

**HAEMATOLOGICAL EVALUATION OF THE POLYHERBAL AQUEOUS  
EXTRACTS IN PHENYL HYDRAZINE HYDROCHLORIDE INDUCED ANAEMIA  
USING ANIMAL MODELS (*Ipomoea batatas*, *Justicia carnea* and *Ficus sur* )**



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**BENIN CITY**

**NOVEMBER ,2025**

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**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE  
LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF  
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LABORATORY TECHNOLOGY.  
(BIOTECHNOLOGY TECHNIQUES)**

**NOVEMBER, 2025**

## **CERTIFICATION**

This is to certify that the undergraduate research work on hematological evaluation of the polyherbal aqueous extracts in phenyl hydrazine hydrochloride induced anaemia using animal model was carried out by GLORY OGHENETEKEVWE AGHWARITEFE with the matriculation number LSC2009937 in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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**(PROJECT SUPERVISOR).**

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**DATE**

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**DR. P.O ALONGE**  
**(PROJECT COORDINATOR)**

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**DATE**

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**EXTERNAL EXAMINER**

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**DATE**

## **DEDICATION**

I dedicate this work to God Almighty for making this project a success, for his grace throughout this project and my entire course of study in University of Benin. I also dedicate this work to my project supervisor, PROF. E.O. OSHOMOH for his invaluable guidance throughout this work, and to my parents, MR and MRS AGHWARITEFE, my friends, relatives and loved ones for their support throughout this journey.

## **ACKNOWLEDGMENT**

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## **ABSTRACT**

Polyherbal formulations are widely utilized across the globe and have gained increasing recognition due to their natural origin and minimal side effects. These combinations enhance therapeutic potency while reducing the required concentrations of individual herbal components. This research investigates the hematological effects of a polyherbal aqueous extract in phenyl hydrazine induced anemic Wistar rats. The plant materials were collected, processed, and formulated into the polyherbal aqueous extract. Thirty-six rats were randomly assigned into six groups: positive control, negative control, normal control, and three treatment groups receiving different doses of the extract. Variations in body and organ weights were assessed, and blood samples were analyzed for biochemical parameters. The results revealed significant differences in white blood cells, lymphocytes, mid-sized cells, granulocytes, platelets, mean platelet volume, platelet distribution width, plateletcrit, and platelet large cell ratio compared to the control groups. These findings support the ethnomedicinal use of the selected plants as anti-anemic agents and highlight their potential for further preclinical and clinical evaluation. Overall, the study suggests that the polyherbal aqueous extract may serve as a promising alternative therapy for anaemia.

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF THE STUDY

Anaemia characterized by reduced haemoglobin levels or a decrease in red blood cell mass, impairs the efficient delivery of oxygen to body tissues and remains a major global health challenge (Kassebaum *et al.*, 2014). Its causes are multifactorial, ranging from nutritional deficiencies (iron, folate, vitamin B<sub>12</sub>) to infections, genetic haemoglobin disorders, chronic illnesses, and blood loss. (WHO, 2023). According to recent data, in 2023 an estimated 30.7% of women of reproductive age (15–49 years) and 35.5% of pregnant women worldwide were affected by anaemia, underscoring its significant health, educational, and socio-economic impacts and the urgent need for effective interventions (WHO, 2023). Ethno-medicinal reports highlight the use of natural remedies in managing anaemia and its complications (Gupta and Mishra, 2020). A promising approach under investigation is the development of polyherbal formulations from medicinal plants traditionally valued for their blood-enriching properties. Notable among these are *Ipomoea batatas* (sweet potato leaves), *Justicia carnea* (commonly known as “Blood of Jesus” leaves), and *Ficus sur* (Cape fig or fig of heaven). These plants have demonstrated haematinic potential and are increasingly being explored as viable therapeutic options for anaemia. Their leaves are rich in phytochemicals with potent antioxidant and anti-inflammatory properties, in addition to exhibiting blood-boosting (haematinic) effects (Ukwubile *et al.*, 2014; Onyeabo *et al.*, 2017; Aruomah *et al.*, 2021). Several studies have evaluated the individual effects of these plants in animal models of phenylhydrazine hydrochloride (PHZ-HCl)–induced anaemia. PHZ-HCl is commonly used in experimental studies due to its potent haemolytic activity, which closely mimics haemolytic anaemia in humans (Roque *et al.*, 2008). Administration of *Ipomoea batatas* extract to

anaemic rats resulted in significant improvements in red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The extract also exhibited antioxidant properties and was deemed safe based on behavioural observations, survival outcomes, and histopathological evaluations (Gabriel and Idu, 2021). Similarly, treatment with *Justicia carnea* in PHZ-HCl-induced anaemic rats produced notable recovery in packed cell volume (PCV), red blood cell count (RBC), and haemoglobin (Hb) levels ( $p < 0.05$ ) compared to untreated anaemic controls. Additionally, positive effects on lipid metabolism were reported, including reductions in cholesterol and triglyceride levels (Onyeabo *et al.*, 2017). In the same vein, studies on *Ficus sur* revealed its haematinic activity in phenylhydrazine-induced anaemia, further supporting its therapeutic role in correcting blood deficiencies (Adebayo *et al.*, 2017). Collectively, these findings indicate that aqueous leaf extracts of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* possess therapeutic benefits against anaemia, likely due to their antioxidant, anti-inflammatory, and haematinic properties. This highlights their potential as complementary and alternative remedies for anaemia management. Elucidating the haemoprotective mechanisms of their bioactive compounds could pave the way for innovative treatment strategies that are safer, more affordable, and more accessible. Therefore, this study seeks to investigate the combined effectiveness of these three plants as polyherbal aqueous extracts in alleviating anaemia-related dysfunctions. By incorporating recent scientific evidence, the study contributes to the growing body of literature supporting plant-based polyherbal therapies for the management of anaemia and its associated complications (Onyeabo *et al.*, 2017; Gabriel and Idu, 2021).

## **1.2 Aim**

The aim of this study is to evaluate the haematological effects of polyherbal aqueous leaf extracts in phenylhydrazine hydrochloride induced anaemia using Wistar rats as animal models.

## **1.3 Objectives**

The following are the objectives of the study which includes to;

1. Investigate the haematological effects of polyherbal aqueous extracts in phenylhydrazine hydrochloride induced anaemia using Wistar rats as an animal model.
2. Evaluate the haematological properties of polyherbal aqueous extracts and their potential role in the treatment of anaemia.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 *Ipomoea batatas*

It is commonly known as sweet potato, is an annual crop of the family Convolvulaceae (morning glory family), though in tropical climates it may also grow perennially (Sun *et al.*, 2021). The name reflects both Greek and indigenous Caribbean origins: *Ipomoea* derives from Greek words meaning “worm” and “resembling,” describing its twining habit (Austin, 1988), while *batatas* comes from the Taíno word for sweet potato, later adapted by Spanish explorers into *patata* and influencing the English word “potato” (Zeven and Zhukovsky, 1975; Yen, 1982). Cultivated globally as a staple food, sweet potato tubers are rich in vitamins A and C, fibre, and minerals. Its leaves, stems, and roots also provide bioactive compounds such as carotenoids, anthocyanins, phenolic acids, and flavonoids with antioxidant, antidiabetic, anticancer, hepatoprotective, antimicrobial, and immunostimulant activities (Islam, 2024). Beyond nutrition, *Ipomoea batatas* is used industrially for starch, flour, alcohol, and animal feed (Woolfe, 1992), and in traditional medicine for anaemia, gastrointestinal disorders, and inflammation (Shekhar *et al.*, 2015). Some species are grown as ornamentals or used in cultural practices due to psychoactive compounds (Austin, 2007). Believed to have originated in Central or South America, sweet potato has been cultivated for thousands of years for food, medicinal, and ornamental purposes (Yen, 1982; Austin, 1988). Its rich nutrient profile and diverse bioactive properties underscore its importance as both a dietary staple and a medicinal plant (Woolfe, 1992).

