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Immunoprotective Effects of the Polyherbal Aqueous Leaf Extract in Phenylhydrazine-Induced Immune-Suppressed Rats

Emmanuel Oshomoh[1], Igbinore Eghianruwa Lydia[2], Iguodala Osahenrumwen Christabel[3], Omiachi Jemimah Ojoachele[4], Emoghene Oghenetega Christiana[5], Aghahowa Etinosa Godspower[6], Aghwaritefe Oghenetekevwe Glory[7], Precious Osarobo Igbinakenzua[8], Queen R. Okoro[9], Benjamin Ogunma Gabriel[10], Abinokhauno Kingsley[11]

[1,2,3,4,5,6,7,8,9,10]University of Benin, Edo State, Nigeria
[11]Stella Obasanjo Hospital, Edo State, Nigeria
benjamin.gabriel@uniben.edu

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Abstract

The immune system protects against infection and oxidative damage, making the preservation of immune function an important therapeutic consideration. This study assessed the immunoprotective effects of a polyherbal aqueous leaf extract (PHALE) in phenylhydrazine (PHZ)-treated Wistar rats. Thirty-six rats were divided into six groups of six animals each. Anemia was induced using PHZ at 40 mg/kg, and treatment groups received oral folic acid at 10 mg/kg or PHALE at 25, 50, or 100 mg/kg for 14 days. Blood samples were assessed for CD4, CD8, total and differential white blood cell counts, platelet counts, and platelet indices. Data were expressed as mean ± standard error of the mean and analyzed using analysis of variance. PHZ administration reduced CD4, CD8, white blood cells, lymphocytes, and platelets. By day 14, PHALE treatment showed dose-dependent restoration of CD4 and CD8 toward normal levels, alongside improved white blood cell counts and lymphocyte percentages of 83.0–84.0%. Platelet counts also increased following treatment at 100 mg/kg. Platelet distribution width and platelet large-cell ratio decreased, while plateletcrit and mean platelet volume remained normal. The most pronounced recovery occurred at 50–100 mg/kg. These findings suggest that PHALE has immunoprotective and hematoprotective

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potential in this experimental model of PHZ-induced anemia and associated immune alterations. The study provides preliminary preclinical evidence supporting further investigation of the extract's potential therapeutic applications.

Keywords: Polyherbal Extract; Immunoprotection; Phenylhydrazine; Anemia; Wistar Rats

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). 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 anemia. It is characterized by reduced red blood cell count or haemoglobin concentration and 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 anemia further suppresses immune efficiency (Nairz and Weiss, 2021). In particular, hemolytic anemia, caused by accelerated destruction of red blood cells, often involves immune and oxidative mechanisms that damage erythrocyte membranes. Addressing both immunosuppression and anaemia often requires interventions that support immune function, protect against oxidative damage, and enhance haematopoiesis. 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).

MATERIALS AND METHODS

Plant Collection and Identification

The three plants (*Ficus sur L.* (Syn. *Ficus capensis* Thunb., *Justicia carnea* Lindl. (Synonym: *Justicia secunda* Vahl.) and *Ipomoea batatas* (L.) are the species that were used in this research and were found in the Egor Local Government Area of Edo State in Nigeria. Prof. H. A. Akinnibosun, a plant Taxonomist in the Department of Plant Biology and Biotechnology, Herbarium Unit, Faculty of Life Sciences, University of Benin, Benin City, Edo State, identified them. The *Ficus sur*, *Justicia carnea,* and *Ipomoea batata* leaves were taken, processed, and stored in the herbarium with the voucher numbers UBH-F331, UBH-J386, and UBH-I493, respectively (Ani et al., 2020).

Extraction Preparation

Freshly obtained *Ficus sur*, *Justicia carnea,* and *Ipomoea batata* leaves were washed with tap water, then allowed to dry at room temperature. The dried samples were finely ground in an industrial grinder and kept in an airtight container. 93 g of each ground sample of the leaves (1:1:1) was dissolved in 1900 ml of distilled water in a soaking jar. The maceration was left to stand for 72 hours. The macerate was filtered with the use of a muslin cloth. A water bath at 40 °C was used to concentrate the filtrate, followed by an oven, until a semi-solid extract was obtained. The extract was then placed in a labelled sterile bottle and stored in the refrigerator for further use. The percentage yield of the extract was 15.6%. This calculation determined the precise volume of the stock solution to be administered (Obazelu and Williams, 2024).

