

**IMMUNOPROTECTIVE EFFECTS OF THE POLYHERBAL AQUEOUS LEAF
EXTRACT IN PHENYLHYDRAZINE INDUCED IMMUNOSUPPRESSED WISTAR
RATS**



BY

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LSC1707055

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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BIOTECHNOLOGY OPTION

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE
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LABORATORY TECHNOLOGY.**

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project titled, Immunoprotective effects of the aqueous leaf polyherbal extract in phenylhydrazine induced immunosuppressed Wistar rats, was carried out and submitted by IGUODALA OSAHENRUMWEN CHRISTABEL, with matriculation number LSC1707055, in partial fulfillment of the requirements of the degree, Bachelor of Science laboratory Technology at the University of Benin, Edo State.

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DEDICATION

I dedicate this work to Almighty God, the giver of life for His abundance mercies, grace and unfailingly love throughout the course of my work and duration of my study at the University of Benin.

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ABSTRACT

The immune system plays a vital role in protecting the body from infections, toxin, and oxidative damage. However, it can be weakened or impaired by chronic infections, autoimmune diseases, malnutrition, exposure to chemical agents, or certain medications. In recent years, plant-based immunomodulators have gained global attention as promising alternatives to synthetic drugs such as *Ipomoea batatas*, *Ficus carpensis* and *Justica carnea*, being utilised as immunoprotective agents. The aim is to assess the Immunoprotective effects of the aqueous polyherbal leaf extract of equal amount of *Ipomoea batatas*, *Ficus carpensis*, and *Justica carnea*, in phenylhydrazine induced immunosuppressed Wistar rats. The method used for this analysis were evaluated using standard and established method. Oral administration of polyherbal extract at doses 25, 50, 100 mg/kg significantly increase blood levels compared to untreated group. CD_4^+ and CD_8^+ T-lymphocyte counts were monitored for 24 hours(1 day), 7 days, and 14 days. Results showed a dose dependent restoration of immune cell counts, with 50 mg/kg group exhibiting the most significant improvement in ($CD_4^+ = 7.95 \pm 0.12$ cells/mm³; $CD_8^+ = 3.11 \pm 0.0$ cells/mm³) when compared to the untreated group. This study demonstrates the efficacy of the polyherbal leaf extract of equal amount of *Ipomoea batatas*, *Ficus carpensis*, and *Justica carnea* as an immunoprotective agent at 50 mg/kg of the extract as the best. This validates its traditional use and such insights as a promising alternative for the development of innovative treatments for metabolic disorders.

CHAPTER ONE

1.1 INTRODUCTION

The immune system is a complex and dynamic defense network composed of organs, cells, and molecules working together to protect the host from infections, toxins, and other harmful stimuli. This system is divided into the innate and adaptive arms. The innate immune system provides the first line of defense through barriers, phagocytic cells, natural killer cells, and the complement system, while the adaptive immune system involves antigen-specific T and B lymphocytes, producing long-lasting protective immunity (Jantan *et al.*, 2022; Gasmi *et al.*, 2023). A well-functioning immune system is critical for survival, as even minor deficiencies can increase vulnerability to infections, malignancies, and autoimmune dysfunctions and maintain physiological stability.

Immunosuppression occurs when this protective system is weakened or impaired. This can result from chronic infections, autoimmune diseases, malnutrition, exposure to chemical agents, or certain medications. When the immune system is compromised, the body becomes more susceptible to opportunistic infections and systemic disorders. Beyond increased infection risk, immunosuppression also has far-reaching effects on various physiological systems—including the hematopoietic system responsible for blood cell formation and oxygen transport (Kumar *et al.*, 2022; Patel *et al.*, 2025).

One of the notable consequences and aggravating factors of immune dysfunction is anaemia. Anaemia, characterized by reduced red blood cell count or hemoglobin concentration, can develop from chronic infections, nutritional deficiencies, or immune-mediated destruction of blood cells. It not only results from a weakened immune response but can also exacerbate immune compromise by impairing oxygen delivery to immune tissues and organs. This creates a

vicious cycle, where immune weakness fuels anemia and anaemia further suppresses immune efficiency (Nairz *et al.*, 2021). In particular, hemolytic anemia, caused by accelerated destruction of red blood cells, often involves immune and oxidative mechanisms that damage erythrocyte membranes. Worldwide, anaemia affects about 800 million including children and women, while in sub-Saharan Africa, an estimate of 83.5 million people are affected by this haematological disorder (WHO, 2017).

In experimental research, phenylhydrazine (PHZ) has been widely used to induce hemolytic anaemia and oxidative stress in laboratory animals. PHZ causes oxidative modification of hemoglobin, leading to red blood cell lysis, splenic congestion, and secondary immune system compromise (Ousaid *et al.*, 2022). PHZ-induced anaemia provides a reliable and reproducible model for studying immunosuppression, as it mimics conditions of oxidative stress and immune imbalance seen in many human disorders.

Addressing both immunosuppression and anaemia often requires interventions that support immune function, protect against oxidative damage, and enhance hematopoiesis. In recent years, plant-based immunomodulators have gained global attention as promising alternatives or adjuncts to synthetic drugs. Traditional medical systems, particularly in Africa and Asia, have long relied on herbal remedies to boost general body resistance—a concept now aligned with immunomodulation and immunoprotection (Patel *et al.*, 2025). Many plants are rich in bioactive phytochemicals such as flavonoids, alkaloids, saponins, and tannins, which can modulate immune function by increasing immune cell proliferation, enhancing antioxidant defenses, stimulating cytokine production, and improving red blood cell regeneration (Gasmi *et al.*, 2023; Kumar *et al.*, 2022).

Polyherbal formulations, which combine multiple plant species, are particularly valued for their synergistic effects, offering broader pharmacological activity than single herbs (Sheth *et al.*, 2021). Aqueous leaf extracts of plants such as *Ficus capensis*, *Ipomoea batatas*, and *Justicia carnea*—commonly used in African traditional medicine—have been shown to exhibit hematopoietic, antioxidant, and immunoprotective properties (Akomolafe *et al.*, 2016; Ezeigwe *et al.*, 2020; Enaohwo *et al.*, 2025). These properties make them ideal candidates for evaluation in PHZ-induced immunosuppressed and anaemic models.

This study, therefore, aims to investigate the immunoprotective effects of an aqueous leaf polyherbal extract in phenylhydrazine-induced immunosuppressed rats. By evaluating hematological indices, immune cell profiles, antioxidant status, and histological changes in immune organs, the research seeks to provide scientific evidence supporting the traditional use of medicinal plants in boosting immunity and managing anaemia. If successful, the findings could contribute to developing affordable and accessible plant-based immunoprotective agents, especially valuable in low-resource settings where both anaemia and immune-related disorders are prevalent.

1.2 STATEMENT OF THE PROBLEM

This study will help evaluate and analyze the immunoprotective potential of the combined effects of *Ipomoea batatas*, *Ficus capensis* and *Justica carnea*, extract in the treatment, and management in Phenylhydrazine-induced immunosuppressed rats. Understanding their efficacy could support the development of affordable, accessible, and safer natural interventions to improve immune health with focus on anaemic conditions.

1.3 AIM OF STUDY

In this research, I conducted an analysis on Immunoprotective effects of the aqueous polyherbal leaf extract of equal amount of *Ipomoea batatas*, *Ficus capensis*, and *Justica carnea*, in phenylhydrazine induced immunosuppressed Wistar rats.

1.4 OBJECTIVE OF THE STUDY

1. To prepare and combine the aqueous leaf polyherbal extract of *Ipomoea batatas*, *Ficus capensis* and *Justica carnea*.
2. To induce anaemia in Wistar albino rats using phenylhydrazine as a chemical agent.
3. To evaluate the combined effects of *Ipomoea batatas*, *Ficus capensis* and *Justica carnea*, on hematological parameters(e.g., red blood cell count, hemoglobin, white blood cell count, and differential count) in immunosuppressed rats.
4. To examine histopathological changes in immune-related organs (e.g, Kidneys, Spleen, Liver) of treated and untreated groups. To compare the efficacy of the polyherbal extract with standard immunomodulatory treatments.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Ipomoea batatas*

Ipomoea batatas (L.) Lam. is a hexaploid root and vine crop of the Convolvulaceae family cultivated across tropical and subtropical regions for its storage roots and edible leaves. Over the last decade, it has gained renewed attention as a climate-resilient staple and “functional food,” owing to its high nutrient density and abundant bioactives (Alam, 2021). From a global nutrition perspective, sweet potato contributes significantly to vitamin and mineral intake, with orange-fleshed types (OFSP) notable for provitamin A carotenoids; across root and tuber crops it delivers comparatively higher carbohydrate, selected vitamins (A and C), and key minerals, making it a strategic crop for low-resource settings and food-based nutrition programs. (Laveriano-Santos *et al.*, 2022).