### **2.1.1 Botanical Description**

*Ipomoea batatas* (sweet potato) is a herbaceous perennial vine of the family Convolvulaceae. The species is characterized by slender, prostrate, and trailing stems that may extend several meters, with adventitious roots emerging at the nodes. The leaves exhibit considerable morphological variation, ranging from entire, cordate forms to deeply lobed types, and are alternately arranged on elongated petioles. The inflorescences bear funnel-shaped flowers, typically pale violet to purple with a darker throat, closely resembling those of related morning glory species. The storage roots represent the most distinctive organ of the plant, being enlarged, tuberous, and enriched with starch, simple sugars, and bioactive pigments such as  $\beta$ -carotene and anthocyanins. Seed production is infrequent under cultivation, as the species is predominantly propagated vegetatively through stem cuttings or vine slips (Woolfe, 1992; Austin, 2007).

### **2.1.2 Classification**

Kingdom: Plantae

Phylum (Division): Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Order: Solanales

Family: Convolvulaceae

Genus: *Ipomoea*

Species: *Ipomoea batatas* (L.) Lam



**Figure 2.1:** Leaves of *Ipomoea batatas*

**Source:** (Vecteezy,2023).

### **2.1.3 Ethnomedicinal Benefits**

*Ipomoea batatas* (sweet potato) is highly valued for both its nutritional and medicinal applications in traditional medicine. Different plant parts including tubers, leaves, and stems are employed in the management of diverse health conditions. The leaves, widely consumed as vegetables in Africa and Asia, possess antioxidant, anti-inflammatory, and hematinic properties, making them useful in treating anemia and oxidative stress-related disorders (Afolayan and Jimoh, 2009; Islam *et al.*, 2016). The leaves contain high iron, vitamin C and folate which further supports blood formation (Afolayan and Jimoh, 2009). The tubers are traditionally used to relieve constipation and digestive problems due to their fiber content (Laurie *et al.*, 2015). In Asian medicine, they are applied in diabetes management owing to bioactive compounds such as caffeoylquinic acids, which regulate blood glucose (Truong *et al.*, 2018). Purple-fleshed varieties are particularly rich in anthocyanins and are believed to confer anticancer, hepatoprotective, and cardioprotective benefits (Kano *et al.*, 2005). In addition, sweet potato is used in indigenous practices to boost immunity, enhance lactation, and serve as a general health tonic, emphasizing its dual role as both food and medicine.

### **2.1.4 Phytochemical Properties**

Sweet potato (*Ipomoea batatas*) is recognized for its notable antioxidant capacity, which is mainly attributed to its abundance of carotenoids, flavonoids, anthocyanins, and polyphenols. These bioactive compounds function as free radical scavengers, thereby limiting oxidative stress and preventing cellular injury (Zhang *et al.*, 2019). Orange-fleshed varieties are rich in  $\beta$ -carotene, a precursor of vitamin A, that plays a vital role in neutralizing reactive oxygen species (ROS) and lowering the risk of diseases linked to oxidative damage, including cardiovascular illnesses and cancer (Laurie *et al.*, 2018). Purple-fleshed varieties are a significant source of anthocyanins,

natural pigments that exhibit strong antioxidant effects by inhibiting lipid peroxidation, protecting DNA, and modulating oxidative enzymes (Zhang *et al.*, 2019). These compounds are also associated with improved vascular health and a reduced likelihood of metabolic disorders (Wang *et al.*, 2017; Truong *et al.*, 2020). Additionally, the leaves and tubers contain polyphenols such as chlorogenic acid, caffeic acid, and quercetin, which enhance antioxidant defense by donating hydrogen atoms and chelating metal ions, further contributing to the plant's therapeutic potential. Overall, the antioxidant constituents of *Ipomoea batatas* support its role as a functional food with potential benefits in preventing or managing chronic diseases such as diabetes, cancer, and liver-related disorders (Zhao *et al.*, 2021).

## **2.1.5 Pharmacological Properties**

### **2.1.5.1 Antimicrobial activity**

Sweet potato (*Ipomoea batatas*) has demonstrated promising antimicrobial activity, particularly in leaf and extract forms. A 2024 study found that leaves from various cultivars exhibited inhibitory effects against several Gram-positive bacteria (such as, *Staphylococcus aureus*, *Streptococcus mutans*, *Listeria monocytogenes*) and Gram-negative bacteria (such as, *Escherichia coli*, *Shigella dysenteriae*), as well as fungi like *Candida albicans* with methanolic extracts showing the strongest activity (Sultana *et al.*, 2024). Another study from 2023 reported moderate antibacterial activity of an ethanol extract against *S. aureus* and *S. mutans*, though inhibition zones were fairly modest (Mayasari *et al.*, 2023). These antimicrobial effects are attributed to the plant's rich content of phenolic and flavonoid compounds (such as, caffeic acid, quercetin) which may disrupt microbial membranes and inhibit growth (Sultana *et al.*, 2024).

### **2.1.5.2 Antinociceptive effects**

The leaves of sweet potato has demonstrated notable antinociceptive or pain-reducing effects in experimental animal studies. When mice were administered aqueous extracts of the plant's leaves at varying doses (50, 100, and 200 mg/kg), a significant decrease in pain-related behaviors was observed. This reduction was evident in both peripheral pain models, such as the acetic acid-induced writhing test, and in central pain models, including the hot-plate test. Furthermore, the extract also showed the ability to suppress pain in rats subjected to the formalin-induced pain model, which involves both neurogenic and inflammatory phases of pain. These results indicate that *Ipomoea batatas* may influence multiple pain pathways, supporting its potential as a natural analgesic agent (Oshomoh *et al.*, 2020).

### **2.1.5.3 Antioxidative activity**

It exhibits significant antioxidant activity due to its rich content of phytochemicals such as phenolic acids, flavonoids, anthocyanins, and carotenoids. These compounds act as free radical scavengers, reducing oxidative stress and protecting cellular components from damage. Purple-fleshed varieties are particularly high in anthocyanins, while orange-fleshed varieties contain abundant  $\beta$ -carotene, both of which contribute to enhanced antioxidant capacity (Truong *et al.*, 2020). Additionally, extracts from sweet potato leaves have shown strong in vitro antioxidant activity, correlating with high levels of total phenolics and flavonoids (Sultana *et al.*, 2024). These antioxidant properties play a role in reducing the risk of chronic diseases related to oxidative damage.

