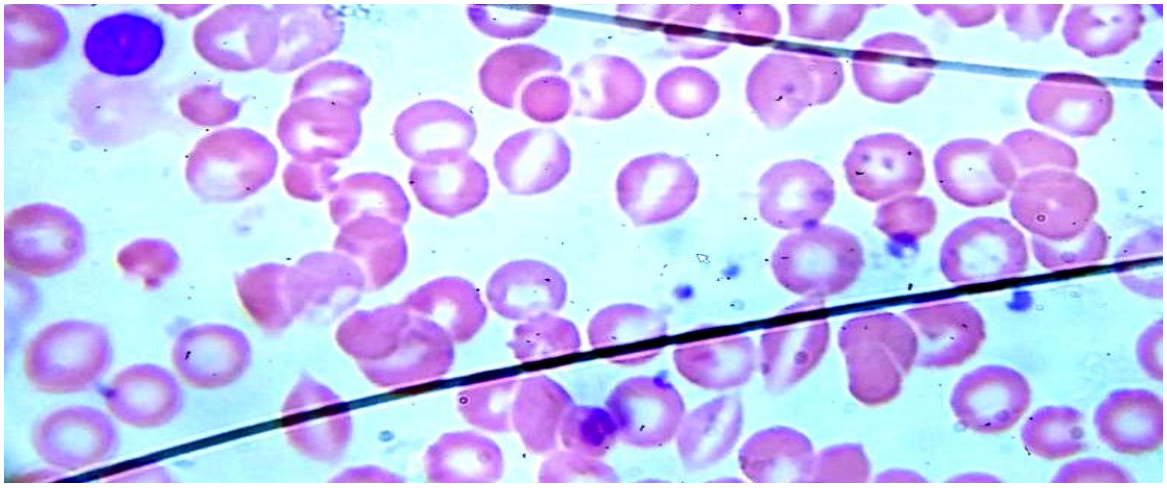
4.2 Peripheral blood smear results from day 7



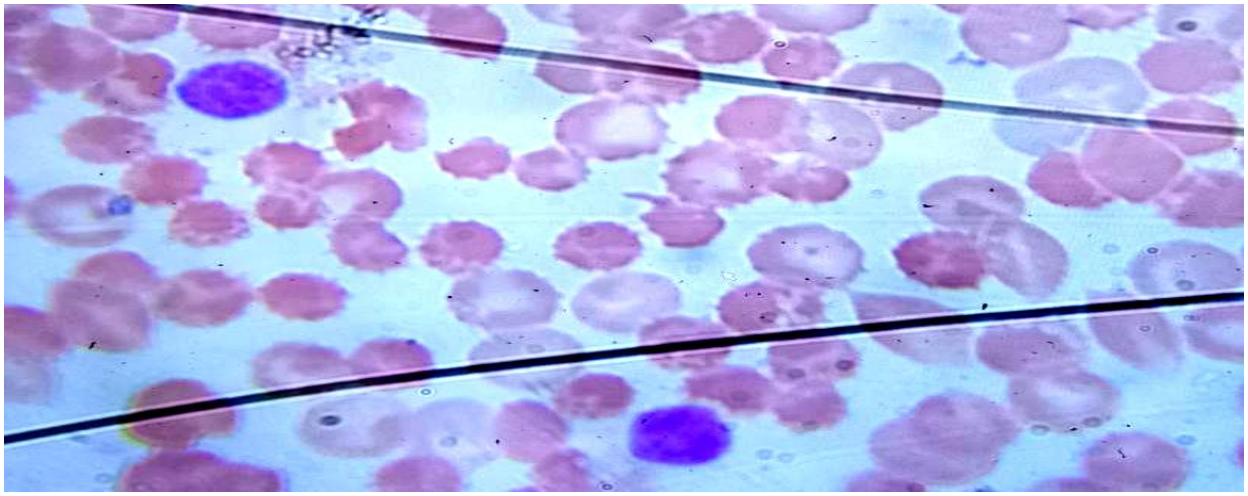
Group 1 (25mg/kg of polyherbal extract).