2.1.1 Scientific Classification

Kingdom: Plantae

Division: Tracheophyta

Subdivision: Spermatophyta

Clade: Magnoliopsida

Order: Solanales

Family: Convolvulaceae

Genus: *Ipomoea*

Species: *Ipomoea batatas*

The genus *Ipomoea* encompasses over 600 species, with *I. batatas* being one of the most economically significant. . It is only distantly related to potato (*Solanum tuberosum*), which is in the Nightshade family, Solanaceae. It is an ancient food from tropical America and the Pacific Island. It was originated in Central America is now extensively cultivated and consumed throughout the world. China is the leading producer of sweet potato followed by Nigeria and Tanzania, Indonesia, and Uganda. It is mainly cultivated from the tubers, used as vegetables, eaten boiled, baked, fried and grounded into flour to make biscuit, bread and other pastries. The leaves of sweet potato is used as potherb in Southeast Asia, they can also be used as forage and the leaf has rich protein content that helps to fill the nutritional gap. The leaf decoction of sweet potato is used in folk remedies for the tumour of the mouth and throat. It can also be used as an alternative for astringent, tonic, laxative, fungicide. Sweet potatoes merit a place in tropical gardens because it is easy to cultivate and yield edible tubers as well as leaves. The leaves are rich sources of protein, carbohydrates and fat. (Osime *et al.*, 2009)

2.1.2 Botanical Description

Ipomoea batatas (L.) Lam., commonly known as sweet potato, belongs to the family Convolvulaceae and is a perennial, herbaceous, dicotyledonous plant primarily cultivated as an annual crop. It is only distantly related to potato (*Solanum tuberosum*), which is in the Nightshade family, Solanaceae. It is characterized by trailing or twining slender stems that can extend up to 4 meters, with adventitious roots forming at the nodes. The plant produces tuberous storage roots, which are typically fusiform or oblong, varying in colour from white, yellow, and orange to deep purple, depending on the cultivar and anthocyanin or carotenoid content (Islam, 2024; Laveriano-Santos *et al.*, 2022). It can be grown in well-drained loamy soil, which is rich in

humus to provide a warm and moist environment to the root. If grown as a vine, it needs training, but when grown as a ground cover, it spreads quickly by runners. Propagation is through root cutting. It is susceptible to frost so it is considered an annual, but it can be propagated from tuber cuttings planted after the last frost. It is resistant to drought and dry soil but is frequently damaged by deer. The edible tuberous roots generally have a burgundy skin and orange flesh though they are found in a wide variety of colours. They are edible raw or cooked. Ornamental cultivars of sweet potato vine are frequently used in mixed containers for their vibrant colours. Also consider planting in an edible, pollinator, water, or rock garden. It is an attractant for bees and pollinators.

The leaves are simple, alternate, and borne on long petioles, with shapes ranging from heart-shaped to lobed forms, and a smooth or slightly pubescent texture. The leaf colour varies from light to deep green, sometimes with purple pigmentation along the veins. The flowers are funnel-shaped, bisexual, and typically pink to pale lavender, resembling those of morning glory (*Ipomoea purpurea*), with a five-lobed corolla and an inferior ovary containing two ovules (Nguyen *et al.*, 2021). The plant is cross-pollinated mainly by insects but can also self-pollinate under controlled conditions. Its fruit is a small, two-celled capsule enclosing one to four brownish-black seeds.

2.1.3 Nutritional Composition

Sweet potato roots provide complex carbohydrates, dietary fibre, and a spectrum of vitamins (notably provitamin A carotenoids in orange-fleshed cultivars) and minerals. Systematic evidence suggests consumption improves vitamin A status, glycaemic indices, blood pressure, and several clinical biomarkers in different populations, though effect sizes vary with cultivar,

dose, and background diet (Yuxin Qin *et al.*, 2022). Comparative work highlights a partitioning of nutrients and bioactives: leaves typically exhibit higher phenolic and antioxidant capacity, while roots—especially orange-fleshed—concentrate β -carotene (Hapke *et al.*, 2024). The leaves themselves are increasingly consumed as a green vegetable or processed into powders/extracts. Recent in-vitro and ex-vivo studies document substantial total polyphenols/flavonoids, with cultivar-dependent variability and demonstrable antioxidant and antimicrobial activity (Sultana *et al.*, 2024) Beyond proximate nutrition, leaves and purple-fleshed roots contribute anthocyanins and diverse phenolics that underlie many reported bioactivities (Nguyen *et al.*, 2021).



Figure 2.1. The leaves of *Ipomoea batatas*

Source: (Google images)

2.1.4 Phytochemical Properties

Ipomoea batatas accumulates diverse phytochemicals across its organs. Roots (especially orange-fleshed cultivars) are rich in carotenoids (β -carotene, β -cryptoxanthin), while purple-fleshed roots concentrate acylated anthocyanins (mainly peonidin/cyanidin derivatives). Leaves provide high total phenolics (phenolic acids and flavonoids), often exceeding many common vegetables in polyphenol content. Carotenoids are found in roots, phenolics in leaves, anthocyanins in purple flesh. *Ipomoea batatas* have gained significant attention in pharmaceutical and biomedical research owing to their rich phytochemical profile and demonstrated bioactivities.

2.1.5 Anti-diabetic and metabolic effects

Extracts from sweet potato have been shown in preclinical and some clinical settings to improve glycaemic control, increase insulin sensitivity, reduce fasting and post-prandial glucose levels, and modulate lipid profiles. For example, tuber extracts (white sweet potato “Caiapo”) were used in human studies to reduce HbA1c and improve insulin sensitivity. The mechanisms include enhancement of glucose uptake, inhibition of carbohydrate-digesting enzymes, and modulation

of adiponectin/leptin signalling. Given the global burden of type 2 diabetes, sweet potato presents as a plant-based adjunct in metabolic disorder management (Elgabry *et al.*, 2023).

2.1.6 Antioxidant, anti-inflammatory & hepatoprotective effects

The high content of carotenoids, anthocyanins and phenolic compounds in sweet potato contribute to potent antioxidant activity. These effects translate into protection against oxidative stress, mitigation of inflammation, and hepatoprotective outcomes (reduced liver enzyme release, reduced lipid peroxidation) in animal models (Sharma and Sharma, 2023). For instance, purple-fleshed sweet potato extracts suppress oxidative damage in liver injury models. These properties are relevant in pharmaceutical contexts such as chronic inflammatory diseases, liver disorders and ageing-related deficits.

2.1.7 Anticancer and anti-proliferative activities

Various *in vitro* and *in vivo* studies indicate that sweet potato leaf and tuber extracts inhibit proliferation of cancer cell lines (breast, prostate, and colon), induce apoptosis, and modulate cell cycle arrest. These effects are attributed primarily to phenolic acids (such as caffeoylquinic acids), anthocyanins and flavonoids (Elgabry *et al.*, 2023). Although human clinical trials remain scarce, these preclinical findings suggest a promising pharmaceutical adjunct role in oncology supportive therapy or chemoprevention.

2.1.8 Antimicrobial, anti-ulcer and immunomodulatory effects

Sweet potato extracts demonstrate antimicrobial properties against bacteria such as *Staphylococcus aureus*, *E. coli*, and *Salmonella* species, likely due to phenolics and flavonoids in

leaves and roots (Sharma and Sharma, 2023). In gastrointestinal research, certain sweet potato root extracts show anti-ulcer activity (protecting gastric mucosa under stress models) (Elgabry *et al.*, 2023). In immunology, polysaccharide fractions from sweet potato have shown immunostimulant potential—augmenting immune cell activity and modulating cytokine release—in animal models (Elgabry *et al.*, 2023). These multiple activities open opportunities for formulation of nutraceutical or pharmaceutical products deriving from sweet potato components.

2.1.9 Hematological Effects Of *Ipomoea Batatas*

1. Increase in Hemoglobin and Hematocrit Levels

A study involving multiparous pregnant women demonstrated that consuming sweet potato-based interventions, in conjunction with iron tablets, led to a significant increase in haemoglobin (Hb) levels (from 10.46 to 11.16 g/dL) and Hematocrit (Hct) levels (from 32.5% to 35.5%) after 2 weeks. (Marniati *et al.*, 2020).

2. Improved Blood Parameter in Mice

In an animal model, administration of purple sweet potato pollen (*Ipomoea batatas*) to sodium nitrite-induced anemic mice resulted in normalization of blood cells components, including red and white blood cell count, Hb concentration, and hematocrit levels. (Rismawati *et al.*, 2020).