Experimental Animals

A total of thirty-six (36) Wistar rats weighing 180 – 260 g of either sex were sourced from the University of Benin, Department of Biochemistry animal house. The animals were kept in the animal house of the phytomedicine unit in comfortable separate cages. They were provided with free access to pelleted food and water for 14 days of acclimatization. The

animal treatment complied with the guidelines for the use of laboratory animals (Ani et al., 2020). It was approved by the ethical committee of Science Laboratory Technology with a reference number UNIBEN/ FSLT/ 00070

Anemia Induction of Wistar Rats.

Phenylhydrazine Hydrochloride (PHZ-HCL) in distilled water (40 mg/kg for 3 days) was used to induce anemia. The induction was confirmed by the manifestation of characteristic anemic symptoms, including paleness or dullness of the eyes, ears, tails, and paws, labored breathing, gasping, lethargy characterized by prolonged immobility, and reduced appetite (Sheth et al., 2021). The animals were weighed using a weighing balance weekly.

Experimental design

After the establishment of anemia, the weight of all the animals was measured, and the rats were randomly allocated to six groups of six rats each: Group 1: Anemic rats administered 25 mg/kg of the polyherbal extract. Group 2: Anemic rats administered 50 mg/kg of the polyherbal extract. Group 3: Anemic rats administered 100 mg/kg of the polyherbal extract. Group 4: Anemic rats administered 10 mg/kg folic acid (positive control). Group 5: Non-anemic rats, no treatment (normal control). Group 6: Untreated anemic rats (negative control). Gastric intubation was used to treat the rats orally 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 (Obazelu and Odionyenma 2024)

Sacrifice and Collection of Samples.

At the end of the treatment period, animals were fasted overnight and anaesthetized according to institutional guidelines before sacrifice. The animals were euthanized under chloroform anesthesia. 5 ml of blood was collected via cardiac puncture; 2 mL was transferred into an EDTA container and 3 mL into plane container for biochemical analysis. Serum was separated by centrifugation at 3000 rpm for 10 min using a Hettich EBA-21 centrifuge (Melini et al., 2026)

Hematological analysis

Hematological indices of the blood samples were carried out at the University of Benin Teaching Hospital with the help of an automated URIT-2900 Plus 3 Differential Hematological Analyzer. Determined parameters are White Blood Cell count (WBC),

lymphocyte count (LYM), mid-sized cell (MID), granulocyte count (GRM), platelet count (PLT), Platelet Distribution Width (PDW), Plateletcrit, Platelet Large Cell Ratio (P-LCR), and Mean Platelet Volume (MPV) (Akintimehin et al., 2021).

Procedure for CD4 and CD8 Count

Whole blood was collected in EDTA tubes, incubated with anti-CD4 and anti-CD8 antibodies (e.g BD Multisets), RBCs lysed, and cells fixed. A flow cytometer was used; T-cells CD4+ and CD8+ were studied (Oladipo et al., 2017; Dhakre *et al.*, 2023).

Data Analysis

All hematological and biochemical data obtained were presented as Mean ± Standard Error of the Mean (SEM). Statistical evaluation was performed using the Statistical Package for the Social Sciences (SPSS), version 16.0 software. One-way analysis of variance (ANOVA) was employed to detect significant variations across experimental groups. In instances where ANOVA indicated significant differences, Duncan's Multiple Range Test was implemented as a post-hoc procedure to identify specific group differences. Results were deemed statistically significant when the probability value was equal to or less than 0.05 ($p \leq 0.05$).

RESULTS

As shown in Table 1, the results obtained showed that the PHZ administration resulted in a significant reduction in CD_4 count on Day 1 in the PHZ-only, folic acid, and 25 mg/kg extract groups compared to the normal control ($p < 0.05$). However, the 50 mg/kg and 100 mg/kg extract groups showed values of 6.79 ± 0.02 and 6.38 ± 0.02 cells/µL, which were not significantly different from the normal control (6.52± 0.05). By Day 7, the normal control and 50 mg/kg extract groups maintained significantly higher CD_4 counts of 6.48 ± 0.13 and 6.50 ± 0.01 cells/µL, respectively. The remaining groups remained significantly lower, with values ranging from 5.70 to 5.88 cells/µL. On Day 14, all extract-treated groups demonstrated recovery, attaining CD_4 counts of 7.69 ± 0.10, 7.95 ± 0.12, and 7.94 ± 0.09 cells/µL for the 25, 50, and 100 mg/kg doses, respectively. These values were not significantly different from the normal control at 7.96 ± 0.10 cells/µL. The PHZ-only and folic acid groups also increased to 7.23 ± 0.08 and 6.91 ± 0.07 cells/µL, but remained significantly lower than the normal control and extract-treated groups.