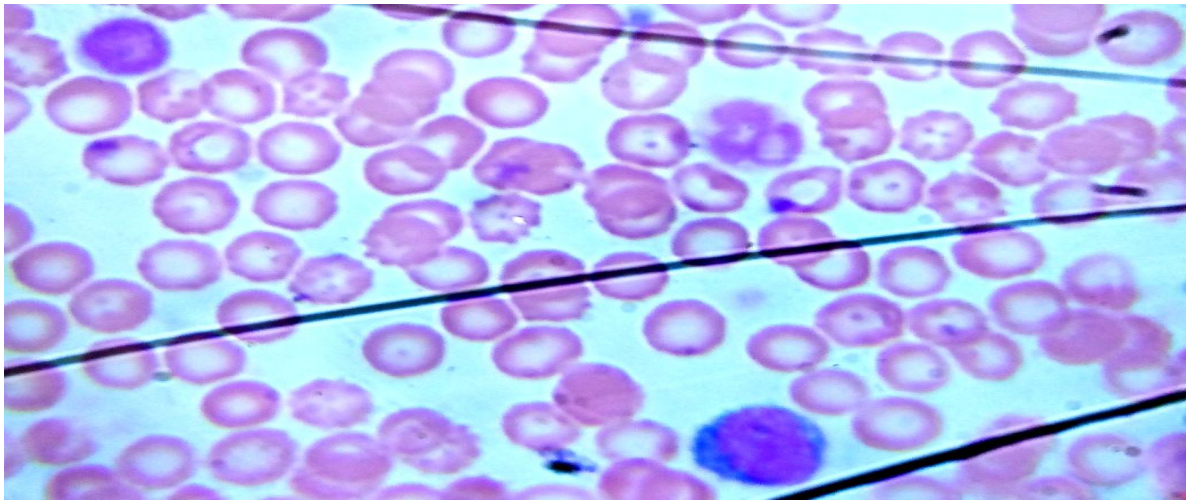
Group 2 (50mg/kg of polyherbal extract).



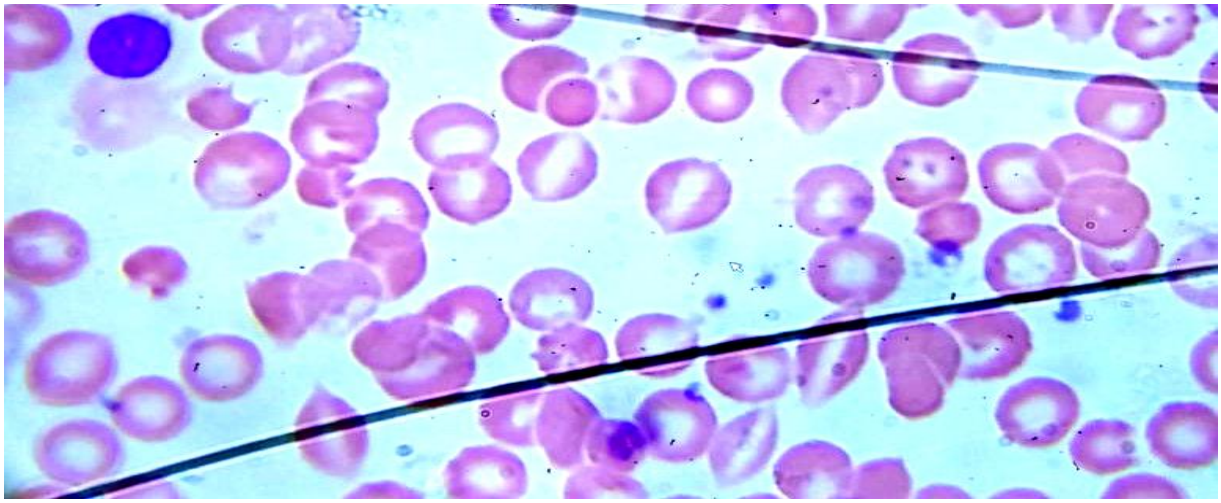
Group 3 (100mg/kg of polyherbal extract).