3. Enhanced Iron Absorption

Biofortified orange-fleshed sweet potatoes have shown increased iron absorption. A study found that women consuming iron-biofortified sweet potatoes absorbed 1.9 times more iron than those

consuming regular varieties, potentially aiding in the prevention and treatment of iron-deficiency anemia. (Jongstra, 2020).

4. Positive Effects in Adolescent Girls

A study on adolescent girls revealed that consuming sweet potato-based cookies (UJU cookies) led to a significant increase in Hb levels (by 0.52 g/dL) and Hct levels (by 4.04%) over a 60-day period. (Rismawati *et al.*, 2020).

2.2 Ficus capensis

The species *Ficus capensis* Thunb. is classified within the family Moraceae, genus Ficus. The genus Ficus is one of the largest angiosperm genera, comprising roughly 750 species worldwide (Madrigal-Santillán *et al.* 2024). Within the genus, *F. capensis* is native to parts of tropical and subtropical Africa, often found in savannah and woodland habitats. Some taxonomic treatments have treated *F. capensis* as a synonym of *Ficus sur* Forssk., but in many African floras it remains recognized as *F. capensis* Thunb (Nweze *et al.*, 2014) .The correct binomial therefore is *Ficus capensis* Thunb., although local nomenclature may vary.

2.2.1 Scientific Classification

Kingdom: Plantae

Phylum: Tracheophyta

Clade: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *Ficus sur*

Plants from the genus *Ficus* (family Moraceae) have long been valued in traditional medicine, ethnobotany and nutrition. Among them is *Ficus capensis* (commonly called “Cape fig” or “bush fig”) stands out due to its widespread use in Africa for both foods and therapeutic applications.

2.2.2 Botanical Description

Ficus capensis is typically a medium-to-large evergreen tree or large shrub. It can reach heights of 10–20 m or more, with a broad, spreading crown and often prominent buttress roots in mature trees. The bark is smooth and pale when young, becoming greyish and fissured with age. The leaves are alternate, simple, leathery, and vary in shape from ovate to elliptic; the margin is often entire or slightly toothed, with a glossy upper surface and paler underside. The petiole is distinct and the leaf lamina may display prominent venation. The figs (syconia) are borne in leaf axils or on short lateral branches; they are globose to subglobose, approximately 1–2 cm in diameter when ripe, and change colour from green to purplish or reddish. The latex is present in stems and leaves, exuding when damaged. The root system supports adventitious rooting and sometimes aerial buttresses, especially in shallow soils. The species is frequently found in riparian zones and woodland margins, indicating some adaptability (Madrigal-Santillán *et al.* 2024). Morphologically, *F. capensis* shows considerable plasticity, with variations in leaf size, shape, crown form and root buttress development depending on site conditions (soil depth, moisture, exposure). The tree is found from Cape Verde and Senegambia, across tropical West Africa to Cameroon and the Central Africa Republic, then eastwards to Eritrea, Northern Somalia and Yemen, and southwards through all tropical eastern and southern African countries.



Figure 2.2.1 The leaves of *Ficus capensis*

Source: (Ani et al., 2024)

2.2.3 Nutritional Composition

Beyond its structural and ecological characteristics, *F. capensis* has been increasingly studied for its nutritional composition, particularly of leaves, stems and fruits, reflecting its usage as both food and medicine in traditional societies. Proximate analyses of leaves have reported high carbohydrate content (for example ~73.8 % in one study), moderate protein (~6.3 %), low lipids (\approx 1.8 %) and moisture >100 % on fresh weight basis in some sample (Achi *et al.*, 2017). Mineral analyses reveal significant levels of calcium, potassium, iron, magnesium and trace minerals: e.g.,

a study of the stem showed Ca ~34.01 mg/kg, K ~21.40 mg/kg, Fe ~0.89 mg/kg and other elements like Zn and Mn present though in low amounts (Ani *et al.*, 2024). Vitamins have also been quantified: in one 2024 study of stem material, vitamins A (0.57 mg/g), β -carotene (1.27 mg/g), B-complex (B1, B2, B3, B6, B9) and vitamins C (0.81 mg/g), D (0.26 mg/g), E (0.85 mg/g), K (1.10 mg/g) were reported (Ani *et al.*, 2024). These data collectively indicate that *F. capensis* may serve as a food-nutrient source in regions where it grows naturally, and support its ethnomedicinal use as a “blood-tonic” or nutritive leafy vegetable.

2.2.4 Phytochemical Properties

2.2.5 Antioxidant & anti-inflammatory (core mechanisms)

Aqueous leaf extracts reduce oxidative damage and inflammatory mediators *in vivo*, indicating robust redox and anti-inflammatory effects that underpin many downstream benefits. In a carboplatin-injury model, lyophilized aqueous leaf extract restored hepatic antioxidants and suppressed inflammation (Josiah *et al.*, 2024).

2.2.6 Hepatoprotective Effects

In rats with carboplatin-induced liver injury, *F. capensis* leaf extract significantly improved serum liver enzymes and histo-architecture via antioxidant/inflammation modulation—supporting hepatoprotective potential and possible adjuvant use during cytotoxic therapy (Josiah *et al.*, 2024).

2.2.7 Anti-anaemic Effects

Both aqueous and ethanolic leaf extracts reversed phenylhydrazine (PHZ)-induced anaemia, improving RBC indices and oxidative stress markers; an aqueous co-formulation with *Cnidoscolus aconitifolius* showed dose-responsive benefits. These results align with traditional “blood-building” uses (Ibe *et al.*, 2022).

2.2.8 Antihypertensive / cardiometabolic enzyme targeting

Leaf extracts inhibit ACE and arginase (enzymes linked to vascular tone and erectile function), and also inhibit acetylcholinesterase (AChE). Diets enriched with fig leaves in hypertensive rat models reduced systolic/diastolic BP and ACE/arginase activities while improving antioxidant status—collectively supporting vascular benefits for the *Ficus* leaves cohort that includes *F. capensis* (Seun, *et al.*, 2016).

2.2.9 Hematological Effects Of *Ficus Capensis*

1. Packed Cell Volume (PCV) and Hemoglobin (Hb) Levels:

Studies have consistently shown that *F. capensis* extracts lead to significant increases in PCV and Hb levels in anemic rats. For instance, a combination of 400 mg/kg aqueous extracts of *F. capensis* and *Cnidoscolus aconitifolius* resulted in a PCV of 41.94% and Hb concentration of 13.97 g/dL, compared to 36.31% and 12.06 g/dL, respectively, in rats treated with *F. capensis* alone (Obiajulu, *et al.* 2022).

2. Red Blood Cell (RBC) Count:

Treatment with *F. capensis* extracts has led to significant increases in RBC counts. For example, rats administered 200 mg/kg of the extract exhibited RBC counts of 8.39×10^6 cells/ μL , compared to 6.44×10^6 cells/ μL in the control group (Njoku-Oji, 2016)

3. White Blood Cell (WBC) Count:

Increases in WBC counts have been observed, indicating potential immunostimulatory effects. Rats treated with higher doses of *F. capensis* extracts showed elevated WBC counts, suggesting an enhancement in immune response (Njoku-Oji, 2016)

4. Platelet Count and Differential Leukocyte Count:

While specific data on platelet counts are limited, studies have reported changes in differential leukocyte counts, such as neutrophilia and lymphopenia, following administration of *F. capensis* extracts. These alterations may reflect an adaptive immune response (Njoku-Oji, 2016).

2.3 *Justicia carnea*

Plants used in traditional medicine serve as an important bridge between ethnobotany and modern pharmacology. Among such species is *Justicia carnea*, a member of the Acanthaceae family, which has been used traditionally in several countries for a broad range of health conditions—from anaemia and infections to inflammatory and metabolic disorders. This introduction provides a detailed overview of *J. carnea*: first covering its taxonomy; then its botanical characteristics; followed by its nutritional composition; and finally examining its phytochemistry and pharmaceutical importance. Together, this sets the foundation for more detailed study of its immunoprotective or therapeutic potentials (Carneiro *et al.*, 2023)

2.3.1 Scientific Classification

Kingdom: Plantae

Clade: Angiosperms

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia*

Species: *Justicia carnea* Lindl.

Synonyms include *Jacobinia carnea* (Lindl.) Nicholson and other historic names. Common names include Brazilian plume flower, plume flower, flamingo flower, and Jacobinia (especially in horticultural contexts). The species is native to the Atlantic Forest region of eastern Brazil and has been cultivated and introduced elsewhere in tropical and subtropical climates. Its taxonomic identity is therefore well established and its genus widely recognized in phytochemical and ethnopharmacological research.

