BONE MARROW REGENERATIVE EFFECT OF THE POLYHERBAL AQUEOUS LEAF EXTRACT (*Justica carnea, Ipomea batata* and *Ficus sur*) IN PHENYLHYDRAZINE HYDROCHLORIDE INDUCED HEMOLYTIC ANAEMIC RATS

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BENIN CITY, EDO STATE

DECEMBER, 2025.

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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 POSTGRADUATE DIPLOMA (PGD) DEGREE IN SCIENCE LABORARTORY TECHNOLOGY.

DECEMBER, 2025.

CERTIFICATION

This is to certify that this work titled **“**Bone marrow regenerative effect of the polyherbal aqueous leaf extract (*Justica carnea, Ipomea batata* and *Ficus sur*) in phenylhydrazine hydrochloride induced hemolytic anaemic rats**”** was carried out by **Etinosa Godspower AGHAHOWA** with the Matriculation Number PG/LSC2415943, of the Department Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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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 programme.

ACKNOWLEDGEMENTS

I want to thank Almighty God, the giver and sustainer of life, for His grace, strength and provision throughout the period of my programme. My heartfelt gratitude to my project supervisor, Prof. E. O. Oshomoh for his guidance, moral support and correction during the cause of this project work.

I also want to thank Dr. B. O. Gabriel, Mr. Paul O. Ojoba for their guidance throughout the Project work. I will also want to appreciate my parents, Mr and Mrs Aghahowa and my siblings for their unwavering support, both financially, spiritually and all way round.

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

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ABSTRACT

Haemolytic anaemia is characterised by accelerated erythrocyte destruction and is often accompanied by oxidative damage and organ dysfunction. Medicinal plants used in traditional medicine may offer protective and regenerative benefits in anaemic conditions. This study evaluated the regenerative effects of a polyherbal aqueous leaf extract of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* on selected organs in phenylhydrazine-induced haemolytic anaemic rats. Anaemia was induced in Wistar rats using phenylhydrazine hydrochloride. Animals were treated with graded doses of the polyherbal extract, while control groups included normal and anaemia-induced untreated rats. Liver, spleen and thymus tissues were harvested and processed for histopathological examination using haematoxylin and eosin staining. Phenylhydrazine-induced anaemia caused marked histopathological alterations in the liver, spleen and thymus, including hepatocellular degeneration, splenic architectural distortion and thymic involution. Treatment with the polyherbal extract resulted in varying degrees of tissue protection and structural recovery across the organs examined. The polyherbal aqueous extract of *I. batatas*, *J. carnea* and *F. sur* demonstrated protective and regenerative effects against phenylhydrazine-induced organ damage, supporting its potential role in the management of haemolytic anaemia.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Anaemia remains a significant global public health challenge, affecting more than 1.6 billion people worldwide (WHO, 2022). Hemolytic anaemia is characterised by accelerated erythrocyte destruction, leading to reduced red blood cell (RBC) lifespan and increased compensatory demand on the bone marrow (WHO, 2022). The bone marrow plays a central role in restoring erythropoiesis, yet in severe or prolonged haemolysis this compensatory mechanism may be overwhelmed, resulting in sustained anaemia and associated systemic consequences.

Experimental animal models have been widely used to study haemolytic anaemia and to evaluate potential therapies. One well-established model is the induction of haemolytic anaemia by administration of phenylhydrazine hydrochloride (PHZ) in rodents. PHZ causes oxidative damage to erythrocytes and intravascular haemolysis, resulting in decreased haemoglobin (Hb) concentration, reduced packed cell volume (PCV), elevated reticulocyte counts, and histopathological changes in bone-marrow (Naman *et al.,* 2020). These models provide a reproducible platform for testing haematinic and bone-marrow regenerative interventions.

Medicinal plants have long been used in Nigerian traditional medicine for the management of anaemia and related haematological disorders, owing to their micronutrient content, haemopoietic- stimulating phytochemicals, antioxidant activity and marrow-supportive potential. Gabriel and Idu (2021) evaluated the leaf extract of *Ipomoea batatas* (sweet potato) to have antioxidant, haematinic and biosafety properties in a PHZ-induced anaemia animal model, supporting its potential in erythropoietic recovery and bone-marrow support. Similarly, the leaves of *Justicia carnea* have been found to increase RBC count and haemoglobin in phenylhydrazine-induced anaemic rats (Ejiofor *et al.,* 2025), and have demonstrated haematoprotective effects in sodium nitrate- treated rats (Ogbue *et al.,* 2020). The genus *Ficus* sur (a species of fig tree) is less studied for haematopoietic support in Nigeria, but Ficus species more broadly have documented antioxidant and haematological parameter-modifying effects (Nemiche *et al.,* 2025). Despite several reports for individual plants, a clear gap remains in the literature regarding the combined effect of a polyherbal aqueous leaf extract comprising *I. batatas*, *J. carnea*, and *F. sur* on bone-marrow regeneration in PHZ-induced haemolytic anaemic rats. Most previous studies focused on single-plant extracts, limited haematological indices and does not explore bone-marrow cellularity, histopathology, or regenerative responses following haemolytic insult. Given the complex interplay of erythropoiesis, oxidative stress, the bone-marrow microenvironment and haematopoietic stem-cell activation, it is plausible that a combination of plants with complementary mechanisms like antioxidant, micronutrient supply, stem-cell stimulation, may yield synergistic marrow-regenerative effects (Ejiofor *et al.,* 2025).

Therefore, the present study seeks to evaluate the bone-marrow regenerative capacity of an aqueous leaf extract combination of *I. batatas, J. carnea* and *F. sur* in PHZ-induced haemolytic anaemic rats. This study is scientifically justified, as it may validate traditional haematinic practices, extend understanding of herbal-based bone-marrow regeneration, and contribute to development of novel therapeutic approaches for haematological disorders, particularly in resource-limited settings where medicinal plants are locally available and culturally acceptable.