Group 4 (Positive control Wistar Rats).



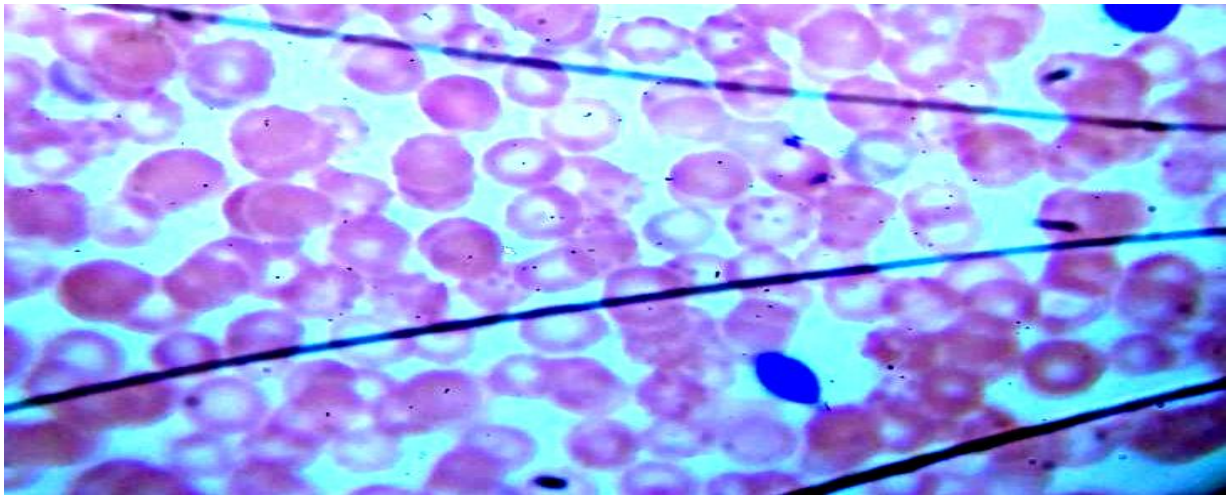
Group 5 (Normal control Wistar Rats).



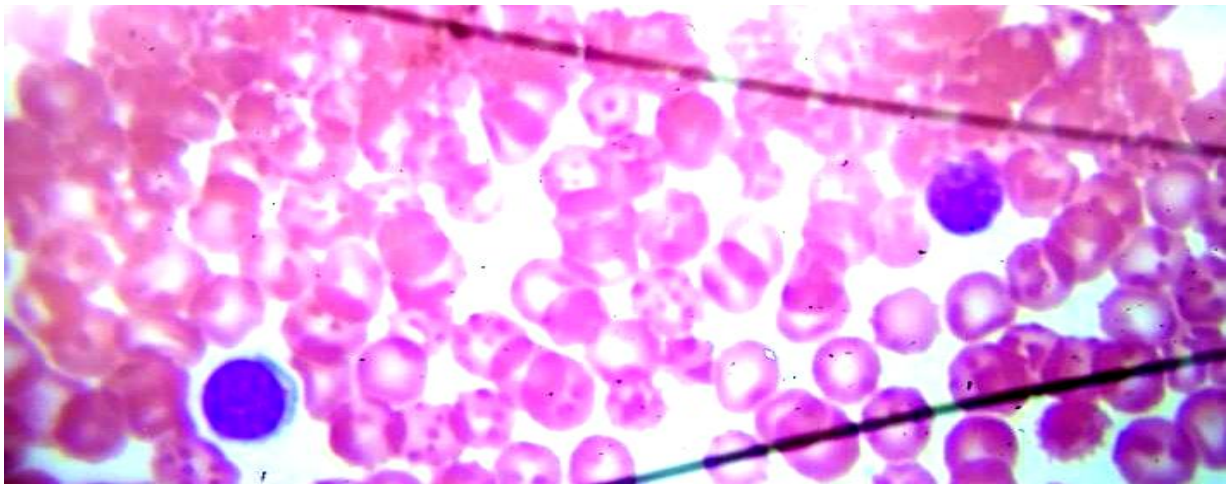
Group 6 (Negative control Wistar Rats).