2.3.2 Botanical Description

Justicia carnea is an evergreen shrub (or sometimes perennial sub-shrub) which is appreciated horticulturally for its showy flowers and lush foliage, but it also exhibits morphological features

relevant to its botanical and pharmacological study. The plant typically grows to about 1.2-1.8 m tall and 0.6-1.2 m wide in favourable conditions. Leaves are large, dark green, glossy, and have prominent veins; lengths of up to 20 cm have been reported, with pointed ends and coarse texture. The stems are erect, soft-wooded, and branch to form a bushy habit. The plant also produces dense terminal spikes (“plumes”) of tubular flowers that vary in colour (typically rose-pink, but cultivars may be red, apricot, white, or orange) and appear from early summer through fall—or year-round in suitable climates. Botanically, the flowers are bilateral (zygomorphic), tubular, and arranged in a spike-like inflorescence; the calyx is tubular and lobed, the corolla flared at the mouth, usually 3–4 cm long, often attracting hummingbirds or butterflies (in its native or horticultural settings) due to their nectar. The plant thrives in moist, well-drained soils in partly shaded to semi-shade conditions; it is not frost-tolerant in cooler climates. Fruits are capsules typical of the Acanthaceae, containing numerous small seeds, although in many cultivated settings the ornamental value overshadows seed production. Morphological variability among cultivars (flower-color forms, leaf-shape variations) is known, which can impact phytochemical content (as leaves vs flowers may vary in metabolite accumulation). Therefore, in pharmacological-nutritional study, specifying the plant part and cultivar is important.



Figure 2.3.1 The leaves of *Ficus capensis*

Source: (Abosedo *et al.*, 2022)

2.3.3 Nutritional Composition

The nutritional profile of *J. carnea* has gained attention more recently, as researchers recognise its potential both as a leafy vegetable (in regions where it is consumed) and as a source of nutrients for medicinal applications. Several studies have evaluated the proximate composition (moisture, protein, fat, fibre, ash, and carbohydrate), mineral and vitamin content, along with bioactive phytochemicals. A study comparing leaf meals of *J. carnea* reported crude protein circa 22.3 g/100 g, fibre ~42.5 g/100 g, ash ~15.6 g/100 g, lipid ~8.0 g/100 g, moisture ~13.6%

(though note this was a dry-basis leaf meal study) in one sample. Ajuru *et al.*, 2021). Another nutritional investigation of an aqueous leaf extract found it “rich in proximate composition, phytochemicals, minerals and vitamins” and safe in animal studies of anaemia (Anthonia *et al.*, 2019). One biochemical analysis reported leaves with vitamin C ~232.32 mg/100g, vitamin A ~22.16 mg/kg, and significant iron levels (8.61 mg/kg) and other micronutrients (Asiwe *et al.*, 2023). A more recent layered-extract study found that different aqueous extract layers of *J. carnea* contained varying vitamins and minerals, with the “down-layer” having higher vitamins/minerals content (Ani *et al.*, 2025). There is also a presence of high levels of flavonoids, alkaloids, saponins, phenolics, and other secondary metabolites confers functional nutritional value (nutraceutical value) to *J. carnea* leaves. For example, these bioactive compounds may enhance micronutrient uptake, support antioxidant defence, or modulate metabolism.

2.3.4 Phytochemical Properties

2.3.5 Phytochemistry Effects

Multiple analyses have identified a broad spectrum of secondary metabolites. For instance, a recent GC-FID/GC-MS study of the ethanol leaf extract of *J. carnea* reported abundant compounds such as spartein (9.17 µg/ml), epihedrine (7.35 µg/ml), naringenin (7.20 µg/ml), phytate (6.86 µg/ml), kaempferol (6.43 µg/ml), dihydrocytisine (5.73 µg/ml), sapogenin (4.42 µg/ml), flavone (3.90 µg/ml) and anthocyanin (3.86 µg/ml) among others; and 86 volatile bioactive compounds were identified by GC-MS (Asiwe *et al.*, 2023). Another recent study using aqueous leaf extract and GC-MS indicated higher-resolution structural elucidation of a cassette of bioactives including flavonoid, alkaloid and fatty acid constituents (OMEJE *et al.*, 2025).

2.3.6 Anti-anaemic & haematological effects

Several *in vivo* studies have shown that *J. carnea* leaf extracts reverse anaemia (induced in animal models) by improving RBC count, haemoglobin, PCV and other haematological indices. One nutritional study showed that aqueous leaf extract, administered at graded doses, significantly reverted anaemic rats within 4 to 8 days and did not adversely impact liver or kidney function (Oboma *et al.*, 2024).

2.3.7 Antioxidant / neuroprotective / anti-inflammatory activity

A study on *J. carnea* leaf extract in mice exposed to TNBS (an oxidative stress/inflammatory insult) found that treatment significantly increased antioxidant enzyme activities in Superoxide Dismutase (SOD), Glutathione Peroxide (GPx), Catalase (CAT), Glutathione (GSH), decreased Malondialdehyde (MDA), Nitric acid (NO) and C- reactive protein (CRP), and protected against cortical necrosis and neuronal degeneration—indicating strong neuroprotective potential (Enaohwo *et al.*, 2025). More broadly, reviews of the genus *Justicia* highlight that species (including *J. carnea*) show antioxidant, antimicrobial, anti-diabetic, antihyperglycaemic, and anti-inflammatory activities (Carneiro *et al.*, 2023).

2.3.8 Antimicrobial / antibacterial

A paper reported isolation of chlorogenic acid, quinaldic acid, oleic acid from leaf extracts of *J. carnea*, and antibacterial activity (zones of inhibition \geq ~11 mm) against several pathogens (*S. aureus*, *E. coli*, *Salmonella typhi*, etc.) (Ijoma *et al.*, 2025).

2.3.9 Hematological Effects Of *Justica carnea*

1. Reversal of experimental anemia (PHZ model)

Ethanol or methanol leaf extracts significantly increase Hb, PCV/Hct, and RBC counts and normalize red-cell indices in phenylhydrazine-induced anaemic rats; several studies also report improved lipid profile alongside hematinic action (Onyeabo *et al.*, 2017).

2. Protection in toxin models

Aqueous (and aqueous-ethanol) leaf extracts attenuate TAA-induced haematological disruptions (restoring Hb, PCV, RBC; modulating WBC), supporting use against haemolytic/oxidative injury (Ebhoon *et al.*, 2023).

3. Mitigation of heavy-metal effects

In lead-acetate exposure, aqueous leaf extract elevates Haemoglobin (Hb), Red Blood Cells (RBC), Packed Cell Volume (PCV), White Blood Cell (WBC) and platelets versus controls, indicating hematinic and marrow-supportive activity even under toxic stress (Onyekwere *et al.*, 2023)

4. Nutritive (hematinic) basis

Quantitative profiles show iron, vitamins (notably vitamin C), amino acids, and flavonoids; authors argue these contribute to the plant's "blood-building" reputation and measured hematological improvements (Andrew *et al.*, 2024).

2.4 Immunoprotection

Immunoprotection refers to the mechanisms and interventions through which an organism's immune system is safeguarded, stimulated, or restored to resist infectious agents, toxins, and immunosuppressive conditions. In biomedical research, immunoprotection is studied to evaluate how drugs, nutrients, or plant-derived compounds modulate immune responses at both the innate and adaptive levels. Several phytochemicals—particularly flavonoids, alkaloids, saponins, and phenolics—have been shown to contribute to immunoprotective effects by mitigating oxidative stress, stimulating hematopoiesis, and maintaining immune organ integrity (Madrigal-Santillán *et al.*, 2024).

2.4.1 Mechanisms of Immunoprotection

The immune system's efficiency depends on a delicate balance between pro-oxidant and antioxidant mechanisms. Excessive oxidative stress weakens immune cells and promotes inflammation. Natural antioxidants in medicinal plants, including vitamins A, C, E and bioactive polyphenols, help restore this balance. They scavenge reactive oxygen species (ROS), enhance endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and reduce lipid peroxidation (Nwadibia *et al.*, 2024). Furthermore, plant-based immunoprotective compounds can modulate cytokine expression, suppress inflammatory mediators such as TNF- α and IL-6, and promote lymphocyte proliferation and macrophage activation. These mechanisms together maintain immune competence, particularly in immunocompromised states caused by toxins, infections, or drug exposure.

2.4.2 Hematological and Organ-Protective Effects

In experimental models, immunoprotection is often reflected in improvements in hematological parameters—such as packed cell volume (PCV), hemoglobin (Hb) concentration, red and white blood cell counts—and in the preservation of lymphoid organ histology. For example, *Justicia carnea* and *Ficus capensis* extracts have demonstrated significant hematopoietic and immunoprotective activities. (Onyeabo *et al.*, 2017) reported that *J. carnea* leaf extract reversed phenylhydrazine-induced immunosuppressive anemia in rats by restoring Hb, RBC, WBC, and PCV values to near-normal levels. Similarly, aqueous leaf extract of *F. capensis* has shown to be ameliorated carboplatin-induced oxidative and inflammatory liver injury, which secondarily improved leukocyte counts and immune status. (Nwadiibia *et al.*, 2024). These findings indicate that antioxidant and anti-inflammatory properties indirectly contribute to immune recovery, since oxidative stress is a major cause of immune cell dysfunction.