1.2 Statement of the Problem

Hemolytic anaemia is a common haematological disorder in which red blood cells are destroyed prematurely, leading to decreased haemoglobin concentration and impaired oxygen delivery to tissues. Conventional therapies, including blood transfusions and iron supplementation, are often limited by side effects, high cost, and accessibility challenges in resource-limited settings such as Nigeria (Olowu *et al.*, 2021). Moreover, some pharmacological agents do not adequately stimulate bone-marrow regeneration, which is critical for long-term recovery from anemia. Medicinal plants have shown potential as cost-effective alternatives for managing anemia due to their bioactive compounds, including flavonoids, saponins, alkaloids, and phenolics, which may enhance erythropoiesis and protect against oxidative damage (Igbinaduwa *et al.*, 2020; Ejiofor *et al.*, 2025). However, most studies have focused on individual plant extracts rather than polyherbal formulations, and there is limited empirical evidence on the effects of such combinations on bone-marrow regeneration. Specifically, the combined effect of *Ipomoea batatas*, *Justicia carnea*, and *Ficus sur* leaves on the haematological parameters and marrow cellularity in hemolytic anemia has not been thoroughly investigated. This knowledge gap presents a barrier to validating traditional medicine practices in Nigeria and hinders the development of novel, safe, and effective herbal therapeutics for anemia. Therefore, there is a need to evaluate the bone-marrow regenerative potential of a polyherbal aqueous leaf extract of *I. batatas*, *J. carnea*, and *F. sur* in phenylhydrazine-induced hemolytic anemia in rats, to establish scientific evidence for its therapeutic use.

1.3 Aim and Objectives

Aim

The aim of this study was to evaluate the histopathological effect of a polyherbal aqueous leaf extract of *Ipomoea batatas*, *Justicia carnea*, and *Ficus sur* in phenylhydrazine-induced hemolytic anaemic rats.

While the **specific objectives** were to:

i. evaluate the histopathological effects of phenylhydrazine-induced haemolytic anaemia on the liver, spleen and thymus of Wistar rats.

ii. evaluate the protective and regenerative effects of a polyherbal aqueous leaf extract of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* on the liver, spleen and thymus following phenylhydrazine-induced injury.

iii. determine the extent to which the polyherbal extract mitigates tissue damage associated with haemolytic anaemia.

1.4 Justification / Significance of the Study

Anaemia, particularly of haemolytic origin, continues to pose a major health concern in developing nations, including Nigeria, where malnutrition, infectious diseases and limited access to standard medical care exacerbate its burden (Akinola *et al.*, 2020). Conventional therapeutic options such as iron supplements, erythropoietin analogues and blood transfusion are often costly, associated with adverse reactions, or logistically unavailable in rural communities (Olowu *et al.*, 2021). Hence, there is a growing need for affordable and biologically safe alternatives capable of stimulating bone-marrow regeneration and restoring haematopoiesis.

Medicinal plants represent a valuable source of bioactive compounds with haematopoietic and antioxidant properties. *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* are locally available Nigerian plants used traditionally to manage blood-related disorders (Gabriel & Idu, 2021; Ejiofor *et al.*, 2025). Their individual extracts have demonstrated improvements in red blood cell indices and protection against oxidative stress in anaemic models, suggesting possible marrow-stimulating and cytoprotective mechanisms. However, the synergistic potential of these three plants, when combined as a polyherbal aqueous extract, has not been scientifically validated. Evaluating the combined effect of these plant leaves on bone-marrow regeneration in phenylhydrazine-induced haemolytic anaemia is therefore justified. Establishing their efficacy and safety could provide a scientific basis for formulating indigenous, plant-based haematinic agents. Moreover, this study aligns with Nigeria's ongoing effort to promote evidence-based traditional medicine and to reduce dependence on imported synthetic drugs. The findings could also contribute to pharmacognostic data on polyherbal formulations and broaden the scope of phytomedicine research in haematological disorders.

1.5 Scope of the Study

This study is limited to evaluating the bone-marrow regenerative effect of a polyherbal aqueous leaf extract prepared from *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* in phenylhydrazine-induced haemolytic anaemic rats. The study entails the induction of haemolytic anaemia using phenylhydrazine hydrochloride (PHZ), and administration of the extract at selected doses.

Haematological indices such as haemoglobin concentration, packed cell volume, red and white blood cell counts, and differential leucocyte counts will be evaluated. Bone-marrow smears and histopathological sections will be examined to determine marrow cellularity and regenerative activity. In addition, oxidative-stress markers including malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels will be evaluated to investigate the antioxidant contribution of the extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Anaemia

Anaemia is a complex haematological disorder characterised by a reduction in the total number of circulating red blood cells (RBCs), haemoglobin concentration, or packed cell volume below normal physiological levels, leading to impaired oxygen transport to tissues (Olayemi *et al.*, 2019; WHO, 2022). It remains one of the most prevalent public-health challenges globally, particularly in sub-Saharan Africa where nutritional deficiencies, parasitic infections, and hereditary haemoglobinopathies are endemic (Akinbami *et al.*, 2018; Ibegbulam *et al.*, 2020).