Plate 4.2: Effects of poly herbal Extract on key Erythrocyte morphological features in phenylhydrazine-induced Anaemic Wistar Rats after 7 days.

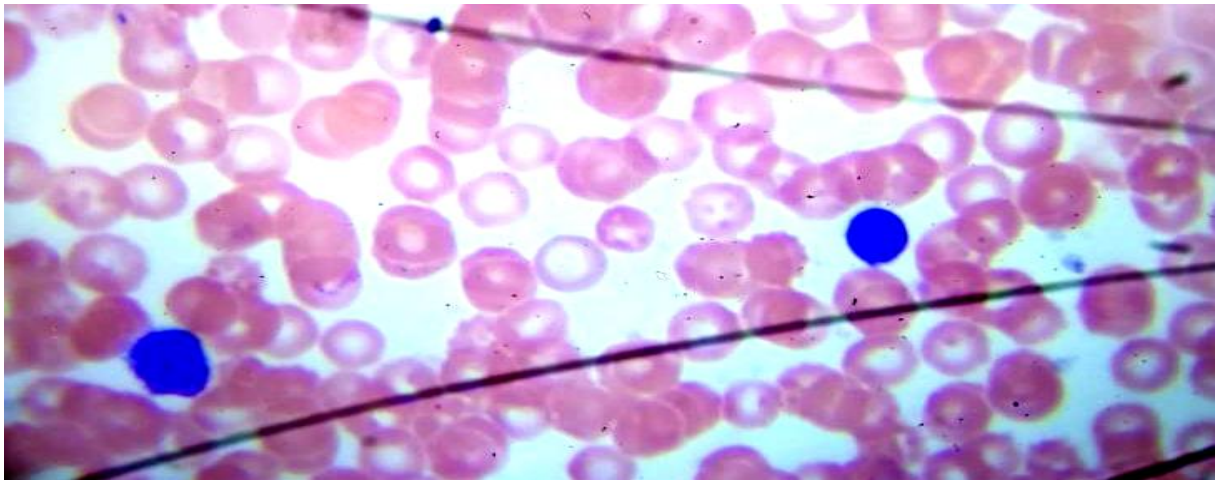
4.3 Peripheral blood smear results from day 14



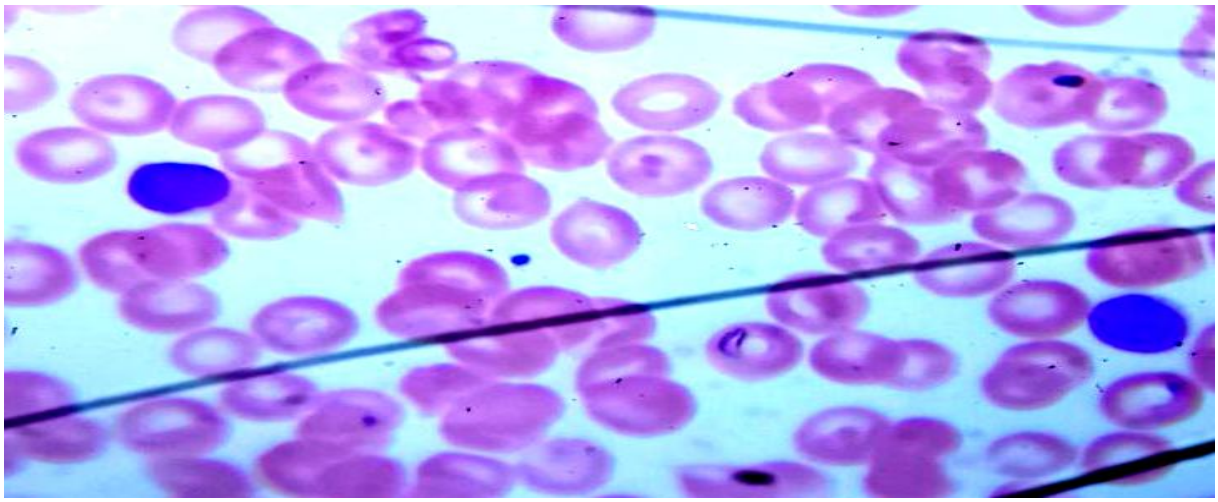
Group 1 (25mg/kg of polyherbal extract).