2.4.3 Cytokine Modulation and Antioxidant Pathways

Immunoprotective effects also involve molecular regulation of immune mediators. Studies on *Justicia carnea* leaf extracts have shown up-regulation of antioxidant genes and down-regulation of inflammatory cytokines in toxin-induced models (Ebhothon *et al.*, 2023). The increase in antioxidant enzyme activity (superoxide dismutase (*SOD*), catalase (*CAT*), glutathione peroxidase (*GPX*)), and suppression of nitric oxide (NO) and C-reactive protein (CRP) confirm that *J. carnea* acts through both enzymatic and non-enzymatic antioxidant pathways. These antioxidant pathways preserve macrophage and lymphocyte function and protect immune tissues such as the spleen and thymus from oxidative necrosis.

2.4.4 Nutrient Contribution to Immunoprotection

Beyond phytochemicals, nutrient composition plays a vital role in immune regulation. Leaves of *J. carnea* and *F. capensis* contain appreciable amounts of iron, calcium, vitamin C, and β -carotene (Andrew *et al.*, 2024). Iron is required for immune cell proliferation and for haemoglobin synthesis, which maintains oxygen supply to immune tissues. Vitamin C enhances phagocytosis and neutrophil activity, while β -carotene (a precursor of vitamin A) supports mucosal immunity. The combined action of these nutrients and phytochemicals explains why such plant extracts are often described as “blood-builders” or natural immunotonics in African traditional medicine.

2.4.5 Pharmacological Implications

The immunoprotective potential of these plants suggests promising applications in managing conditions characterized by immune suppression—such as chemotherapy-induced neutropenia, viral infections, or malnutrition-related anaemia. Pharmacological evaluation of these extracts can lead to the development of standardized herbal immunostimulants or adjuncts to conventional therapy. However, differences in extraction solvent, dosage, and duration significantly influence outcomes. For instance, hydro-ethanolic extracts of *J. carnea* at higher doses have shown mild hepatic enzyme elevation, highlighting the need for dose standardization and toxicity screening (Akintimehin *et al.*, 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and authentication of *Ipomoea batatas*, *Ficus capensis*, *Justica carnea*

The leaves were obtained from in July 2025 from Ugbowo in Egor Local Government Area of Edo State, Nigeria and authenticated by a plant taxonomist at University of Benin as *Ipomoea batatas*, *Ficus capensis*, *Justica carnea* with herbarium number UBH No 1493, UBH No F331, UBH No J386 respectively.

3.2 Extract Preparation

Fresh leaves of *Ipomoea batatas*, *Ficus capensis*, and *Justica carnea*, were washed and dried under shade for two weeks (14 days) at room temperature to preserve their phytochemical components. After properly drying, all plants samples were subjected to oven drying at 45⁰C for 30 minutes. Once the samples was dried, they were finely grounded using an industrial blender. 93g of the powdered samples of each plant was weighed with an analytical balance. A total of 279 g of plant material was obtained (approximately 93g of each plant species). The measured quantity of the plant powder was placed into a clean soaking jar. Distilled water was used as the extraction solvent. First, 1000mL of distilled water was poured into the jar and the content was thoroughly shaken. An additional 500mL and 400mL were subsequently added to make up a total volume of 1900mL of solvent. The jar was covered and left to stand for three (3) days for maceration. The macerate was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a water bath at 40⁰C until a semi-solid extract was obtained with a percentage yield of 15.6%.

3.3 Haemolytic Anaemia Induction

Thirty-six Male and Female Wistar rats were purchased and kept under 12:2 h light and dark cycle at $25 \pm 2^\circ\text{C}$ for to acclimatize to the new environment. After which, thirty Wistar rats was induced with Haemolytic Anaemia using freshly prepared Phenylhydrazine hydrochloride (PHZ-HCL) with the aid of orogastric Tube. The animals were fed with high fat diet and water ad libitum throughout the treatment period of 30 days. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use.

3.4 Experimental Design

The experimental setup involved the categorization of rats into different group, each comprising six individual groups. Within this framework, Group 1 through 3 were administered increasing doses of the polyherbal drug (Group 1 received 25mg/kg, Group 2 received 50mg/kg, Group 3 received 100mg/kg). Group 4 was designated as the control group. Group 5 was designated as the negative control and Group 6 was administered Folic acid. The ethical code for animal handling was obtained via University of Benin ethical committee and the ethical number is LS24317.

3.5 Data Analysis

Data were analysed by one-way ANOVA tests using GraphPad Prism 6. Data were presented as means of \pm SEM (n=3). Statistical significant difference was considered at $p \leq 0.05$.

CHAPTER FOUR

RESULTS

The results of the 24 hours across CD₄ and CD₈ Count as shown in Table 4.1 had a significant increases across medium and highest doses of the polyherbal extract specifically at 50 mg/kg of the aqueous extracts when compared with the untreated control that elicited a significant reduction CD₄ and CD₈ Count.

Table 4.1: Effect of 24 hours Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic Wistar rats

Treatment	Doses mg/kg	24 hours CD₄ Count (Cells/mm³)	24 hours CD₈ Count (Cells/mm³)
PHZ	40	6.08 ± 0.01 ^a	1.83 ± 0.05 ^a
Folic acid	10	6.07 ± 0.04 ^a	1.82 ± 0.01 ^a
Normal Control	-	6.52 ± 0.05 ^b	1.96 ± 0.01 ^b
Polyherbal	25	5.93 ± 0.04 ^a	1.78 ± 0.01 ^a
Polyherbal	50	6.79 ± 0.02 ^b	2.04 ± 0.01 ^b
Polyherbal	100	6.38 ± 0.02 ^b	1.92 ± 0.01 ^b

KEY

The data were analyzed using Mean \pm SEM and analysis of variance; where p -value $>^a = 0.05$, $^b = 0.1$ and $^d = 0.001$. The % inhibition taken indicated at what % the plant exhibits its anti-inflammatory effect.