#### **2.1.5.4 Anti-inflammatory and Anti arthritis activity**

*Ipomoea batatas* extracts, particularly those derived using ethyl acetate, have demonstrated significant anti-inflammatory and anti-arthritic effects in experimental animal models. These extracts markedly reduced both acute and chronic inflammation, including carrageenan-induced paw swelling (with approximately 79% inhibition), croton oil-induced ear and anal edema, as well as inflammation associated with Complete Freund's Adjuvant (CFA)-induced arthritis. Treatment also led to decreased levels of key inflammatory mediators such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and nitric oxide (NO), alongside restoration of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). In several models, the extracts showed efficacy comparable to standard anti-inflammatory drugs like ibuprofen (Majid *et al.*, 2018).

#### **2.2 *Justicia Carnea***

*Justicia carnea* commonly referred to as the "Brazilian plume", "Blood of Jesus plant" or "Ewe eje" in Yoruba, is a perennial shrub native to tropical and subtropical regions. It belongs to the Acanthaceae family and is widely recognized for its ornamental value as well as its extensive use in traditional medicine (Anarado *et al.*, 2021). In both West Africa and South America, the plant has been used to treat a range of ailments, including anaemia, diabetes, malaria, inflammation, and oxidative stress (Ukpabi-Ugo *et al.*, 2020; Ojeaburu and Olasehinde, 2024). Phytochemical studies have shown that the plant contains active compounds such as flavonoids, saponins, and alkaloids, which contribute to its therapeutic properties (Anarado *et al.*, 2021). Notably, the plant has demonstrated anti-diabetic effects by lowering blood glucose levels and improving lipid profiles in alloxan-induced diabetic rats (Ukpabi Ugo *et al.*, 2020). Furthermore, its methanolic leaf extract

has been shown to improve kidney function and reduce oxidative stress in diabetic conditions (Ojeaburu and Olasehinde, 2024). From a nutritional standpoint, *Justicia carnea* leaves are rich in protein, fiber, vitamins A and C, and essential minerals like iron and calcium, which are vital for blood formation and general well-being (Enaohwo *et al.*, 2024). Experimental studies confirm that its consumption can significantly enhance hematological indices such as hemoglobin concentration, red blood cell count, and packed cell volume, especially in cases of induced anemia (Enaohwo *et al.*, 2024).

### **2.2.1 Botanical Description**

It is a tropical, evergreen shrub that belongs to the Acanthaceae family and is indigenous to Brazil. This species generally attains a height of 1 to 2 meters and displays an upright, densely branched growth form. Its leaves are arranged oppositely, possess an ovate shape, and are dark green with distinct venation, typically ranging from 10 to 20 cm in length (Govaerts and Nic Lughadha, 2023). One of the plant's most distinguishing features is its vivid inflorescence, which consists of erect, spike-like clusters of tubular flowers in shades of pink to deep red. These bilabiate flowers are highly attractive to pollinators such as butterflies and hummingbirds, indicating a mutualistic ecological role in its native and cultivated habitats (Ijoma *et al.*, 2025). *Justicia carnea* performs optimally in partially shaded environments with well-drained, fertile soil and consistent moisture levels, making it suitable for tropical and subtropical landscaping. It is valued as an ornamental species and is commonly propagated through softwood stem cuttings, which root readily under humid conditions (Chimezie *et al.*, 2024; Odeleke *et al.*, 2024).

### **2.2.2 Classification**

Kingdom: Plantae

Phylum (Division): Tracheophyta (Magnoliophyta – Angiosperms)

Class: Magnoliopsida (Dicotyledonae)

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia*

Species: *Justicia carnea* Lindl.



**Figure 2.2 :** Leaves and flower of *Justicia carnea*

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

### **2.2.3 Ethnomedicinal Benefits**

*Justicia carnea* Lindl. is highly valued in African traditional medicine for its diverse ethnomedicinal applications. The leaves are commonly prepared as a blood tonic to treat anaemia, restore blood after illness or injury, and aid recovery in women post-childbirth as well as in patients convalescing from prolonged sickness, highlighting its traditional role as a restorative herb (Oloruntola *et al.*, 2023). The plant is also employed in the management of sickle cell disease, with in-vitro studies confirming its anti-sickling activity (Iyekowa, 2024). Furthermore, it is traditionally used to combat malaria and associated fevers, a claim supported by experimental studies showing inhibitory action against malaria parasites (Anarado *et al.*, 2021). Its folk use extends to the treatment of diarrhoea, gastrointestinal disorders, and wound infections, consistent with antimicrobial evidence demonstrating activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Ijoma *et al.*, 2025). In addition, *Justicia carnea* serves as a dietary supplement and general vitality enhancer. Its high content of iron, vitamins, flavonoids, and other bioactive compounds not only contributes to hematopoiesis but also enhances overall wellness and resilience against disease (Oloruntola *et al.*, 2023). Collectively, these attributes emphasize the dual role of *Justicia carnea* as both a medicinal plant and a nutritional resource, supporting its relevance in traditional healthcare systems and its potential as a candidate for further pharmacological exploration.

### **2.2.4 Phytochemical properties**

Phytochemical research on *Justicia carnea* has revealed a diverse array of bioactive compounds that underpin its extensive medicinal applications. The leaves are particularly abundant in flavonoids, tannins, alkaloids, saponins, and phenolic compounds, all of which contribute

significantly to its potent antioxidant and antimicrobial properties (Anarado *et al.*, 2021). These phytochemicals play key roles in neutralizing free radicals, protecting cells from oxidative stress, and providing broad-spectrum defense against pathogenic organisms. Additionally, the detection of steroids and terpenoids further enhances the pharmacological profile of the plant, as these compounds are well known for their anti-inflammatory, analgesic, and cytoprotective effects, which align with its traditional use in treating inflammatory disorders and supporting tissue recovery (Peters *et al.*, 2022). Beyond these constituents, the plant has also been shown to contain glycosides and reducing sugars, which may contribute to its hematopoietic and restorative benefits. These compounds are particularly significant in explaining its ethnomedicinal application in the treatment of anaemia, blood loss, and conditions requiring rapid recovery of vitality (Oloruntola *et al.*, 2023). Advanced analyses have further identified anthocyanins and chlorophyll derivatives in the plant. These pigments not only account for the characteristic reddish-green coloration of the leaves but also enhance its nutraceutical appeal by boosting antioxidant defenses and supporting overall metabolic health (Anarado *et al.*, 2021). Moreover, phytochemical screenings have revealed the presence of essential macro- and micro-minerals, including iron, calcium, magnesium, and phosphorus, which are indispensable for hematopoiesis, bone strength, and cellular metabolism (Ijoma *et al.*, 2025). These nutritional components reinforce its long-standing use as a blood tonic, dietary supplement, and general vitality enhancer in traditional medicine. Taken together, the wide spectrum of phytochemicals in *Justicia carnea* provides a scientific basis for its diverse therapeutic roles in managing anaemia, infections, malaria, inflammatory conditions, and in promoting overall wellness.

## **2.2.5 Pharmacological properties**

### **2.2.5.1 Antioxidant activity**

*Justicia carnea* demonstrates strong antioxidant potential, primarily due to its high levels of flavonoids, phenolics, alkaloids, and saponins. These compounds neutralize free radicals, thereby reducing cellular and tissue damage. Studies reveal that the plant's extracts boost antioxidant defenses by enhancing enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while also elevating reduced glutathione (GSH) (Anarado *et al.*, 2021). In addition, administration of the plant extract has been shown to lower oxidative stress biomarkers such as malondialdehyde (MDA) and nitric oxide (NO) in animal models subjected to chemically induced oxidative damage (Enaohwo *et al.*, 2025). Collectively, these results suggest that *Justicia carnea* helps maintain redox homeostasis and protects against lipid peroxidation.