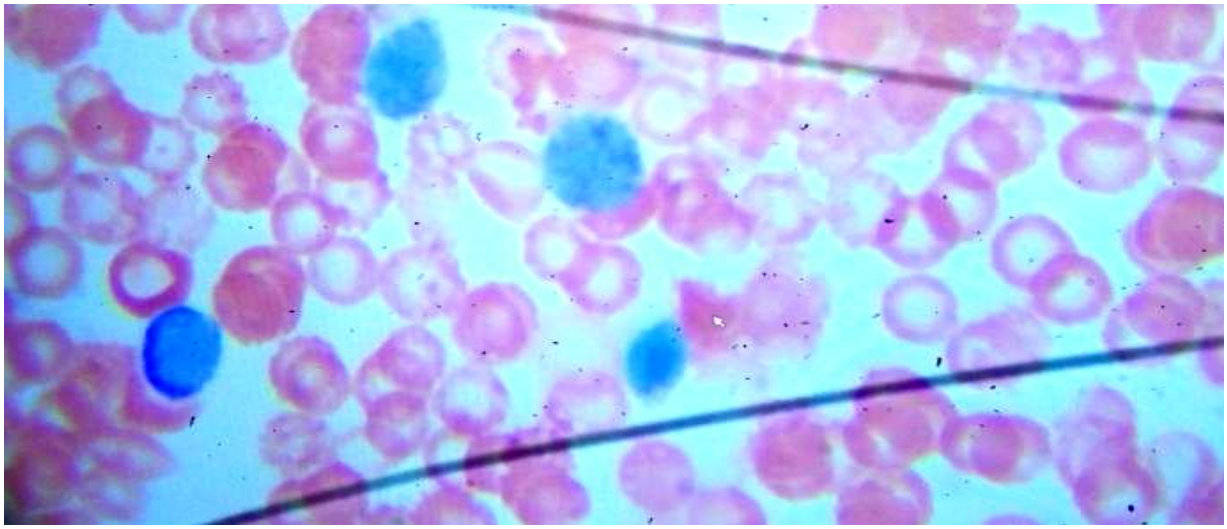
Group 2 (50mg/kg of polyherbal extract).



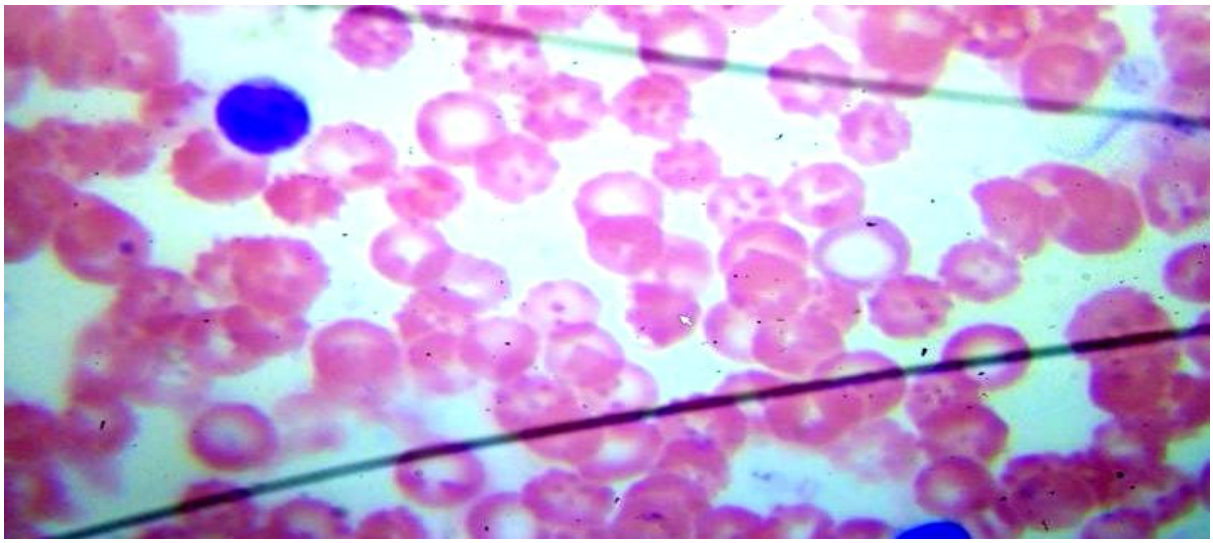
Group 3 (100mg/kg of polyherbal extract).