CD₄ is Helper T-cell

CD₈ is Killer T-cell

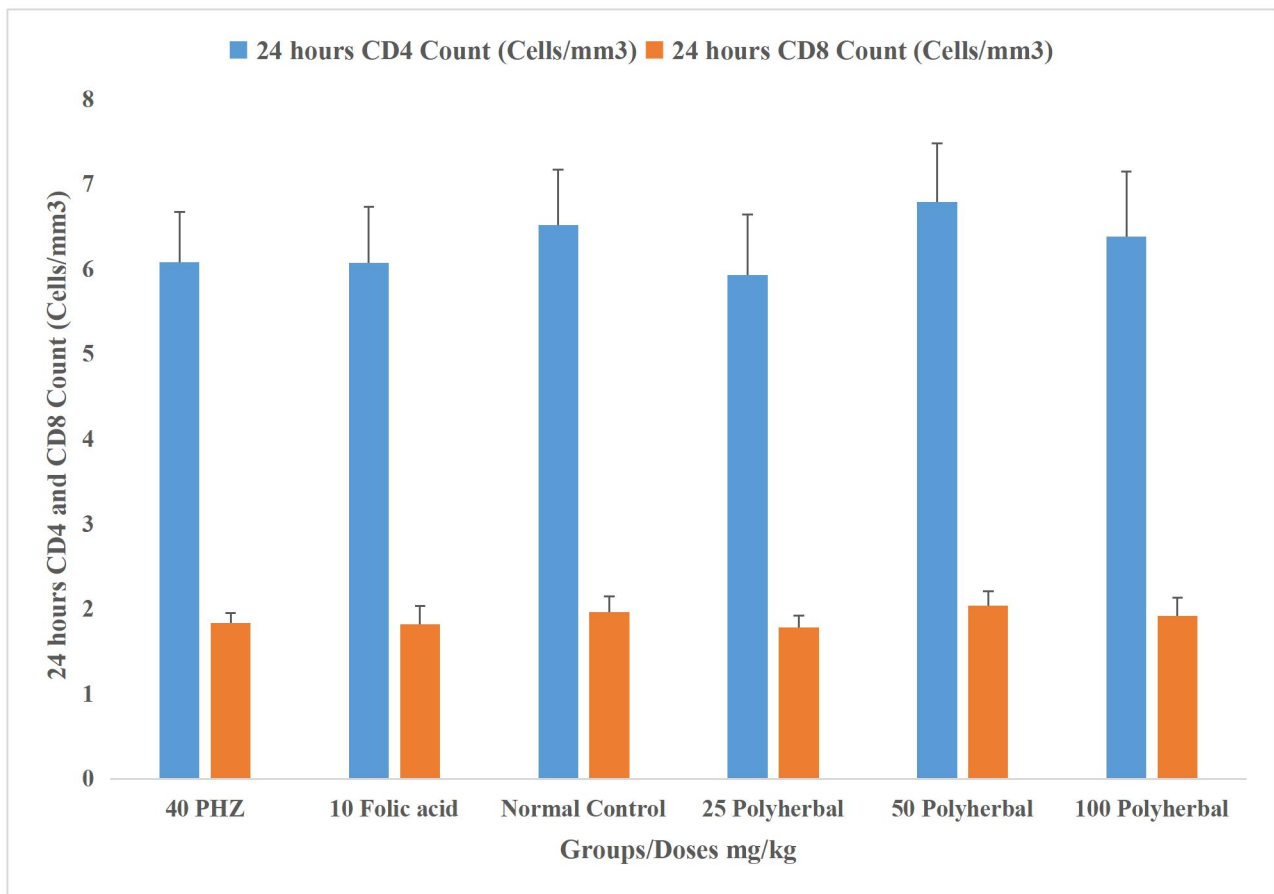


Figure 1: Effect of 24 hours Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic Wistar rats

The results obtained in day 7 of CD₄ and CD₈ Count displayed a slight increases in the treatment groups (polyherbal extract) with 50 mg/kg of the aqueous extracts elicited a significant increase in CD₄ and CD₈ Count when compared with the untreated control that elicited a significant reduction.

Treatment	Doses mg/kg	Day 7 CD ₄ Count (Cells/mm ³)	Day 7 CD ₈ Count (Cells/mm ³)
PHZ	40	5.70 ± 0.05 ^a	1.71 ± 0.01 ^a
Folic acid	10	5.88 ± 0.05 ^a	1.77 ± 0.02 ^b
Normal Control	-	6.48 ± 0.13 ^b	1.95 ± 0.04 ^b
Polyherbal	25	5.72 ± 0.03 ^a	1.72 ± 0.01 ^a
Polyherbal	50	6.50 ± 0.01 ^b	1.95 ± 0.00 ^b
Polyherbal	100	5.85 ± 0.06 ^a	1.75 ± 0.02 ^a

Table 2: Effect of 7 days Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic Wistar rats

KEY

The data were analyzed using Mean±SEM and analysis of variance; where *p-value* > ^a = 0.05, ^b = 0.1 and ^d = 0.001. The % inhibition taken indicated at what % the plant exhibits its anti-inflammatory effect.

CD₄ is Helper T-cell

CD₈ is Killer T-cell

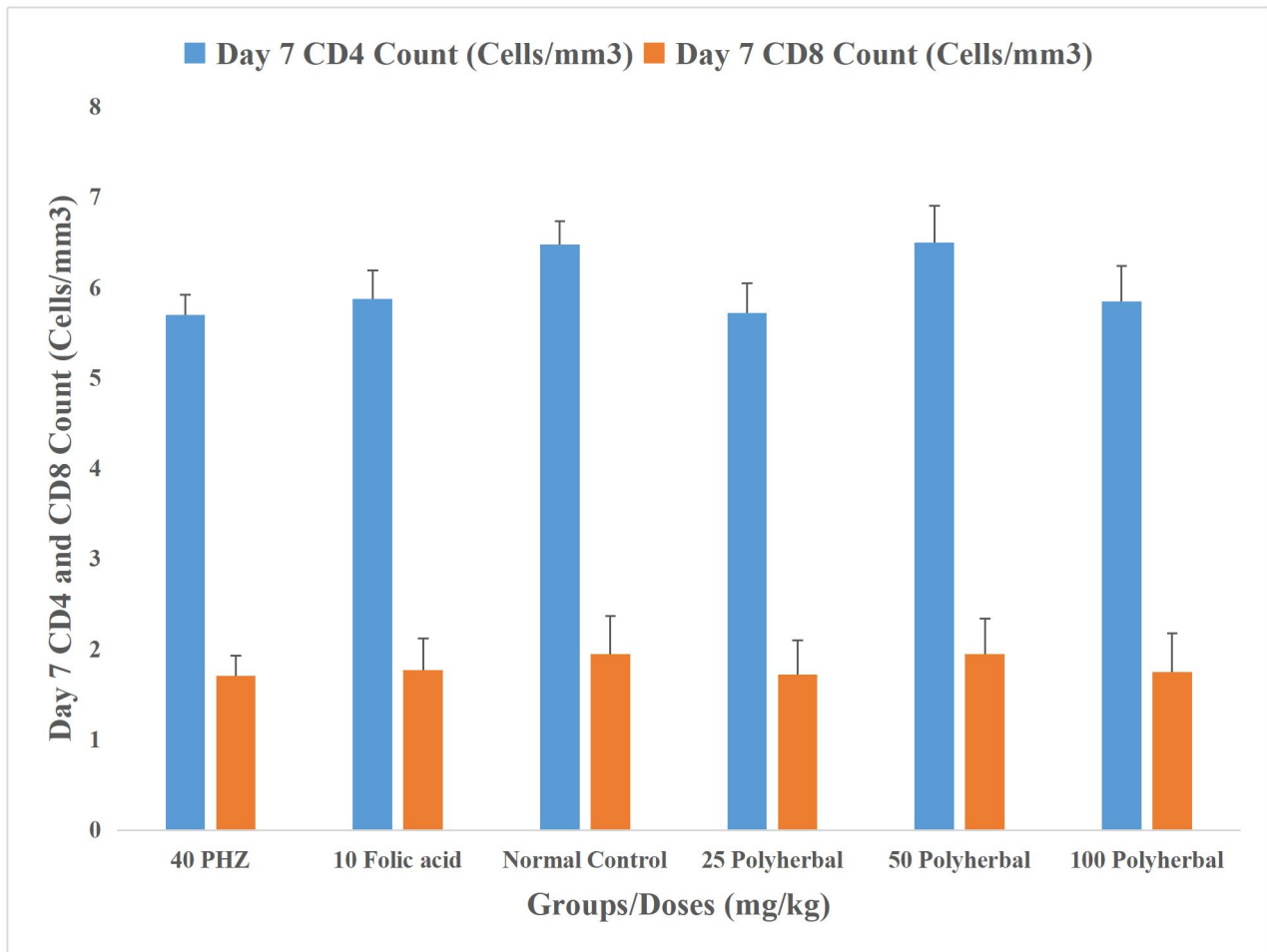


Figure 2: Effect of 7 days Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic Wistar rats

Treatment	Doses mg/kg	Day 14 CD ₄ Count (Cells/mm ³)	Day 14 CD ₈ Count (Cells/mm ³)
PHZ	40	7.23 ± 0.08 ^a	2.17 ± 0.02 ^a
Folic acid	10	6.91 ± 0.07 ^a	2.08 ± 0.03 ^a
Normal Control	-	7.96 ± 0.10 ^b	3.15 ± 0.03 ^b
Polyherbal	25	7.69 ± 0.10 ^b	3.13 ± 0.03 ^b
Polyherbal	50	7.95 ± 0.12 ^b	3.11 ± 0.04 ^b
Polyherbal	100	7.94 ± 0.09 ^b	3.09 ± 0.05 ^b

The results obtained in day 14 of the CD₄ and CD₈ Count displayed a slight significant increases in the treatment groups (polyherbal extract) in CD₄ and CD₈ Count when compared with the untreated control that elicited a significant reduction.

Table 3: Effect of 14 days Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic rats

KEY

The data were analyzed using Mean±SEM and analysis of variance; where *p-value* > ^a = 0.05, ^b = 0.1 and ^d = 0.001. The % inhibition taken indicated at what % the plant exhibits its anti-inflammatory effect.