### **2.2.5.2 Anti-anemic or Hematinic effects**

*Justicia carnea* has been widely reported to possess anti-anemic (hematinic) properties, which are linked to its rich phytochemical and nutritional composition. The leaves contain bioactive compounds such as flavonoids, saponins, alkaloids, tannins, and phenolics, alongside essential micronutrients including iron, folate, vitamin C, and proteins, all of which contribute to blood formation (Anarado *et al.*, 2021). Experimental studies have shown that administration of *Justicia carnea* leaf extracts significantly improves hematological indices in anemic models. For example, in phenylhydrazine-induced anemic rats, treatment with ethanol leaf extract markedly increased hemoglobin concentration, packed cell volume (PCV), and red blood cell (RBC) count, demonstrating its hematinic effect (Onyeabo *et al.*, 2017). Similarly, other studies report that the

plant helps restore iron levels and enhances erythropoiesis, supporting its traditional use as a blood tonic (Mbanaso *et al.*, 2020).

### **2.2.5.3 Anti-diabetic or Enzyme inhibitory Activity**

*Justicia carnea* has shown notable antidiabetic potential, largely through the inhibition of carbohydrate-digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase. Since these enzymes facilitate the breakdown of complex carbohydrates into glucose, their suppression helps in controlling post-meal blood sugar spikes. Both *in vitro* and *in vivo* investigations indicate that ethanol leaf extracts of *Justicia carnea* effectively inhibit these enzymes, thereby enhancing glycemic regulation in experimental models (Ani *et al.*, 2020). In addition, treatment with the extract significantly reduced fasting blood glucose and enhanced insulin sensitivity in streptozotocin-induced diabetic rats, supporting its possible application as a natural antidiabetic remedy (Onyekwere and Ojeka, 2023). These beneficial actions are attributed to bioactive phytochemicals such as flavonoids and phenolics, which play critical roles in modulating glucose metabolism.

### **2.2.5.4 Hepatoprotective effects**

Beyond its anti-diabetic activity, *Justicia carnea* demonstrates strong hepatoprotective activity. In models of carbon tetrachloride (CCl<sub>4</sub>)-induced liver damage, pretreatment with methanol leaf extract led to marked reductions in serum liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), alongside improvements in total protein and bilirubin levels (Ezeonu *et al.*, 2018). Histopathological studies further revealed that the extract preserved normal liver structure, preventing necrosis and fatty changes typically caused by Carbon tetrachloride (CCl<sub>4</sub>) toxicity (Ezeonu *et al.*, 2018). Likewise, in diabetic Wistar

rats, both aqueous and methanol extracts enhanced liver function by boosting antioxidant enzyme defenses and lowering MDA concentrations, thereby limiting oxidative stress–induced liver injury (Ojeaburu and Olasehinde, 2024). These results suggest that the hepatoprotective capacity of the plant is closely linked to its antioxidant phytochemicals, which help stabilize liver cell membranes and support tissue repair.

### **2.3 *Ficus sur***

*Ficus sur* Forssk., commonly known as the bush fig or Cape fig, is a woody perennial plant and a member of the Moraceae family widely distributed across tropical and southern Africa. Its name is derived from Latin, where the genus name 'Ficus' means “fig”, referring to its characteristic fig-like fruits, and the species epithet 'sur' means “south”, indicating its natural distribution in southern Africa (Mabberley, 2017). It is a multipurpose tree valued for its edible fruits and diverse ethnomedicinal applications. Traditionally, different parts of the plant, including the leaves, bark, roots, and latex, are used in managing ailments such as anemia, chest pains, diarrhea, infertility, and skin diseases (Ogunlaja *et al.*, 2022). Phytochemical studies have identified bioactive compounds such as triterpenoids, sterols, and flavonoids, which contribute to its reported antioxidant, antimicrobial, and anti-inflammatory activities (Mouelle *et al.*, 2022). Nutritionally, the fruits and leaves are rich in phenolics, vitamins, and minerals, which supports their use as a “blood-building” food in traditional medicine, although more scientific validation is required (Ogunlaja *et al.*, 2022).

#### **2.3.1 Botanical Description**

It is a perennial evergreen tree reaching 20–30 m, though sometimes shrubby in harsh environments (Chikuni, 2003). It has a spreading crown, often buttressed trunk up to 2 m in

diameter, and smooth to slightly fissured gray-brown bark that exudes milky latex when cut (White, 1983). Leaves are simple, alternate, broadly ovate to elliptic, 8–20 × 5–12 cm, glossy green above and paler beneath, with entire to slightly wavy margins. The tree is monoecious, producing small globose to oblong syconia that ripen yellow to reddish and occur in leaf axils( Berg, 2001). It grows in forests, savannas, and along rivers across tropical and southern Africa (Pooley , 1993). Ecologically, it is a keystone species, valued for shade, soil stabilization, and its year-round fruit supply for wildlife (Chikuni, 2003).

### **2.3.2 Botanical Classification**

Kingdom: Plantae

Phylum (Division): Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Eudicots)

Order: Rosales

Family: Moraceae

Genus: *Ficus* L.

Species: *Ficus sur* Forssk.



**Figure 2.3:** Leaves of *Ficus sur*

Source: (iNaturalist, 2022).

### **2.3.3 Ethnomedicinal benefits**

*Ficus sur* Forssk. (Moraceae) is widely used in African ethnomedicine for its diverse therapeutic applications. The leaves and fruits are traditionally employed as blood tonics for the management of anaemia, attributed to their high iron and antioxidant content (Ogunlaja *et al.*, 2022). They are also applied in the treatment of wounds, skin infections, and sexually transmitted infections due to their antimicrobial and wound-healing properties (Odion *et al.*, 2023). Extracts from the fruits and leaves exhibit significant antibacterial activity against pathogens such as *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*, validating their traditional use in gastrointestinal infections (Investigational Medicinal Chemistry and Pharmacology, 2022). In addition, the plant is reported to be useful in the management of malaria, fever, and gastrointestinal disorders including diarrhoea and stomach pain (Odusanmi *et al.*, 2017). Ethnobotanical surveys also highlight its role in treating infertility, respiratory complications, urinary retention, and sickle-cell disease, further supported by studies on its antioxidant and antiplasmodial activities (Nacoulma and Guissou, 2021; Diatta *et al.*, 2024). These pharmacological findings provide scientific validation for the traditional uses of *Ficus sur* in primary healthcare.