Table 1: Effect of Polyherbal aqueous extract on CD_4 count in phenylhydrazine hydrochloride-induced anemic rats

Treatment	Doses (mg/kg)	Day 1	Day 7	Day 14
PHZ	40	6.08 ±0.01^{a}	5.70 ± 0.05^{a}	7.23 ± 0.08^{a}
Folic acid	10	6.07 ±0.04^{a}	5.88 ± 0.05^{a}	6.91 ± 0.07^{a}
Normal Control	-	6.52 ± 0.05^{b}	6.48 ± 0.13^{b}	7.96 ± 0.10^{b}
Extract	25	5.93 ± 0.04^{a}	5.72 ± 0.03^{a}	7.69 ± 0.10^{b}
Extract	50	6.79 ± 0.02^{b}	6.50 ± 0.01^{b}	7.95 ± 0.12^{b}
Extract	100	6.38 ± 0.02^{b}	5.85 ± 0.06^{a}	7.94 ± 0.09^{b}

Values are presented as mean ± SEM; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

Table 2 showed that treatment with the PHZ administration caused a significant reduction in CD_8 count on Day 1 in the PHZ-only, folic acid, and 25 mg/kg extract groups compared to the normal control ($p < 0.05$). The 50 mg/kg and 100 mg/kg extract groups showed counts of 2.04 ± 0.01 and 1.92 ± 0.01 cells/µL, which were not significantly different from the normal control (1.96 ± 0.01**)**. Day 7, the normal control and 50 mg/kg extract groups maintained significantly higher CD_8 counts of 1.95 ± 0.04 and 1.95 ± 0.00 cells/µL, respectively. By Day 14, all extract-treated groups showed marked recovery, attaining CD_8 counts of 3.13 ± 0.03, 3.11 ± 0.04, and 3.09 ± 0.05 cells/µL for the 25, 50, and 100 mg/kg doses, respectively. These values were not significantly different from the normal control at 3.15 ± 0.03 cells/µL. The PHZ-only and folic acid groups also increased to 2.17 ± 0.02 and 2.08 ± 0.03 cells/µL, but remained significantly lower than the normal control and extract-treated groups.

Table 2: Effect of the Polyherbal aqueous leaf extract on CD_8 count in phenylhydrazine hydrochloride induced anemic rats

Treatment	Doses mg/kg	Day1	Day 7	Day 14
PHZ	40	1.83 ± 0.05^{a}	1.71 ± 0.01^{a}	2.17 ± 0.02^{a}
Folic acid	10	1.82 ± 0.01^{a}	1.77 ± 0.02^{b}	2.08 ± 0.03^{a}
Normal Control	-	1.96 ± 0.01^{b}	1.95 ± 0.04^{b}	3.15 ± 0.03^{b}
Extract	25	1.78 ± 0.01^{a}	1.72 ± 0.01^{a}	3.13 ± 0.03^{b}
Extract	50	2.04 ± 0.01^{b}	1.95 ± 0.00^{b}	3.11 ± 0.04^{b}
Extract	100	1.92 ±0.01^{b}	1.75 ±0.02^{a}	3.09 ± 0.05^{b}

Values are presented as mean ± SEM; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

PHZ administration induced significant alterations in total white blood cell count across the experimental period in Table 3. On day one, there was a significant drop in WBC count across all treated groups compared to the normal control. The normal control was 20.35 ± 3.46 $\times 10^3/\mu L$, while the PHZ-only group dropped to 15.85 ± 2.76, and the extract groups ranged from 10.05 to 16.30. The lowest was the 50 mg/kg group at 10.05 ± 0.78. On day 7, the normal control dropped to 10.35 ± 1.34. The PHZ-only group recovered to 8.40 ± 2.12, and the extract groups ranged from 7.40 to 8.05. There was no significant difference between the PHZ-only and the extract-treated groups. On Day 14, WBC counts further stabilized across all groups. The PHZ-only group recorded 3.35 ± 0.78 $\times 10^3/\mu L$, while the extract-treated groups recorded 5.00 ± 0.85, 4.30 ± 0.28, and 4.20 ± 0.42 $\times 10^3/\mu L$ for 25, 50, and 100 mg/kg, respectively. The 25 mg/kg group had the highest value and was significantly different from the other groups.