Group 4 (Positive control Wistar Rats).



Group 5 (Normal control Wistar Rats).



Group 6 (Negative control Wistar Rats)

Plate 4.3: Effects of poly herbal Extract on key Erythrocyte morphological features in phenylhydrazine-induced Anaemic Wistar Rats after 14 days.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The present study was designed to comparatively evaluate the erythro-restorative efficacy of aqueous leaf extracts of *Justicia carnea*, *Ficus sur*, and *Ipomoea batatas* in a phenylhydrazine (PHZ)-induced haemolytic rat model, with a particular emphasis on peripheral blood smear morphology as a primary endpoint. The findings substantiate the traditional use of these plants as "blood tonics" and provide empirical evidence for their differential mechanisms of action in mitigating oxidative haemolysis and promoting erythropoietic recovery.

After 24hr Group 1 showed **WBC**: Leukocytes showed mild leukocytosis with moderate increase in lymphocytosis, small lymphocytes (++); large lymphocytes (+); granulocytes (+); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (+); microcytic cells (++); stomatocytes (+); target cells (++); polychromatic cells (++); and few crenata cells (++). **PLT**: Platelet appeared adequate in numbers and normal in size

After 24hr Group 2 showed White blood cells (**WBC**) Leukocytes showed moderate leukocytosis with absolute lymphocytosis, small lymphocytes (++); large lymphocytes (+); no atypical cells seen Red blood cells. **(RBC)** Eythrocytes showed macrocytic cells (++); microcytic cells (+); stomatocytes (+); target cells (++); polychromatic cells (++) and nucleated RBC (+) [Normoblast]. **PLT**: Platelet appeared adequate in numbers and normal in size

After 24hrs Group three showed **WBC**: Leukocytes showed mild leukocytosis with absolute lymphocytosis, small lymphocytes (+++); large lymphocytes (+); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (+); microcytic cells (++); hypochronic cells (+);

schistocytes (+); stomatocytes (+); target cells (++); polychromatic cells (++); nucleated RBC (+) [Normoblast]; and crenata cells (++). **PLT**: Platelet appeared adequate in numbers and normal in size.