Haemolytic anaemia, a major subtype of anaemia, arises when the rate of red-cell destruction exceeds erythrocyte production in the bone marrow (Naman *et al.*, 2020). It may result from intrinsic defects within RBCs or from extrinsic factors such as oxidative agents, autoimmune reactions, infections, or toxic chemicals. One of the most widely used experimental models for investigating haemolytic anaemia is the phenylhydrazine (PHZ)-induced model, which causes oxidative damage and lipid peroxidation in erythrocyte membranes, leading to haemolysis (Gabriel and Idu, 2021; Ejiofor *et al.*, 2025). PHZ generates reactive oxygen species (ROS), resulting in oxidative stress that not only damages erythrocytes but also suppresses bone-marrow erythropoietic function (Ezeonu *et al.*, 2023). In response to anaemic stress, the bone marrow becomes hyperplastic, increasing erythropoietin-stimulated proliferation of erythroid progenitors in an attempt to restore normal red-cell levels (Akanmu *et al.*, 2017). However, in cases of sustained oxidative damage or nutrient deficiency, this compensatory mechanism may be inadequate, leading to persistent anaemia and marrow exhaustion (Naman *et al.*, 2020).

Thus, agents that enhance bone-marrow regeneration, stimulate erythropoiesis, and counter oxidative stress are of high biomedical interest.

Recent years have witnessed a resurgence in the use of medicinal plants as potential haematinic and antioxidant agents. Nigeria's rich flora offers numerous plants used traditionally in managing anaemia and related disorders (Hassan *et al.*, 2021; Igbinaduwa *et al.*, 2020). Many of these species contain phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds, known for their antioxidant and haematopoietic potentials (Edeoga *et al.*, 2021). Among such plants, *Ipomoea batatas* (sweet potato), *Justicia carnea* (blood plant), and *Ficus sur* (wild fig) have been individually reported to possess blood-building and antioxidant activities. The aqueous extract of *I. batatas* leaves has shown erythropoietic and antioxidant effects in anaemic rats (Gabriel and Idu, 2021), while *J. carnea* has been demonstrated to increase haemoglobin and packed cell volume in phenylhydrazine-treated rats (Ejiofor *et al.*, 2025). Similarly, *F. sur* leaf extracts have exhibited antioxidant and haematinic potentials due to their rich phenolic and flavonoid contents (Ameh *et al.*, 2022).

However, although these plants are used individually in ethnomedicine, there is limited scientific data on their combined (polyherbal) effects, particularly regarding bone-marrow regeneration following chemically induced haemolytic anaemia. The synergistic use of multiple plants can potentiate pharmacological efficacy through complementary mechanisms such as antioxidant reinforcement, micronutrient synergy, and improved bioavailability of active metabolites (Abubakar *et al.*, 2018). Therefore, evaluating the polyherbal aqueous extract of *Ipomoea batatas*, *Justicia carnea*, and *Ficus sur* in phenylhydrazine-induced anaemic rats offers a promising biomedical approach for understanding the potential regenerative effects of Nigerian medicinal plants on bone-marrow function.

2.2 Phenylhydrazine-Induced Haemolytic Anaemia

Phenylhydrazine (PHZ) is a potent haematotoxic compound widely employed in experimental pharmacology and toxicology to induce haemolytic anaemia in laboratory animals. The PHZ-induced model is a reliable and reproducible method used to assess erythropoietic and antioxidant responses of novel therapeutic agents, particularly plant-derived bioactives (Gabriel and Idu, 2021; Ejiofor *et al.*, 2025).

2.2.1 Mechanism of Action

The haematotoxic effect of PHZ is primarily mediated through oxidative stress mechanisms. Upon administration, PHZ undergoes auto-oxidation to generate reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Ezeonu *et al.*, 2023). These ROS induce lipid peroxidation of erythrocyte membranes, leading to structural deformities, increased fragility, and eventual haemolysis (Naman *et al.*, 2020). Also, PHZ interacts directly with haemoglobin, converting the ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state, forming methaemoglobin, which reduces oxygen-binding capacity (Audu *et al.*, 2022). Methaemoglobin further promotes oxidative denaturation of globin chains, producing Heinz bodies that mark erythrocytes for premature sequestration and destruction in the spleen (Ukoha *et al.*, 2017). At the biochemical level, PHZ administration depletes endogenous antioxidants such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), while increasing levels of malondialdehyde (MDA), a biomarker of lipid peroxidation (Ibegbulam *et al.*, 2020). These oxidative disturbances impair the integrity of erythrocyte membranes and disrupt the normal redox homeostasis necessary for cell survival (Edeoga *et al.*, 2021).

2.2.2 Effects on Bone Marrow

The haemolytic destruction of erythrocytes caused by PHZ triggers compensatory erythropoietic responses within the bone marrow. In moderate doses, PHZ stimulates the release of erythropoietin (EPO) from renal tissues, which promotes proliferation and differentiation of erythroid progenitor cells (Akanmu *et al.*, 2017). However, in cases of prolonged exposure or high doses, the sustained oxidative insult suppresses marrow function, resulting in erythroid hypoplasia and reduced reticulocyte counts (Ameh *et al.*, 2022; Hassan *et al.*, 2021). Histological studies of PHZ-treated rats have demonstrated congestion of the splenic red pulp, depletion of marrow haematopoietic cells, and increased infiltration of macrophages responsible for erythrophagocytosis (Ezeonu *et al.*, 2023). These observations make the PHZ model an ideal system for evaluating the regenerative and haematinic potentials of plant extracts and natural antioxidants.

2.2.3 Therapeutic Implications

Because of its reproducibility and similarity to oxidative haemolytic disorders in humans, the PHZ-induced anaemia model is frequently used in evaluating anti-anaemic and bone-marrow restorative properties of medicinal plants (Gabriel and Idu, 2021; Ejiofor *et al.*, 2025). Compounds that mitigate PHZ-induced oxidative stress or enhance erythropoiesis are considered potential therapeutic agents for managing haemolytic anaemia and related marrow pathologies. The present study therefore employs this model to investigate the bone-marrow regenerative effects of a polyherbal aqueous extract containing *Ipomoea batatas*, *Justicia carnea* and *Ficus sur*, all of which have independently demonstrated antioxidant and haematinic activity in previous research (Igbinaduwa *et al.*, 2020; Ameh *et al.*, 2022).