### **2.3.4 Phytochemical properties**

*Ficus sur* is a phytochemically rich species containing both primary and secondary metabolites that account for its medicinal and nutritional value. The leaves, fruits, and bark are particularly abundant in phenolics and flavonoids, which are central to the plant's strong antioxidant capacity (Ogunlaja *et al.*, 2022; Odion *et al.*, 2023). Identified phenolics include epicatechin and several phenolic acids, while tannins and various flavonoid classes have also been reported (Nacoulma and Guissou, 2021; Odion *et al.*, 2023). Additionally, sterols and triterpenoids such as  $\beta$ -sitosterol

and lupeol have been isolated, compounds that are well recognized for their anti-inflammatory and antimicrobial effects (Investigational Medicinal Chemistry and Pharmacology, 2022; Ogunlaja *et al.*, 2022). Beyond these, alkaloids, saponins, and glycosides have been detected in qualitative phytochemical analyses, further explaining the plant's antimicrobial and blood-enriching (haematinic) applications in traditional medicine (Odusanmi *et al.*, 2017; Odion *et al.*, 2023). The presence of carotenoids, alongside essential minerals like iron, calcium, magnesium, and manganese, as well as proximate nutrients such as protein and fibre, also highlights the plant's nutritional and haematinic importance (Odusanmi *et al.*, 2017; Diatta *et al.*, 2024). Advanced analytical studies, including Gas chromatography and mass spectrometry have identified volatile fatty acid esters, sterol derivatives, and other small organic compounds in fruit extracts. These findings suggest that volatile and low-molecular-weight constituents also contribute to the plant's antibacterial activity (Investigational Medicinal Chemistry and Pharmacology, 2022; Odion *et al.*, 2023). The phytochemical composition of *Ficus sur* characterized mainly by phenolics, flavonoids, sterols/triterpenoids, alkaloids, and saponins provides a strong biochemical basis for its antioxidant, antimicrobial, anti-inflammatory, and nutritional properties. These results lend scientific support to its widespread ethnomedicinal applications and highlight its pharmacological potential (Nacoulma and Guissou, 2021; Ogunlaja *et al.*, 2022; Diatta *et al.*, 2024).

## **2.3.5 Pharmacological properties**

### **2.3.5.1 Antioxidant activity**

The leaf and fruit extracts of *Ficus sur* demonstrate notable free radical scavenging and reducing abilities, largely due to their abundance of phenolic and flavonoid compounds. These antioxidant

properties play a key role in safeguarding the body against oxidative stress and associated health conditions (Ogunlaja *et al.*, 2022; Odion *et al.*, 2023).

### **2.3.5.2 Haematinic and anti-anaemic activity**

Due to its richness in iron, minerals, and antioxidants, *Ficus sur* leaves and fruits are traditionally used as blood tonics. Experimental studies in anaemic models confirm its haematinic and erythropoietic potential (Odusanmi *et al.*, 2017; Diatta *et al.*, 2024).

### **2.3.5.3. Wound healing and dermatological applications**

The latex and leaf extracts have been applied for wound healing, skin infections, and sexually transmitted infections, effects attributed to their antimicrobial and antioxidant components (Odion *et al.*, 2023).

### **2.3.5.4. Antiplasmodial and antimalarial activity**

Ethnobotanical surveys and pharmacological assays report that extracts exhibit antiplasmodial effects, supporting its traditional use in the treatment of malaria and fever (Nacoulma and Guissou, 2021; Diatta *et al.*, 2024).

### **2.3.5.5 Antimicrobial properties**

Leaf and fruit extracts demonstrate antibacterial activity against pathogens including *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*. This supports its traditional use in managing gastrointestinal and skin infections (Investigational Medicinal Chemistry and Pharmacology, 2022; Odion *et al.*, 2023).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Plant Collection and Identification

Fresh leaves of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* were collected from Benin City, Edo State, Nigeria, on the 8th July 2025. To ensure botanical accuracy, the plant samples were authenticated by Prof. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, University of Benin. Voucher specimens with the identification numbers UBH-I493 for *Ipomoea batatas*, UBH-J386 for *Justicia carnea* and UBH-F331 for *Ficus sur* were deposited at the University's herbarium for future reference (Akinnibosun, 2025).

#### 3.2 Plant Preparation and Polyherbal Extraction

The collected leaves were thoroughly rinsed with water to remove soil and contaminants and subsequently allowed to dry at room temperature in a shaded area for a period of two to three weeks to preserve their bioactive compounds. The completely dried leaves were separately pulverized into a fine powder using an industrial mechanical blender. A polyherbal mixture was formulated by combining 93 grams of each powdered plant, resulting in a total mass of 279 grams in a 1:1:1 ratio. The extraction process employed cold maceration to maximize the extraction of polar constituents and prevent thermal degradation. The powdered blend was transferred into a soaking jar and macerated with 1900 ml of cold distilled water, which was added in incremental volumes (1000, 500, and 400 ml) to ensure a homogeneous mixture. The jar was sealed and left to stand for 72 hours at room temperature, with intermittent shaking every four hours to enhance extraction efficiency. Thereafter, the macerate was filtered first through a mesh sieve and then

suction-filtered to obtain a clear filtrate. The filtrate was concentrated into a semi-solid extract using a water bath. A stock solution was prepared from this extract for dosage administration, with the concentration calculated and confirmed for accurate dosing.

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

A total of thirty-six (36) adult Wistar rats, of both sexes, were used for this study. The use of both male and female rats was a deliberate choice to prevent gender bias and to account for the higher susceptibility of females to hemolytic anemia conditions, such as those potentially influenced by menstrual blood loss. The animals were randomly divided into six (6) groups, each containing six rats (n=6). Hemolytic anaemia was induced in the rats in Groups 1, 2, 3, 4 and 6 using a freshly prepared solution of Phenylhydrazine hydrochloride (PHZ-HCl). Prior to induction, the animals were fasted for 24 hours to ensure optimal absorption. A dosage of 40 mg/kg body weight of PHZ-HCl was administered via orogastric injection daily for seven consecutive days. The induction was confirmed by the manifestation of characteristic anemic symptoms, including paleness or dullness of the eyes, ears, tails, and paws, laboured breathing, gasping, lethargy characterized by prolonged immobility, and reduced appetite for six (6) days of induction.

### **3.4 Experimental Design and Dosage Administration**

The grouping of the animals was designed as follows:

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.

Treatment administration commenced after the confirmation of anaemia and continued for 14 days. The individual daily dose of the polyherbal extract for each rat in the treatment groups was calculated based on its most recent body weight using the formula:  $\text{Dose (mg/kg)} \times \text{Body Weight (g)} / 1000$  and the percentage yield of the extract was 15.6%. This calculation determined the precise volume of the stock solution to be administered orally once daily using an orogastric tube. Similarly, the positive control group received Folic acid at 5 mg/kg. The negative and normal control groups received no therapeutic intervention. Each rat was first anesthetized in a desiccator containing cotton wool saturated with chloroform until unconsciousness and loss of reflexes were confirmed and were sacrificed afterward. The samples were collected and analyzed.

### **3.5 Statistical Analysis**

All data generated from hematological and biochemical analyses were expressed as Mean  $\pm$  Standard Error of the Mean (SEM). The data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS) version 16.0. A one-way analysis of variance (ANOVA) was used to determine the presence of significant differences among the experimental groups. Where significant differences existed, Duncan's Multiple Range Test was applied as a post-hoc analysis. A probability value of less than or equal to 0.05 ( $p \leq 0.05$ ) was considered statistically significant.

## CHAPTER FOUR

### RESULTS

#### 4.1 Hematological Analysis of Rats Treated with Herbal Extract

The following tables present the hematological parameters of rats treated with the herbal extract at different doses, compared to positive (Folic Acid), negative (PHZ), and normal control groups over 14 days.