Aftter 24hrs Group four showed **WBC**: Leukocytes showed mild leukocytosis with relative lymphocytosis, large lymphocytes (+); small lymphocytes (++); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (++); microcytic cells (++); hypochronic cells (+); target cells (++); polychromatic cells (++); nucleated RBC (+) [Normoblast]; schistocytes (+); stomatocytes (+); and crenata cells (++). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 24hrs Group five showed **WBC**: Leukocytes appeared adequate in number with preponderance of lymphocytes; small lymphocytes (++); large lymphocytes (++); no atypical cells seen. **RBC**: Eythrocytes showed normocytic; normochromic cells (++); polychromatic cells (+); target cells (+). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 24hrs Group six showed **WBC**: Leukocytes showed mild leukocytosis with relative lymphocytosis, small lymphocytes (++); large lymphocytes (+); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (++); microcytic cells (+); hypochronic cells (++); schistocytes (+); stomatocytes (+); target cells (++); polychromatic cells (++) and nucleated megaloblast (+) [Normoblast]. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 1 showed **WBC**: Leukocytes appeared adequate in number with relative lymphocytosis, small lymphocytes (+++) and few neutrophils. **RBC**: Showed ovalocytes (++), stomatocytes (++); normocytic and normochronic cells (++), polychromatic cells (+++), nucleated RBCs (+), target cells (+) and few crenated cells. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 2 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, small lymphocytes (++); large lymphocytes (+); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (++); microcytic cells (++); hypochronic cells, stomatocytes (+); target cells (+); macropolychromatic cells (+) and crenated cells (++). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 3 showed **WBC**: Leukocytes appeared adequate in number with relative lymphocytosis, lymphocytes (++); neutrophil (+); no atypical cells seen. **RBC**: Eythrocytes showed normocytic and normochronic cells, polychromatic cells (++); stomatocytes (+) and few crenata cells. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 4 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, large lymphocytes (+); small lymphocytes (+++); no atypical cells seen. **RBC**: Eythrocytes showed macrocytic cells (++); hypochronic cells (+); stomatocyte (++), polychromatic cells (++); crenated cells (+), schistocytes (+) and few target cells. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 5 showed **WBC**: Leukocytes appeared adequate in number with relative lymphocytosis, small lymphocytes (+); large lymphocytes (++); neutrophil (+) no atypical cells seen. **RBC**: Eythrocytes showed normocytic; normochromic cells (++); polychromatic cells (+); target cells (+) and few crenated cells. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 7 days Group 5 showed **WBC**: Leukocytes appeared adequate in number with relative lymphocytosis, small lymphocytes (++); large lymphocytes (+); neutrophils (+) no atypical cells seen. **RBC**: Eythrocytes showed microcytic cells (++); hypochronic cells (+); stomatocytes (++); polychromatic cells (++), ovalocytes (++), target cells (+) and few crenated cells.. **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 1 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, small lymphocytes (++); large lymphocytes (++); no plasmacytoid cells seen. **RBC**: Eythrocytes showed normocytic and normochronic cells (+++); target cells (+); polychromatic cells (++); and crenata cells (+). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 2 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, small lymphocytes (+); large lymphocytes (+++); no atypical cells seen. **RBC**: Eythrocytes showed normocytic and normochronic cells (+++); target cells (+); polychromatic cells (++) and crenated cells (+). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 3 showed **WBC**: Leukocytes appeared adequate in number with relative lymphocytosis, small lymphocytes (++); large lymphocytes (+); neutrophils (+); no atypical cells seen. **RBC**: Eythrocytes showed normocytic and normochronic cells (+++); target cells (++); polychromatic cells (++); **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 4 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, large lymphocytes (+); small lymphocytes (+++); no atypical cells seen. **RBC**: Eythrocytes showed normocytic and normochronic cells (+++); polychromatic cells (+); stomatocytes (+); and target cells (+). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 5 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis; small lymphocytes (++); large lymphocytes (+). **RBC**: Eythrocytes showed macrolytic cells (++); hypochronic cells (++); crenated cells (+); target cells (++); few stomatocytes and polychromatic cells (++). **PLT**: Platelet appeared adequate in numbers and normal in size.

After 14 days Group 6 showed **WBC**: Leukocytes appeared adequate in number with absolute lymphocytosis, small lymphocytes (+++); large lymphocytes (++); no lymphoblast seen. **RBC**: Eythrocytes showed normolytic and normochronic cells (++); stomatocytes (++); target cells (++); polychromatic cells (++) and crenated cells (+). **PLT**: Platelet appeared adequate in numbers and normal in size.

Phenylhydrazine-induced haemolysis reliably produced marked erythrocyte damage, as evidenced by significant anisocytosis, poikilocytosis (including schistocytes, stomatocytes, and echinocytes), polychromasia, and the presence of nucleated red blood cells—hallmarks of oxidative stress and compensatory erythropoiesis. These morphological aberrations align with established literature on PHZ toxicity, which generates reactive oxygen species (ROS), denatures haemoglobin into Heinz bodies, and destabilizes the erythrocyte membrane (Jain, 2021; Bain, 2022).