CD₄ is Helper T-cell

CD₈ is Killer T-cell

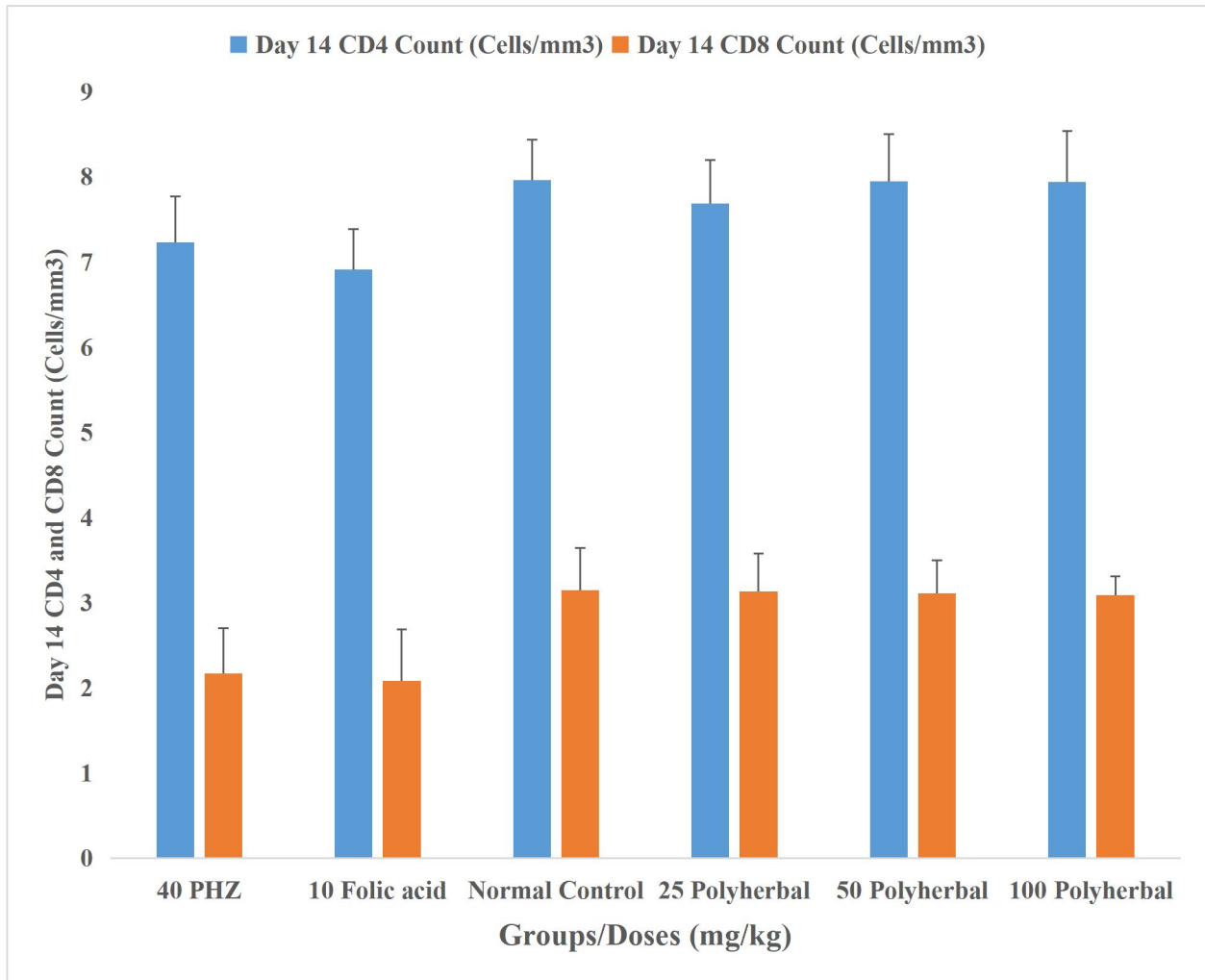


Figure 3: Effect of 14 days Polyherbal aqueous treatment phenylhydrazine hydrochloride induced anemic Wistar rats

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Anaemia is a global public health condition characterized by a decrease in the number of circulating red blood cells (RBCs) or a reduction in hemoglobin concentration below the normal physiological range. This results in decreased oxygen-carrying capacity of the blood, leading to tissue hypoxia and multiple systemic effects (Camaschella, 2015). Beyond its hematological effects, anaemia significantly influences the immune system. The immune system relies on adequate oxygen and essential nutrients, particularly iron, folate, and vitamin B12 for optimal functioning. Deficiency or imbalance of these elements leads to immune suppression, increased vulnerability to infections, and impaired recovery following illness (Weiss and Goodnough, 2015). Anaemia as a result of iron deficiency (IDA) impairs the growth and differentiation of immune cells such as lymphocytes, macrophages, and neutrophils. Iron is an essential cofactor for ribonucleotide reductase, the enzyme responsible for DNA synthesis in rapidly dividing cells. Its deficiency therefore reduces T-cell proliferation and compromises both innate and adaptive immunity (Manco *et al.*, 2021). Furthermore, iron-deficient conditions alter the ratio of helper T cells (CD4⁺) to cytotoxic T cells (CD8⁺), reducing immune response efficiency. The maturation of B cells is also impaired, leading to lower antibody production and weakened humoral immunity (Lopez *et al.*, 2019). Various synthetic drugs are used for the treatment of Anaemia, like folate and Iron supplements. These synthetic drugs are associated with side effects which lead to limitations in their use and create issues in the management of immunosuppression caused by anaemia. The immunoprotection activity of these individual plants combined has been proven by different research groups of *Ipomoea batatas*, *Ficus capensis* and *Justica carnea* and the synergistic effects of these drugs (Floate) is the main essence of this research.

The results of the 24-hour, 7-day, and 14-day CD₄⁺ and CD₈⁺ T-cell counts revealed that treatment with equal amounts of aqueous extract of *Ipomoea batatas*, *Ficus capensis*, and *Justicia carnea* produced a progressive and dose-dependent immunoprotective effect in phenylhydrazine-induced anaemic Wistar rats. At 24 hours, the polyherbal extract, at 25 mg/kg restored CD₄⁺ (5.93 ± 0.04 cells/mm³) and CD₈⁺ (1.78 ± 0.01 cells/mm³) below the untreated group and folic acid groups. By day 7 (168 hours), the polyherbal extract restored both CD₄⁺ (5.72 ± 0.03 cells/mm³) and CD₈⁺ (1.72 ± 0.01 cells/mm³) below the untreated and folic acid groups. By day 14 (336 hours), it significantly restored CD₄⁺ (7.23 ± 0.08 cells/mm³) and CD₈⁺ (3.13 ± 0.03 cells/mm³) below the untreated and folic acid groups. Using 50 mg/kg of the extract; in 24 hours, it restored both CD₄⁺ (6.79 ± 0.02 cells/mm³) and CD₈⁺ (2.04 ± 0.01 cells/mm³) levels above those of the untreated and folic acid groups, indicating early immune stimulation. By day 7, the same dose maintained elevated counts CD₄⁺ (6.50 ± 0.010) and CD₈⁺ (1.95 ± 0.00) comparable to the normal control, while day 14 data showed a marked and sustained increase CD₄⁺ (7.95 ± 0.12) and CD₈⁺ (3.11 ± 0.04), suggesting cumulative enhancement of T-helper and cytotoxic T-cell functions. However, in 24 hours using 100 mg/kg, restored CD₄⁺ (6.38 ± 0.02 cells/mm³) and CD₈⁺ (1.92 ± 0.01 cells/mm³) below the untreated group and above folic acid groups. By day 7, restored CD₄⁺ (5.85 ± 0.06 cells/mm³) and CD₈⁺ (1.75 ± 0.01 cells/mm³) below the untreated group and folic acid groups. By day 14, restored CD₄⁺ (7.94 ± 0.04 cells/mm³) and CD₈⁺ (3.09 ± 0.01 cells/mm³) below the untreated group and above folic acid groups. 50 mg/kg showed best results at day 14 as it restored CD₄⁺ (7.95 ± 0.12) and CD₈⁺ (3.11 ± 0.04), closely followed by 100 mg/kg at day 14 as it restored CD₄⁺ (7.94 ± 0.04 cells/mm³) and CD₈⁺ (3.09 ± 0.01 cells/mm³) above the untreated and folic acid groups.

These findings demonstrate that the polyherbal formulation exerts a synergistic immunorestorative effect, most potent at moderate concentrations (as seen with 50 mg/kg), likely through antioxidant protection and cytokine modulation. Similar studies have confirmed that *Ipomoea batatas* leaves contain β -carotene, polyphenols, and anthocyanins that stimulate lymphocyte proliferation and enhance interleukin (IL-2) secretion (Ekunseitan *et al.*, 2020); *Ficus capensis* leaf extract increases CD_4^+/CD_8^+ ratios and augments macrophage activity via interleukin regulation (Okafor *et al.*, 2018); and *Justicia carnea* promotes hematopoietic stem-cell activation and restores leukocyte counts in phenylhydrazine-induced anaemia (Uchegbu *et al.*, 2021). The combined use of these plants has also been reported to synergistically elevate CD-cell counts and anti-oxidant enzyme levels, providing superior immunoprotection compared to single extracts (Eze and Onah, 2022). These observations align with the current study's outcome, indicating that the polyherbal mixture effectively mitigates oxidative stress and immunosuppression while enhancing adaptive immune responses through complementary phytochemical interactions.