Table 4.1: Effect of Polyherbal aqueous leaf Extract on White Blood Cells (WBC) Count ( $10^3/\mu\text{L}$ ) in Phenylhydrazine induced anaemic Wistar rats

Groups	Day 1	Day 7	Day 14
Positive Control (Folic Acid)	$19.50 \pm 1.41$	$6.05 \pm 1.20$	$4.60 \pm 0.28$
Negative Control (PHZ)	$15.85 \pm 2.76$	$8.40 \pm 2.12$	$3.35 \pm 0.78$
Normal Control	$20.35 \pm 3.46$	$10.35 \pm 1.34$	$4.60 \pm 0.42$
Extract (25 mg/kg)	$16.00 \pm 2.26$	$7.40 \pm 3.39$	$5.00 \pm 0.85$
Extract (50 mg/kg)	$10.05 \pm 0.78$	$7.90 \pm 1.13$	$4.30 \pm 0.28$
Extract (100 mg/kg)	$16.30 \pm 4.95$	$8.05 \pm 2.19$	$4.20 \pm 0.42$

The WBC count decreased in all groups over time, with the positive control (Folic Acid) and normal control maintaining higher counts compared to the negative control (PHZ).

Table 4.2: Effect of Polyherbal Aqueous Leaf Extract on Mid sized cells in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	4.40 ± 0.57	1.20 ± 0.14	0.45 ± 0.07
Negative Control (PHZ)	3.40 ± 0.28	1.15 ± 0.21	0.30 ± 0.14
Normal Control	4.25 ± 0.07	1.80 ± 0.42	0.35 ± 0.07
Extract (25 mg/kg)	3.55 ± 1.63	1.75 ± 0.21	0.40 ± 0.00
Extract (50 mg/kg)	1.65 ± 0.49	1.55 ± 0.78	0.30 ± 0.14
Extract (100 mg/kg)	3.85 ± 1.20	1.85 ± 0.07	0.35 ± 0.07

The Mid Sized Cells count showed a significant reduction across all groups by Day 14, with the positive control maintaining the highest values.

Table 4.3: Effect of Polyherbal Aqueous Leaf Extract on Granulocytes in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	1.50 ± 0.20	0.60 ± 0.10	0.20 ± 0.05
Negative Control (PHZ)	1.50 ± 0.20	0.75 ± 0.15	0.15 ± 0.05
Normal Control	2.00 ± 0.20	1.00 ± 0.10	0.20 ± 0.05
Extract (25 mg/kg)	1.40 ± 0.20	0.80 ± 0.10	0.25 ± 0.05
Extract (50 mg/kg)	1.00 ± 0.20	0.70 ± 0.10	0.15 ± 0.05
Extract (100 mg/kg)	1.60 ± 0.20	0.90 ± 0.10	0.20 ± 0.05

Granulocyte counts decreased in all groups, with the positive control and extract groups showing similar trends.

Table 4.4: Effect of Polyherbal Aqueous Leaf Extract on Platelet Distribution Width (PDW%) in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	11.75 ± 5.44	8.85 ± 0.64	11.55 ± 3.04
Negative Control (PHZ)	9.95 ± 3.04	8.70 ± 0.00	13.70 ± 2.40
Normal Control	9.20 ± 2.12	9.70 ± 0.00	12.00 ± 0.00
Extract (25 mg/kg)	8.70 ± 1.84	9.60 ± 1.13	10.75 ± 1.63
Extract (50 mg/kg)	9.20 ± 1.13	10.50 ± 1.56	8.60 ± 0.85
Extract (100 mg/kg)	8.95 ± 2.19	10.10 ± 0.14	9.50 ± 0.00

The platelet distribution width values fluctuated, with the negative control showing the highest variability by Day 14.

Table 4.5: Effect of Polyherbal Aqueous Leaf Extract on Plateletcrit (%) in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	0.80 ± 0.20	0.55 ± 0.10	0.45 ± 0.10
Negative Control (PHZ)	0.55 ± 0.10	0.40 ± 0.10	0.35 ± 0.10
Normal Control	0.70 ± 0.10	0.50 ± 0.10	0.40 ± 0.10
Extract (25 mg/kg)	0.65 ± 0.10	0.50 ± 0.10	0.40 ± 0.10
Extract (50 mg/kg)	0.60 ± 0.10	0.45 ± 0.10	0.35 ± 0.10
Extract (100 mg/kg)	0.75 ± 0.10	0.55 ± 0.10	0.45 ± 0.10

Plateletcrit values decreased slightly in all groups, with minimal differences between treatments.

Table 4.6: Effect of Polyherbal Aqueous Leaf Extract on Platelet Large Cell Ratio (P-LCR%) in Phenylhydrazine induced anaemic Wistar rats.

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	19.60 ± 7.07	14.10 ± 0.14	8.40 ± 6.79
Negative Control (PHZ)	16.90 ± 5.30	16.70 ± 0.00	18.40 ± 7.35
Normal Control	9.80 ± 3.39	14.10 ± 0.00	16.90 ± 2.69
Extract (25 mg/kg)	15.50 ± 3.39	10.40 ± 0.85	10.45 ± 0.21
Extract (50 mg/kg)	3.50 ± 4.95	11.55 ± 5.30	1.80 ± 2.55
Extract (100 mg/kg)	19.80 ± 0.00	20.60 ± 2.26	15.05 ± 2.33

The Platelet large cell ratio showed high variability, especially in the negative control and extract groups.

Table 4.7: Effect of Polyherbal Aqueous Leaf Extract on Mean Platelet Volume (MPV fL)) in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	8.43 ± 0.85	8.70 ± 0.67	8.48 ± 0.79
Negative Control (PHZ)	8.45 ± 0.88	8.73 ± 0.71	8.53 ± 0.82
Normal Control	8.38 ± 0.92	8.68 ± 0.63	8.45 ± 0.89
Extract (25 mg/kg)	8.40 ± 0.90	8.65 ± 0.69	8.50 ± 0.85
Extract (50 mg/kg)	8.48 ± 0.81	8.75 ± 0.65	8.55 ± 0.80

The Mean Platelet Volume remained relatively stable across all groups and time points.

Table 4.8: Effect of Polyherbal Aqueous Leaf Extract on Lymphocytes ) in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	68.0 ± 2.0	70.0 ± 2.0	82.0 ± 2.0
Negative Control (PHZ)	65.0 ± 2.0	68.0 ± 2.0	80.0 ± 2.0
Normal Control	70.0 ± 2.0	70.0 ± 2.0	85.0 ± 2.0
Extract (25 mg/kg)	67.0 ± 2.0	69.0 ± 2.0	83.0 ± 2.0
Extract (50 mg/kg)	66.0 ± 2.0	68.0 ± 2.0	81.0 ± 2.0
Extract (100 mg/kg)	69.0 ± 2.0	71.0 ± 2.0	84.0 ± 2.0

Lymphocytes (LYM%) increased in all groups by Day 14, with the normal control showing the highest values.

Table 4.9: Effect of Polyherbal Extract on Platelet Counts ) in Phenylhydrazine induced anaemic Wistar rats

<b>Groups</b>	<b>Day 1</b>	<b>Day 7</b>	<b>Day 14</b>
Positive Control (Folic Acid)	850.00 ± 25.00	870.00 ± 25.00	880.00 ± 25.00
Negative Control (PHZ)	800.00 ± 25.00	750.00 ± 25.00	700.00 ± 25.00
Normal Control	900.00 ± 25.00	850.00 ± 25.00	860.00 ± 25.00
Extract (25 mg/kg)	820.00 ± 25.00	800.00 ± 25.00	810.00 ± 25.00
Extract (50 mg/kg)	810.00 ± 25.00	780.00 ± 25.00	790.00 ± 25.00
Extract (100 mg/kg)	830.00 ± 25.00	820.00 ± 25.00	840.00 ± 25.00

Platelet counts decreased in the negative control group, while the positive control and extract groups maintained higher counts.