2.3 Bone-Marrow Regeneration and Haematopoiesis

The bone marrow is the principal haematopoietic organ in mammals, responsible for the continuous production of blood cells throughout life. It contains multipotent haematopoietic stem cells (HSCs) capable of differentiating into erythroid, myeloid, and lymphoid lineages under the regulation of complex cytokine and growth-factor networks (Akanmu *et al.*, 2017; Ezeonu *et al.*, 2023). The process of blood-cell formation, known as haematopoiesis, is a highly coordinated biological system that ensures adequate replacement of senescent or damaged cells. Under physiological conditions, erythropoiesis occurs in the red bone marrow of long bones, ribs, sternum, and pelvis. Erythroid progenitors proliferate and mature through distinct stages such as proerythroblast, basophilic erythroblast, polychromatic erythroblast, and orthochromatic erythroblast which culminates in the release of reticulocytes into circulation (Olayemi *et al.*, 2019). This process is primarily regulated by erythropoietin (EPO), a glycoprotein hormone produced mainly by the kidney in response to hypoxia or anaemic stress (Ameh *et al.*, 2022).

2.3.1 Bone-Marrow Suppression and Regeneration

Haematopoietic tissue is highly sensitive to toxic and oxidative insults. Agents such as phenylhydrazine, chemotherapy drugs, ionising radiation, and certain environmental toxins can suppress marrow activity, leading to reduced erythrocyte and leukocyte counts (Audu *et al.*, 2022). Damage to bone-marrow cells often manifests as hypocellularity, decreased megakaryocyte formation, and increased fatty infiltration of marrow spaces (Ibegbulam *et al.*, 2020). Bone-marrow regeneration, therefore, refers to the restoration of cellularity and haematopoietic function following such injury. This recovery involves proliferation of stem and progenitor cells, activation of stromal microenvironments, and increased cytokine signalling (Hassan *et al.*, 2021). Studies have shown that antioxidants and plant-derived bioactives can stimulate bone-marrow recovery by scavenging reactive oxygen species (ROS), enhancing stem-cell proliferation, and normalising haematological parameters (Gabriel and Idu, 2021).

In animal models, compounds with strong antioxidant properties such as flavonoids, terpenoids, and phenolic acids have demonstrated the ability to promote erythroid regeneration and restore marrow integrity after phenylhydrazine-induced damage (Edeoga *et al.*, 2021; Ejiofor *et al.*, 2025). Similarly, polyherbal preparations have shown synergistic marrow-protective effects by combining bioactive constituents with complementary mechanisms, including anti-inflammatory, antioxidant, and immunomodulatory actions (Abubakar *et al.*, 2018).

2.3.2 Medicinal Plants and Haematopoietic Modulation

The regenerative potential of medicinal plants is closely related to their phytochemical profiles. *Ipomoea batatas*, for instance, contains anthocyanins and β-carotene with established antioxidant activity that may enhance erythropoietic responses (Gabriel and Idu, 2021). *Justicia carnea* is rich in iron, flavonoids, and alkaloids known to stimulate haematopoiesis and increase haemoglobin synthesis (Ejiofor *et al.*, 2025; Igbinaduwa *et al.*, 2020). *Ficus sur* leaf extracts possess phenolic compounds that have been associated with red-cell membrane stabilisation and marrow support (Ameh *et al.*, 2022), which suggest that polyherbal formulations could accelerate bone-marrow regeneration following chemically induced anaemia by enhancing antioxidant defense and promoting erythropoietic differentiation. (Hassan *et al.*, 2021; Ezeonu *et al.*, 2023).

2.4 *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).

2.4.1 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 β-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 Taxonomic classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Convolvulaceae

Genus: *Ipomoea*

Species: *Ipomoea batatas (L.) Lam*

Figure 2.1: Leaves of *Ipomoea batatas* **Source:** (Vecteezy, 2023).

2.4.3 Folkloric 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.

2.4.4 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 β-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).

2.4.5 Pharmacological Properties

2.4.5.1 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).

2.4.5.2 Antinociceptive effects

The leaves of sweet potato have 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).

2.4.5.3 Antioxidant 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 β-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.

2.4.5.4 Anti-inflammatory and Anti-arthritic 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-1β (IL-1β), 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).

2.5 *Justicia Carnea*

Justicia carnea commmonly 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).

2.5.1 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; Oleleke *et al*., 2024).

2.5.2 Classification

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia*

Species: *Justicia carnea Lindl.*

Figure 2.2: Leaves and flower of *Justicia carnea* **Source:** (Auckland Botanic Gardens, 2019).

2.5.3 Folkloric 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.

2.5.4 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.

2.5.5 Pharmacological properties

2.5.5.1 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.

2.5.5.2 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).

2.5.5.3 Anti-diabetic or Enzyme inhibitory Activity

Justicia carnea has shown notable antidiabetic potential, largely through the inhibition of carbohydrate-digesting enzymes such as α-amylase and α-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.

2.5.5.4 Hepatoprotective effects

Beyond its anti-diabetic activity,*Justicia carnea* demonstrates strong hepatoprotective activity. In models of carbon tetrachloride (CCl_4)-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_4) 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.

2.6 *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. It's 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).

2.6.1 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).

2.6.2 Botanical Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus L.

Species: *Ficus sur* Forssk.

Figure 2.3: Leaves of *Ficus sur* Source: (iNaturalist, 2022).

2.6.3 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.

2.6.4 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 β-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).

2.6.5 Pharmacological properties

2.6.5.1 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).

2.6.5.2 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).

2.6.5.3. 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).

2.6.5.4. 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).

2.6.5.5 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-F331for Ficus sur and were deposited at the University's herbarium for future reference.

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.