CONCLUSION

In conclusion, CD₄ and CD₈ T-lymphocyte levels tend to increase over time, indicating some level of metabolic response as indicated in the polyherbal drug extract showing significant effect at moderate dose of the extract. The highly significant increases in both demonstrate robust immune reconstitution and validate the traditional medicinal use of these botanical species for enhancing immune function and managing anaemia. Therefore, this polyherbal formulation offers a promising, affordable, and natural therapeutic option for managing immunosuppression as a result of anaemia.

REFERENCES

- Ajuru, M.G., Williams, L.F. and Ajuru, G. (2021). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *Journal of Food and Nutrition Sciences* 2(5): 198-205.
- Akintimehin, E.S., Omale, S., Aileru, C., Ovo-Mbada, B.A. and Braimah, O. (2021). Sub-acute toxicity study of hydroethanolic extract of *Justicia carnea* leaves in rats. *Journal of Medicinal Plants Research* 15(8): 349-356.
- Ani, O.C., Agu, C.V. and Ezeh, C.O. (2025). Comparative study of the vitamins and mineral contents in different aqueous extract layers of *Justicia carnea* leaf. *Asian Journal of Research in Biochemistry* 15(1): 1-12.
- Anthonia, O.A., Deborah, A.E. and Mary, U.O. (2021). Nutritional, phytochemical and haematological effects of aqueous extract of *Justicia carnea* leaves in phenylhydrazine-induced anaemic rats. *European Journal of Medicinal Plants* 30(3): 1-10.
- Asiwe, E.S., Omaka, O.N. and Obasi, N.A. (2023). Biochemical and mineral analysis of *Justicia carnea* leaves: implications for nutrition and health. *Journal of Medicinal Plants Research* 17(4): 112-118.
- Camaschella, C. (2015) Iron-deficiency anaemia. *New England Journal of Medicine* 372(19): 1832–1843.
- Carneiro, F.M., Silva, M.J.D. and Borges, L.L. (2023). *Justicia carnea*: ethnobotany, phytochemistry and biological activities. *Journal of Pharmacy and Pharmacognosy Research* 11(2): 356-372.

- Ekunseitan, D.A., Adegbite, J.A. and Oyeyemi, A.W. (2020) Immunomodulatory and antioxidant activities of Ipomoea batatas leaf extract in phenylhydrazine-induced rats. *Journal of Applied Pharmaceutical Science* 10(5): 75–83.
- Elgabry, E.A., Shalaby, S.M. and Mohamed, W.S. (2023). Sweet potato (Ipomoea batatas) as a functional food: a review of health benefits and therapeutic applications. *Nutrition and Food Science* 53(6): 1102-1121.
- Eze, C.O. and Onah, I.E. (2022). Synergistic immunoprotective effects of polyherbal extract of Ipomoea batatas, Ficus capensis, and Justicia carnea in immunosuppressed Wistar rats. *Journal of Medicinal Plants Research* 16(6): 187–196.
- Gasmi, A., Mujawdiya, P.K., Noor, S., Lysiuk, R., Darmohray, R., Piscopo, S., Lenchyk, L., Antonyak, H., Dehtiarova, K., Shanaida, M. and Peana, M. (2023). Consciousness of the herbal immunomodulators in infectious diseases. *Current Medicinal Chemistry* 30(1): 5-23.
- Jantan, I., Haque, M.A., Arshad, L., Harikrishnan, H., Septama, A.W. and Mohamed-Hussein, Z.A. (2022). Dietary polyphenols suppress chronic inflammation by modulation of immunometabolism. *Journal of Nutritional Biochemistry* 106: 109016.
- Josiah, S.S., Omoruyi, S.I., Owoeye, O., Okoye, E.I. and Eseimua, E. (2024). Hepatoprotective and antioxidant effects of Ficus capensis leaf extract against carboplatin-induced liver injury in rats. *Journal of Ethnopharmacology* 320: 117385.
- Kumar, A., Tiwari, A. and Agrawal, A. (2022). Role of medicinal plants in immunomodulation: a comprehensive review. *Journal of Herbal Medicine* 34: 100584.

- Laveriano-Santos, E.P., López-Yerena, A., Jaime-Rodríguez, C., González-Coria, J., Lamuela-Raventós, R.M., Vallverdú-Queralt, A. and Romanyà, J. (2022). Sweet potato is not simply an abundant food crop: *a comprehensive review of its phytochemical constituents, biological activities, and the effects of processing*. *Antioxidants* 11(9): 1648.
- Madrigal-Santillán, E., Portillo-Reyes, J., Madrigal-Bujaidar, E., Sánchez-Gutiérrez, M., Izquierdo-Vega, J.A., Izquierdo-Vega, J., Valadez-Vega, C., Morales-González, Á. and Morales-González, J.A. (2024). Ficus genus: *a review of biological activities and potential therapeutic applications*. *Plants* 13(2): 224.
- Marniati, M., Ridfah, R., Sumarni, S. and Nurhaedar, J. (2020). Effect of sweet potato-based biscuits on haemoglobin and hematocrit levels in multiparous pregnant women. International. *Journal of Scientific and Technology Research* 9(3): 6420-6423.
- Mechchate, H., Ousaaid, D., Laaroussi, H., Hano, C., Bakhy, K., Conte, R., Lyoussi, B., Greche, H. and Elarabi, I. (2021). Antioxidant, anti-inflammatory and antidiabetic proprieties of LC-MS/MS identified polyphenols from coriander seeds. *Molecules*. 26(2):487-490.
- Nguyen, H.C., Chen, C.C., Lin, K.H., Chao, P.Y., Lin, H.H. and Huang, M.Y. (2021). Bioactive compounds, antioxidants, and health benefits of sweet potato leaves. *Molecules*. 26(7): 1820.
- Njoku-Oji, N.N., Aguiyi, J.C., Chetty, K.N. and Igbokwe, V.U., (2020). Haematological and biochemical effects of aqueous leaf extract of *Ficus capensis* in phenylhydrazine-induced anaemia in rats. *African Journal of Biotechnology* 19(2): 56-62.
- Obiajulu, C.E., Ibe, C.C., Okafor, P.N., Nwakile, C.D. and Onyeka, I.P. (2022). Anti-anaemic potentials of combined aqueous leaf extracts of *Ficus capensis* and *Cnidioscolus*

aconitifolius in phenylhydrazine-induced anaemic rats. *Journal of Pharmaceutical Research International* 34(48A): 34-44.

Oboma, Y.I., Daberechukwu, O.M. and Chukwuma, E.M. (2024). Haematological effects and safety profile of graded doses of aqueous leaf extract of *Justicia carnea* in phenylhydrazine-induced anaemic rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences* 16(2):10-19.

Okafor, C.E., Anosike, C.A. and Opara, M.N. (2018). Immunoprotective potential of *Ficus capensis* leaf extract in phenylhydrazine-induced anaemia. *African Journal of Traditional, Complementary and Alternative Medicines* 15(4): 1–9.

Ousaaïd, D., Mechchate, H., Laaroussi, H., Hano, C., Bakhy, K., Conte, R., Lyoussi, B. and Elarabi, I. (2022). Phenylhydrazine-induced hemolytic anemia model in rats: oxidative stress and effectiveness of natural antioxidants. *Molecules*, 27(20): 6905.

Patel, S.S., Shah, P.V. and Soni, H.P. (2025). Immunomodulatory potential of natural products and herbal medicines: implications for COVID-19 and beyond. *Phytotherapy Research* 39(1): 95-119.

Rismawati, R., Marniati, M. and Jafar, N. (2020). The effect of consumption of UJU cookies based on sweet potato (*Ipomoea batatas*) flour, purple sweet potato pollen and sesame flour on haemoglobin and hematocrit levels in adolescent girls. *International Journal of Scientific and Technology Research* 9(2): 5125-5129.

Sharma, A. and Sharma, L. (2023). Therapeutic potential of sweet potato (*Ipomoea batatas*) and its bioactive compounds. *Critical Reviews in Food Science and Nutrition* 63(18): 3458-3479.

Sheth, K., Patel, P., Patel, B. and Modasiya, M. (2021). Development and evaluation of polyherbal formulations for immune system. *Journal of Drug Delivery and Therapeutics* 11(3-s): 127-132.

Weiss, G. & Goodnough, L.T. (2015) Anemia of chronic disease. *New England Journal of Medicine* 352(10): 1011–1023.

Uchegbu, R.I., Nwaoguikpe, R.N. and Nwachukwu, I.O. (2021) Hematopoietic and immunomodulatory effects of *Justicia carnea* in phenylhydrazine-treated rats. *Journal of Biological Research & Biotechnology* 19(1): 11–19.