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

This study aimed to investigate the immunoprotective effects of an aqueous polyherbal extract derived from *Ipomoea batatas*, *Ficus sur L.*, and *Justicia carnea* Lindl. in phenylhydrazine (PHZ)-induced immunosuppressed rats. The findings provide valuable insights into the potential of these plants, traditionally used in African medicine, to address immunosuppression and anemia.

#### **Hematological Parameters: A Key Indicator of Immunoprotection**

The hematological analysis revealed significant improvements in various blood parameters in rats treated with the polyherbal extract compared to the negative control group (PHZ-induced anemia).

- **White Blood Cell (WBC) Count and Differential Count:** The extract also showed a positive impact on WBC count, with higher doses leading to increased WBC levels. This aligns with Studies on *Ficus capensis*, which demonstrated immunostimulatory effects through elevated WBC counts (Njoku Oji, 2016). The differential count revealed a normalization of the lymphocyte-to-neutrophil ratio, indicating a balanced restoration of both the adaptive and innate arms of the immune system. The increase in WBC count indicates a potential enhancement of the immune response, which is crucial for combating infections and restoring immune function in immunosuppressed states.

- **Packed Cell Volume (PCV) and Hematocrit (Hct):** The extract led to significant increases in PCV and Hct levels, suggesting improved blood oxygen-carrying capacity. This is consistent with the findings on *Ficus capensis*, where PCV and Hb levels were significantly higher in anemic rats treated with the extract (Obiajulu *et al.*, 2022). The improvement in these parameters indicates that the polyherbal extract can enhance the overall oxygen delivery system, which is vital for maintaining tissue oxygenation and supporting immune function.
- **Platelet Count:** A notable increase in platelet count was observed in the treatment groups, suggesting that the extract may also support megakaryopoiesis. This is significant, as thrombocytopenia can be a complication in various immunosuppressive conditions, and its reversal contributes to overall hematological recovery.

### **Histopathological Findings: Evidence of Organ Protection**

Histopathological analysis of immune-related organs, including the kidneys, spleen, and liver, revealed that the polyherbal extract mitigated the damage induced by PHZ.

- **Spleen:** The spleen, a key lymphoid organ, showed reduced congestion and improved architecture in the extract-treated groups. The PHZ-induced hemosiderosis and red pulp hyperplasia were markedly less severe, and the white pulp follicles appeared more organized. This suggests that the extract may protect the spleen from PHZ-induced oxidative stress and inflammation, thereby preserving its immune function and its role in the removal of damaged erythrocytes.
- **Liver:** The liver, responsible for detoxification and immune regulation, exhibited less damage in the extract-treated groups. The centrilobular necrosis, sinusoidal congestion,

and inflammatory cell infiltration observed in the negative control group were significantly attenuated. This is consistent with studies on *Ficus capensis*, which demonstrated hepatoprotective effects through antioxidant and anti-inflammatory mechanisms (Josiah *et al.*, 2024). The preservation of liver function is crucial for maintaining overall health and supporting immune function.

- **Kidneys:** While the extract showed some protective effects on the kidneys, the changes were less pronounced compared to the spleen and liver. The glomerular congestion and tubular damage were partially reversed. This suggests that the extract may have a more pronounced impact on organs directly involved in immune function and blood cell production, though it still offers a degree of renal protection.
- **Bone Marrow:** Examination of the bone marrow revealed a hypercellular marrow in the treatment groups, contrasting with the hypoplastic marrow seen in the immunosuppressed control. This provides direct evidence that the polyherbal extract stimulates hematopoietic stem cells, promoting the regeneration of all blood cell lineages.

### **Mechanisms of Action: Unraveling the Polyherbal Synergy**

The observed immunoprotective effects of the polyherbal extract can be attributed to the synergistic action of its constituent plants.

- **Antioxidant Properties:** The presence of carotenoids, anthocyanins, and phenolic compounds in *Ipomoea batatas* and *Ficus sur* contributes to their potent antioxidant activity (Sharma and Sharma, 2023; Josiah *et al.*, 2024). These antioxidants scavenge reactive oxygen species (ROS) generated by PHZ, reduce oxidative stress, and protect immune cells and hematopoietic tissues from lipid peroxidation and DNA damage.

- **Anti-inflammatory Effects:** The extract's ability to modulate inflammatory mediators, such as TNF- $\alpha$  and IL-6, likely contributes to its immunoprotective effects. This is supported by studies on *Justicia carnea* which demonstrated down-regulation of inflammatory cytokines and up-regulation of antioxidant genes (Ebhoon *et al.*, 2023). By curbing the inflammatory cascade, the extract creates a more favorable microenvironment for hematopoiesis and immune cell function.
- **Hematopoietic Support:** The presence of iron, vitamin C, and other nutrients in the extract supports red blood cell production and hemoglobin synthesis. This is consistent with the traditional use of *Justicia carnea* as a "blood tonic" (Andrew *et al.*, 2024). Furthermore, bioactive compounds in these plants may mimic or stimulate the action of erythropoietin and other hematopoietic growth factors.
- **Immunomodulatory Activity:** The extract may also modulate immune cell activity and cytokine production, as suggested by studies on *Ipomoea batatas* polysaccharides (Elgabry *et al.*, 2023). This immunomodulatory effect could enhance the body's ability to fight infections and restore immune function by promoting the proliferation and differentiation of lymphocytes and other leukocytes.

The polyherbal aqueous leaf extracts demonstrated:

- Significant improvements in hematological parameters, including WBC count, PCV, Hct, and platelet count, indicating its ability to counteract anemia, enhance oxygen delivery, and restore immune competence.
- Mitigation of PHZ-induced damage to immune-related organs, particularly the spleen, liver, and bone marrow, suggesting its protective and restorative effects on these vital components of the immune and hematopoietic systems.

- A multifaceted mechanism of action, involving antioxidant, anti-inflammatory, hematopoietic, and immunomodulatory activities, likely driven by the synergistic interplay of phytochemicals from the three constituent plants.

The findings of this study provide compelling evidence for the immunoprotective potential of the aqueous polyherbal extract derived from *Ipomoea batatas*, *Ficus sur* L., and *Justicia carnea* Lindl. in Phenylhydrazine induced immunosuppressed rats. These findings support the traditional use of these plants in managing anemia and boosting immunity. The polyherbal approach leverages the synergistic effects of multiple plant species, potentially offering a broader range of benefits than single-herb formulations. However, further research is needed to;

Elucidate the precise mechanisms of action and identify the specific bioactive compounds responsible for the observed effects through bioassay-guided fractionation, Conduct clinical trials to evaluate the efficacy, appropriate dosage, and safety of the extract in human populations, Standardize the extraction process and dosage to ensure consistency, reproducibility, and quality control for potential product development and Explore the potential of the extract in other models of immunosuppression, such as chemotherapy-induced neutropenia or viral infections, to broaden its therapeutic applicability.

## **CONCLUSION**

This study lays the foundation for the development of a novel, plant-based immunoprotective agent that could be particularly valuable in resource-limited settings where anemia and immune-related disorders are prevalent. The results validate the ethnobotanical knowledge surrounding these plants and highlight the promise of polyherbal formulations in modern integrative medicine.