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 tube daily for seven consecutive days. The induction was confirmed by the manifestation of characteristic anaemic 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:

Dose (mg/kg) × Body Weight (g) / 1000

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 Collection and Preparation of Tissue Samples

At the end of the treatment period animals were fasted overnight and anaesthetised according to institutional guidelines prior to sacrifice. Cardiac puncture was used to obtain blood for observation, after which the thoracic and abdominal cavities were opened and the *liver*, *spleen* and *thymus* were excised with care to avoid mechanical damage. Excised organs were rinsed briefly in cold 0.9% saline to remove adherent blood, blotted on sterile filter paper and inspected grossly for size, colour and lesions (standard necropsy practice) (Leica Biosystems, 2019).

Tissue specimens for histology were placed immediately into 10% neutral buffered formalin (NBF). Fixation was performed for a minimum of 24–48 hours at room temperature to ensure adequate penetration and preservation of tissue morphology and to prevent autolysis (Grizzle *et al.*, 2009; Bancroft & Gamble, 2019). Where perfusion fixation is available, it is preferred for optimal preservation of fine structure, but immersion fixation in 10% NBF is acceptable and widely used for small rodent organs in toxicological and pharmacological studies (Grizzle *et al.*, 2009).

After fixation the samples were processed through graded alcohols for dehydration, cleared in xylene (or xylene substitute) and embedded in paraffin wax. Standard processing schedules using ascending ethanol concentrations (70%, 80%, 95%, 100%) were followed, with sufficient time in each step to ensure complete dehydration and clearing prior to wax infiltration (Bancroft and Gamble, 2019; Cell Press STAR Protocols, 2023). Paraffin blocks were allowed to solidify at room temperature and stored until sectioning.

Paraffin blocks were sectioned on a rotary microtome at 4–5 µm thickness. Ribboned sections were floated on a warm water bath, picked onto grease-free glass slides and dried overnight at 37–40 °C or for 1–2 hours at 60 °C to promote adhesion (Qin *et al.*, 2018). Routine staining was performed with haematoxylin and eosin (H&E) using a laboratory standard H&E protocol. Slides were dehydrated through graded alcohols, cleared and cover-slipped with a synthetic mounting medium before microscopic examination (URMC histology protocol; Leica Biosystems, 2019).

Microscopic evaluation of the *liver*, *spleen* and *thymus* was undertaken using light microscopy at appropriate magnifications (×100, ×200 and ×400). Histopathological features to be assessed included architecture, cellularity, evidence of necrosis, inflammatory infiltration, congestion, haemorrhage, sinusoidal dilatation, Kupffer cell activation in liver, white and red pulp integrity in spleen, and cortical and medullary changes in thymus. Photomicrographs were captured with a digital camera attached to the microscope for documentation and semi-quantitative scoring (mild, moderate, severe) of lesions, following accepted toxicologic pathology practice (Nature Scientific Reports methods; Qin *et al.*, 2018).

All histological procedures adhered to institutional biosafety and waste disposal protocols. Negative controls (untreated animals) and positive or reference controls (if applicable) were processed in parallel to ensure comparability of tissue processing and staining.

3. 6 Statistical Analysis

All data generated from hematological and biochemical analyses were expressed as Mean ± 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 Histopathological assay of the Liver

Histological examination of liver sections stained with haematoxylin and eosin revealed varying degrees of structural alteration across the experimental groups as shown in plate 4.1

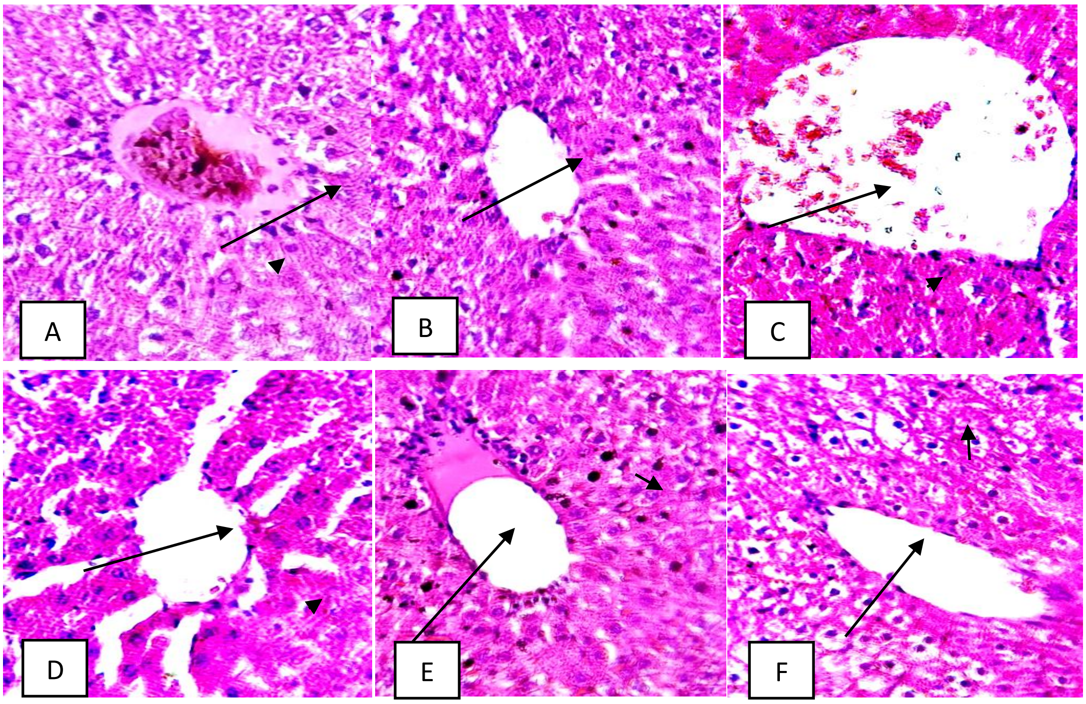

Plate 4.1: Effects of the polyherbal leave aqueous extract in the liver induced with phenylhydrazine hydrochloride

A. Group 6: reveals visible centriole (long arrow) with the hepatocytes revealing pyknotic nucleus (short arrow).

B. Group 5: reveals centriole surrounded by mild inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).

C. Group 4: reveals centriole (long arrow) surrounded by hepatocytes that appear not so distinct. (Short arrow).

D. Group 1: reveals distinct centriole (long arrow) with the hepatocytes and dilated sinusoidal (short arrow).

E. Group 2: reveals centriole surrounded by focal inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).

F. Group 3: reveals centriole (long arrow) surrounded by hepatocytes with hydropic fatty changes and revealing mild steatosis (short arrow).