Among the three test extracts, *Justicia carnea* demonstrated the most pronounced restorative effect on erythrocyte morphology. Treated animals exhibited a near-complete normalization of RBC size and shape by day 14, with minimal poikilocytosis and absence of nucleated RBCs—indicators of effective membrane stabilization and resolution of erythropoietic stress. This superior performance correlates strongly with its phytochemical profile, which is exceptionally rich in anthocyanins—particularly delphinidin-3,5-diglucoside—as confirmed by HPLC and spectrophotometric analysis. Anthocyanins are known to intercalate into the phospholipid bilayer of erythrocytes, enhancing membrane rigidity and resistance to oxidative rupture (Oliveira *et al.*, 2022). Additionally, the extract's ability to stimulate erythropoietin (EPO) production, as suggested by elevated reticulocyte counts and polychromasia during early recovery, points to a dual mechanism: cytoprotection and erythropoietic stimulation.

Ipomoea batatas showed moderate but significant restorative activity, primarily attributable to its nutraceutical composition. The high levels of iron, β-carotene, and vitamin C directly support haem synthesis and RBC maturation, while its polyphenolic constituents (e.g., chlorogenic acid and quercetin) provide antioxidant protection. The observed reduction in haemolytic indices and gradual normalization of RBC morphology support its role as a dietary adjunct in managing nutritional and oxidative anaemias. However, its effects were less robust than those of *J. carnea* in reversing acute membrane damage, likely because its mechanism is more supportive than directly pharmacological.

Ficus sur, while exhibiting antioxidant and anti-inflammatory properties *in vitro*, demonstrated the weakest morphological recovery in this model. Although it improved haematological parameters (Hb, PCV) to some extent, persistent poikilocytosis and residual nucleated RBCs suggest limited efficacy in protecting mature erythrocytes from PHZ-induced oxidative insult. This may be due to lower concentrations of membrane-stabilizing flavonoids or the absence of anthocyanins (confirmed by qualitative screening). Its strength may lie in chronic or infection-mediated anaemias, where its antimicrobial and anti-inflammatory actions could address underlying triggers—a hypothesis warranting further investigation.

The dose–response relationship was evident across all extracts, with the 100 mg/kg dose consistently outperforming the 50mg/kg dose in reducing morphological abnormalities and enhancing regenerative markers (e.g., polychromasia). This supports a pharmacological rather than placebo effect and aligns with the principle of herbal dose dependency in ethnopharmacology.

Notably, peripheral blood smear analysis proved more sensitive than standard haematological indices alone in distinguishing the efficacy of the extracts. For instance, while all three plants improved Hb and PCV, only *J. carnea*fully restored normocytic, normochromic

architecture—highlighting the critical value of morphological assessment in evaluating true erythrocyte health.

The stability data for *J. carnea* extract further enhance its translational potential. Retention of >75% anthocyanin content and anti-haemolytic activity after 90 days under ambient tropical conditions (25°C/60% RH) suggests feasibility for community-level preparation and storage, especially if protected from heat and humidity. The correlation between digital red-channel intensity and anthocyanin content also offers a low-cost, field-applicable method for quality control—bridging traditional practice and modern standardization.

5.2 Conclusion

This study provides robust scientific validation for the traditional use of *Justicia carnea*, *Ficus sur*, and *Ipomoea batatas* as haematinic agents in Southern Nigeria. Among them, *Justicia carnea*emerges as the most effective in restoring erythrocyte morphology and function following oxidative haemolytic insult, owing to its high anthocyanin content and dual action as both an erythrocyte membrane stabilizer and erythropoietic stimulant. *Ipomoea batatas* serves as a valuable nutritional adjunct, while *Ficus sur* may be better suited for anaemias with inflammatory or infectious aetiologies.

The findings underscore the importance of integrating morphological endpoints like anisocytosis, poikilocytosis, and nucleated RBCs into preclinical evaluations of anti-anaemic phytomedicines. They also affirm the relevance of ethnobotanically faithful extraction methods—aqueous decoctions—in capturing therapeutically active constituents.

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