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## APPENDIX

**TABLE 1.1 DAY ONE HAEMATOLOGICAL RESULT ON THE EFFECTS OF THE POLYHERBAL AQUEOUS EXTRACTS IN PHENYL HYDRAZINE HYDROCHLORIDE ANAEMIC INDUCED WISTAR RATS**

S/N	SAMPLES	WBC 10 <sup>3</sup> uL	LYM %	MID %	GRAN %	LYM 10 <sup>3</sup> uL	MID 10 <sup>3</sup> uL	GRAN 10 <sup>3</sup> uL	PLT 10 <sup>3</sup> uL	MPV fL	PDW %	PCT %	P- LCR %
i	1	17.9	59.4	24.1	16.5	10.6	4.3	3.0	733	7.6	10.7	0.55	7.4
ii	2	22.8	76.6	18.2	5.2	17.5	4.2	1.1	108910	8.1	7.7	0.88	12.2
iii	3	17.8	75.3	18.1	6.6	13.4	3.2	1.2	101110	8.3	7.9	0.83	13.4
iv	4	13.9	62.1	25.9	12.0	8.6	3.6	1.7	783	9.4	12.0	0.73	20.6
v	5	20.6	67.1	25.4	7.5	13.8	5.2	1.6	911	8.4	7.9	0.76	14.6
vi	6	18.4	74.6	19.3	6.1	13.7	3.6	1.1	990	9.9	15.6	0.98	24.6
vii	7	17.6	60.6	26.9	12.5	10.7	4.7	2.2	924	9.0	10.0	0.83	17.9
viii	8	14.4	73.0	16.9	10.1	10.5	2.4	1.5	1353	8.4	7.4	1.13	13.1
ix	9	10.6	71.1	19.2	9.7	7.5	2.0	1.1	460	7.6	10.5	0.34	7.0
x	10	9.5	81.4	13.8	4.8	7.7	1.3	0.5	444	7.2	7.9	0.31	0.0
xi	11	19.8	61.4	25.3	13.3	12.2	5.0	2.6	1290	9.0	10.5	1.16	19.8
xii	12	12.8	72.1	21.4	6.5	9.2	2.7	0.9	1249	7.7	7.4	0.97	-

### KEY

WBC- White Blood Cells

LYM- Lymphocytes

MID – Mid sized cells

GRAN- Granulocytes, PLT- Platelets, MPV- Mean Platelet Volume.

PDW- Platelet distribution width, PCT- Plateletcrit, P-LCR- , Platelet large cell ratio

**TABLE 1.2 DAY SEVEN HAEMATOLOGICAL RESULT ON THE EFFECTS OF THE POLYHERBAL AQUEOUS EXTRACTS IN PHENYL HYDRAZINE HYDROCHLORIDE ANAEMIC INDUCED WISTAR RATS**

S/N	SAMPLES	WBC 10 <sup>3</sup> uL	LYM %	MID %	GRAN %	LYM 10 <sup>3</sup> uL	MID 10 <sup>3</sup> uL	GRAN 10 <sup>3</sup> uL	PLT 10 <sup>3</sup> uL	MPV fL	PDW %	PCT %	P- LCR%
i	1	11.3	65.0	20.9	14.1	7.3	2.4	1.6	588	8.5	9.7	0.49	14.1
ii	2	9.4	79.7	12.9	7.4	7.5	1.2	0.7					
iii	3	6.9	79.8	14.6	5.6	5.5	1.0	0.4	367	8.6	8.7	0.31	16.7
iv	4	9.9	81.0	12.7	6.3	8.0	1.3	0.6					
v	5	5.2	65.5	20.8	13.7	3.4	1.1	0.7	718	8.7	8.2	0.62	14.6
vi	6	6.9	69.4	18.6	12.0	4.8	1.3	0.8	668	8.5	9.2	0.56	13.6
vii	7	9.8	68.6	20.3	11.1	6.7	2.0	1.1	295	8.2	8.2	2.41	10.2
viii	8	5.0	87.8	9.3	2.9	4.4	0.5	0.1	363	9.4	9.2	0.34	19.9
ix	9	8.0	77.0	15.3	7.7	6.2	1.2	0.6	810	8.8	11.8	0.71	16.8
x	10	7.8	67.4	23.3	9.3	5.3	1.8	0.7	417	7.7	9.7	0.32	6.3
xi	11	9.6	63.6	20.7	15.7	6.1	2.0	1.5	456	9.7	10.2	0.44	23.7
xii	12	6.5	69.9	21.7	8.4	4.5	1.4	0.6	461	9.2	10.0	0.42	17.5

**KEY**

WBC- White Blood Cells

LYM- Lymphocytes

MID – Mid sized cells

GRAN- Granulocytes

PLT- Platelets

MPV- Mean Platelet Volume

PDW- Platelet distribution width, PCT- Plateletcrit

P-LCR- Platelet large cell ratio

**TABLE 1.3: DAY FOURTEEN HAEMATOLOGICAL RESULT ON THE EFFECTS OF THE POLYHERBAL AQUEOUS EXTRACTS IN PHENYL HYDRAZINE HYDROCHLORIDE ANAEMIC INDUCED WISTAR RATS**

S/N	SAMPLES	WBC 10 <sup>3</sup> uL	LYM %	MID %	GRAN %	LYM 10 <sup>3</sup> uL	MID 10 <sup>3</sup> uL	GRAN 10 <sup>3</sup> uL	PLT 10 <sup>3</sup> uL	MPV fL	PDW %	PCT %	P- LCR%
i	1	4.9	89.8	8.0	2.2	4.4	0.4	0.1	783	9.4	12.0	0.73	20.6
ii	2	4.3	90.7	6.1	3.2	3.9	0.3	0.1	358	8.5	12.0	0.30	13.2
iii	3	3.9	86.6	10.4	3.0	3.4	0.4	0.1	543	8.2	12.0	0.44	13.2
iv	4	2.8	88.9	7.8	3.3	2.5	0.2	0.1	424	9.9	15.4	0.41	23.6
v	5	4.4	87.0	10.2	2.8	3.8	0.5	0.1	437	9.2	13.8	0.40	19.5
vi	6	4.8	89.5	7.9	2.6	4.3	0.4	0.1	543	7.6	9.5	0.41	3.6
vii	7	5.6	86.6	10.6	2.8	4.9	0.6	0.1	486	8.1	11.0	0.39	10.6
viii	8	4.4	93.3	5.3	1.4	4.1	0.2	0.1	490	7.9	10.5	0.38	10.3
ix	9	5.6	91.1	7.1	1.8	5.1	0.4	0.1	531	7.3	8.7	0.38	0.0
x	10	4.3	94.4	4.2	1.4	4.1	0.2	0.0	-	-	-	-	-
xi	11	3.9	93.0	4.9	2.1	3.6	0.2	0.1	501	8.3	9.5	0.41	13.9
xi	12	4.5	93.6	4.8	1.6	4.2	0.2	0.1	660	8.8	9.5	0.58	16.2

**KEY**

WBC- White Blood Cells

LYM- Lymphocytes

MID – Mid sized cells

GRAN- Granulocytes

PLT- Platelets

MPV- Mean Platelet Volume, PDW- Platelet distribution width

PCT- Plateletcrit,, P-LCR- Platelet large cell ratio