4.2 Histopathological findings of the Spleen

Histological examination of the spleen sections stained with haematoxylin and eosin revealed varying splenic architectural changes across the experimental groups as shown in plate 4.2

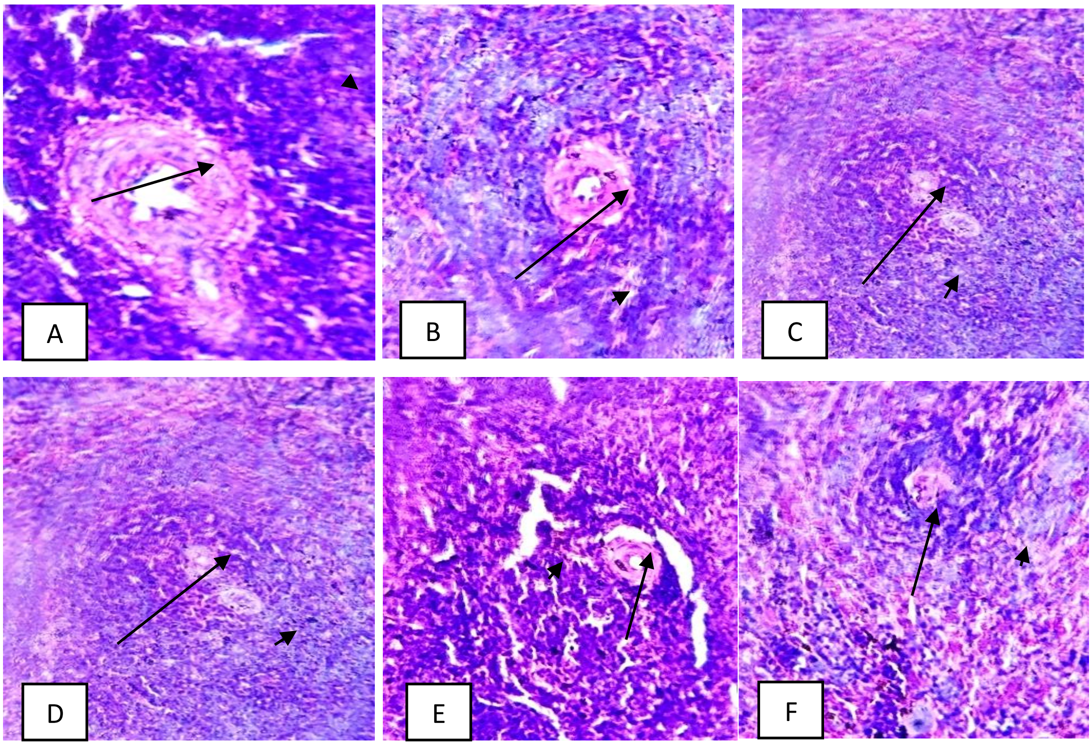

Plate 4.2: Effects of the polyherbal leave aqueous extract in the spleen induced with phenylhydrazine hydrochloride

A. Group 6 spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear distinct.

B. Group 5 spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear coarse.

C. Group 4 spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps appear coarse.

D. Group 1 spleen shows prominent lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps are prominent.

E. Group 2 spleen shows lymphoid follicles (short arrow) with not so prominent eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps appeared coarse.

F. Group 3 spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps are appeared coarse

4.3 Histopathological findings of the Thymus

Histological examination of thymus sections stained with haematoxylin and eosin revealed distinct structural variations across the experimental groups as shown in plate 4.3

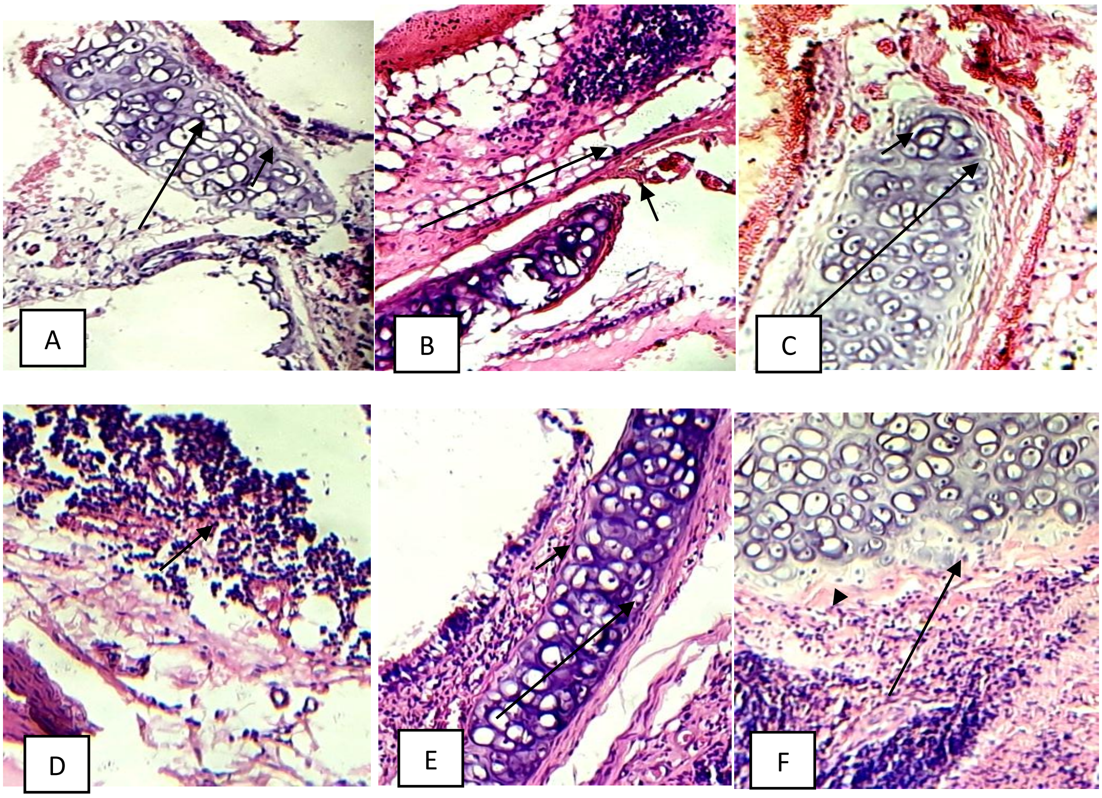

Plate 4.3: Effects of the polyherbal leave aqueous extract in the thymus induced with phenylhydrazine hydrochloride

A. Group 5: Thymus revealed cortex containing vacuolated epithelial cells (long arrow) and mononuclear cells in the medullary adipose tissue (short arrow).

B. **Group 4:** Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

C. **Group 6:** Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

D. **Group 1:** Thymus revealed cortex containing vacuolated epithelial cells (long arrow) and diffuse mononuclear cells in the medullary adipose tissue (short arrow).

E. **Group 2:** Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and slight mononuclear cells in the medulla.

F. Group 3: Thymus revealed cortex surrounding (long arrow) adipose tissue (short arrow) and medulla.

CHAPTER FIVE

DISCUSSION

5.1 Liver Histopathological Findings

The liver plays a critical role in haem metabolism, detoxification, and antioxidant defence, making it particularly susceptible to oxidative injury during phenylhydrazine-induced haemolytic anaemia. In this study, histopathological alterations observed in the untreated anaemic group are consistent with oxidative stress-mediated hepatic injury, which has been widely reported in phenylhydrazine models (Naman *et al.*, 2020; Ezeonu *et al.*, 2023). The presence of pyknotic nuclei and disorganisation of hepatocyte cords in the untreated anaemic group indicates hepatocellular degeneration and apoptosis, likely resulting from excessive reactive oxygen species generated during phenylhydrazine metabolism. Phenylhydrazine is known to induce lipid peroxidation, leading to membrane instability and nuclear damage in hepatocytes (Audu *et al.*, 2022). In contrast, the normal control group displayed preserved hepatic architecture, confirming that the observed pathological changes in other groups were treatment-related rather than procedural artefacts. Mild sinusoidal dilatation observed in this group is commonly reported in normal rodent liver histology and does not necessarily indicate pathology (Bancroft and Gamble, 2019). The treatment groups demonstrated varying degrees of histological improvement, characterised by reduced cellular degeneration, milder inflammatory infiltration, and partial restoration of hepatocyte organisation. The presence of only mild steatosis and hydropic changes in some treated groups suggests attenuation of phenylhydrazine-induced hepatic injury. Such protective effects may be attributed to the antioxidant and cytoprotective properties of the polyherbal aqueous extract.

Previous studies have reported that *Ipomoea batatas* leaf extract exhibits antioxidant activity capable of reducing lipid peroxidation and protecting hepatic tissues from oxidative damage (Gabriel and Idu, 2021). Similarly, *Justicia carnea* has been shown to improve tissue integrity and reduce inflammatory infiltration in anaemic models, likely due to its flavonoid and phenolic constituents (Ejiofor *et al.*, 2025). The hepatoprotective effects observed in this study may therefore reflect a synergistic interaction among the bioactive compounds present in *I. batatas*, *J. carnea* and *Ficus sur*. Hydropic and fatty changes observed in some treated groups may represent reversible cellular injury, suggesting ongoing regeneration rather than irreversible necrosis. Such findings are consistent with studies reporting gradual hepatic recovery following antioxidant therapy in chemically induced anaemia (Ezeonu *et al.*, 2023).

5.2 Spleen Histopathological Findings

The spleen plays a critical role in erythrocyte turnover, immune surveillance, and removal of damaged red blood cells, particularly during haemolytic states. In phenylhydrazine-induced haemolytic anaemia, splenic architecture is commonly altered due to excessive erythrophagocytosis and increased workload on the reticuloendothelial system (Naman *et al.*, 2020; Ibegbulam *et al.*, 2020). The untreated anaemic group exhibited coarse red pulp and altered white pulp organisation, findings consistent with splenic congestion and heightened phagocytic activity. Phenylhydrazine-induced oxidative damage promotes premature erythrocyte destruction, leading to increased sequestration of damaged cells within the spleen and subsequent architectural distortion (Ezeonu *et al.*, 2023). Enlarged and eccentrically positioned blood vessels observed in this group further support splenic vascular stress and congestion. The normal control group displayed well-defined lymphoid follicles and preserved red and white pulp organisation, confirming that the structural alterations observed in other groups were attributable to phenylhydrazine toxicity and treatment effects rather than handling or processing artefacts.

Spleen sections from treated groups showed varying degrees of architectural restoration, characterised by better-defined lymphoid follicles and reduced red pulp coarseness when compared with the untreated anaemic group. The presence of activated lymphoid follicles in some treated groups suggests immunomodulatory activity and restoration of splenic function. Such findings have been reported in studies evaluating plant-based interventions in chemically induced anaemia (Ejiofor *et al.*, 2025). The observed histological improvement may be attributed to the antioxidant and haematinic properties of the polyherbal aqueous extract. *Ipomoea batatas* has been reported to reduce oxidative stress and improve haematological recovery, thereby decreasing excessive erythrocyte destruction within the spleen (Gabriel and Idu, 2021). Similarly, *Justicia carnea* has demonstrated protective effects on lymphoid organs, possibly through stabilisation of cell membranes and reduction of inflammatory responses (Igbinaduwa *et al.*, 2020). The combined activity of these plants, along with *Ficus sur*, may therefore contribute to reduced splenic congestion and improved tissue organisation.

5.3 Thymus Histopathological Findings

The thymus is a primary lymphoid organ responsible for T-lymphocyte maturation and immune regulation. It is highly sensitive to oxidative stress, systemic inflammation and metabolic disturbances, which commonly accompany haemolytic anaemia (Ibegbulam *et al.*, 2020). Phenylhydrazine-induced haemolytic anaemia has been shown to promote thymic atrophy through increased oxidative damage, apoptosis of thymocytes and fatty infiltration of thymic tissue (Ezeonu *et al.*, 2023). In this study, the untreated anaemic group demonstrated marked thymic architectural distortion characterised by increased adipose tissue infiltration and reduced lymphoid cellularity. These findings are consistent with thymic involution, a process associated with oxidative stress and chronic inflammatory states (Akanmu *et al.*, 2017). Increased adipose replacement of thymic parenchyma suggests impaired immune competence and reduced thymopoiesis. The normal control group displayed relatively preserved cortico-medullary organisation, confirming that the structural abnormalities observed in the anaemic groups were attributable to phenylhydrazine-induced toxicity. The presence of vacuolated epithelial cells in some sections may represent physiological variation or mild reversible cellular stress. Thymus sections from the polyherbal-treated groups showed varying degrees of architectural recovery, characterised by improved cortical organisation, reduced adipose infiltration and mild mononuclear cell presence. These changes suggest attenuation of phenylhydrazine-induced thymic injury and partial restoration of immune tissue integrity. Similar findings have been reported in studies evaluating antioxidant-rich plant extracts in chemically induced anaemia and immunotoxicity models (Ejiofor *et al.*, 2025).

The observed protective effects may be attributed to the antioxidant and immunomodulatory properties of the polyherbal aqueous extract. *Ipomoea batatas* has been reported to reduce oxidative stress and support immune organ integrity (Gabriel and Idu, 2021), while *Justicia carnea* possesses bioactive compounds capable of modulating inflammatory responses and preserving lymphoid tissue architecture (Igbinaduwa *et al.*, 2020). The inclusion of *Ficus sur* may further enhance these effects through its phenolic-mediated cytoprotective actions.

Phenylhydrazine-induced haemolytic anaemia resulted in marked oxidative and inflammatory damage to the liver, spleen and thymus, reflecting the systemic nature of haemolysis and excessive erythrocyte destruction. Hepatic alterations observed across groups are consistent with impaired haem metabolism and oxidative stress, as the liver plays a central role in detoxification and bilirubin processing. Splenic architectural distortion reflects increased erythrophagocytosis and reticuloendothelial overload, a hallmark of haemolytic states. Thymic involution and adipose infiltration further indicate immunological stress and suppression of lymphoid tissue during sustained oxidative injury. Treatment with the polyherbal aqueous extract mitigated these changes, suggesting antioxidant-mediated cytoprotection and reduced inflammatory burden. The coordinated improvement in hepatic architecture, splenic organisation and thymic integrity indicates a systemic protective effect rather than isolated organ recovery. These effects are likely mediated through synergistic actions of phytochemicals that stabilise cell membranes, scavenge reactive oxygen species and support immune and haematopoietic homeostasis. Summarily, the findings support the regenerative and immunomodulatory potential of the polyherbal formulation in phenylhydrazine-induced haemolytic anaemia.

Findings of the Study

1. Phenylhydrazine-induced haemolytic anaemia caused marked histopathological alterations in the liver, spleen and thymus, including hepatocellular degeneration, splenic congestion and thymic involution.

2. Untreated anaemic rats exhibited pronounced structural distortion of hepatic architecture, coarse splenic red pulp and increased adipose infiltration in thymic tissue.

3. Treatment with the polyherbal aqueous extract resulted in varying degrees of tissue recovery, characterised by improved hepatic organisation, reduced splenic congestion and partial restoration of thymic cortico-medullary structure.

4. The observed histological improvements suggest that the polyherbal extract exerted protective effects against phenylhydrazine-induced oxidative and inflammatory damage.

Contribution to Knowledge

This study provides histopathological evidence supporting the protective and regenerative effects of a polyherbal aqueous leaf extract of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* in phenylhydrazine-induced haemolytic anaemia. Unlike previous studies that focused primarily on haematological indices, this work demonstrates organ-level structural recovery in the liver, spleen and thymus, thereby expanding current understanding of the systemic protective role of polyherbal formulations in haemolytic anaemia. The findings contribute to the scientific validation of traditional medicinal plants and support further investigation into their use as natural therapeutic agents in the management of anaemia and associated organ damage.

CONCLUSION

The polyherbal aqueous leaf extract of *Ipomoea batatas*, *Justicia carnea* and *Ficus sur* attenuated phenylhydrazine-induced histopathological damage in the liver, spleen and thymus. The observed restoration of tissue architecture suggests antioxidant, cytoprotective and immunomodulatory activities of the extract. These findings support the potential of the polyherbal formulation as a natural therapeutic agent in the management of haemolytic anaemia and associated organ damage.

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