Table 3: Effect of Polyherbal aqueous leaf Extract on White Blood Cells (WBC) Count in Phenylhydrazine-induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	19.50 ± 1.41^d	6.05 ± 1.20a	4.60 ± 0.28^b
PHZ	40	15.85 ± 2.76^c	8.40 ± 2.12^c	3.35 ± 0.78^a
Normal Control	-	20.35 ± 3.46^b	10.35 ± 1.34^d	4.60 ± 0.42^b
Extract	25	16.00 ± 2.26^b	7.40 ± 3.39^b	5.00 ± 0.85^c
Extract	50	10.05 ± 0.78^a	7.90 ± 1.13^b	4.30 ± 0.28^b
Extract	100	16.30 ± 4.95^b	8.05 ± 2.19^c	4.20 ± 0.42^b

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

As shown in Table 4, the administration of PHZ produced changes in mid-sized cell counts across the experimental period. On Day 1, the normal control recorded 4.25 ± 0.07 $\times 10^3/\mu L$, while the PHZ-only group declined to 3.40 ± 0.28 $\times 10^3/\mu L$. The extract-treated groups recorded 3.55 ± 1.63, 1.65 ± 0.49, and 3.85 ± 1.20 $\times 10^3/\mu L$ for 25, 50, and 100 mg/kg, respectively. The 50 mg/kg group had the lowest value and was significantly different from the other groups ($p < 0.05$). The folic acid group recorded 4.40 ± 0.57 $\times 10^3/\mu L$. By Day 7, all groups showed a decline in MID cell counts. On Day 14, MID cell counts stabilized across all

groups between 0.30 ± 0.14 and 0.45 ± 0.07 $\times 10^3/\mu L$. No significant differences were observed among the PHZ-only, normal control, folic acid, and extract-treated groups

Table 4: Effect of Polyherbal Aqueous Leaf Extract on Mid-sized cells in Phenylhydrazine-induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	4.40 ± 0.57^{c}	1.20 ± 0.14^{a}	0.45 ± 0.07^{a}
PHZ	40	3.40 ± 0.28^{b}	1.15 ± 0.21^{a}	0.30 ± 0.14^{a}
Normal Control	-	4.25 ± 0.07^{c}	1.80 ± 0.42^{ab}	0.35 ± 0.07^{a}
Extract	25	3.55 ± 1.63^{b}	1.75 ± 0.21^{ab}	0.40 ± 0.00^{a}
Extract	50	1.65 ± 0.49^{a}	1.55 ± 0.78^{ab}	0.30 ± 0.14^{a}
Extract	100	3.85 ± 1.20^{b}	1.85 ± 0.07^{ab}	0.35 ± 0.07^{a}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

Table 5 shows that Granulocyte counts were altered following PHZ administration and subsequent treatment. On Day 1, the normal control group recorded the highest value of 2.00 ± 0.20 $\times 10^3/\mu L$. The PHZ-only and folic acid groups both recorded 1.50 ± 0.20 $\times 10^3/\mu L$. The extract-treated groups recorded 1.40 ± 0.20, 1.00 ± 0.20, and 1.60 ± 0.20 $\times 10^3/\mu L$ for 25, 50, and 100 mg/kg, respectively. The 25 mg/kg and 50 mg/kg groups were significantly lower than the normal control but not significantly different from the PHZ-only group ($p < 0.05$). By Day 7, granulocyte counts declined in all groups. On Day 14, granulocyte counts further stabilized across all groups, with values ranging from 0.15 ± 0.05 to 0.25 ± 0.05 $\times 10^3/\mu L$. No significant differences were found between the PHZ-only, normal control, folic acid, and extract-treated groups.

Table 5: Effect of Polyherbal Aqueous Leaf Extract on Granulocytes in Phenylhydrazine-induced anemic Wistar rats

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

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

Table 6 showed that the Platelet Distribution Width values varied across treatment groups over the 14-day experimental period. On Day 1, the folic acid group recorded the highest PDW value of 11.75 ± 5.44%, while the PHZ-only group recorded 9.95 ± 3.04%. The normal control recorded 9.20 ± 2.12%. The extract-treated groups recorded 8.70 ± 1.84, 9.20 ± 1.13, and 8.95 ± 2.19% for 25, 50, and 100 mg/kg, respectively. The 25 mg/kg and 100 mg/kg groups were significantly lower than the folic acid and PHZ-only groups ($p < 0.05$). By Day 7, PDW values ranged from 8.70 ± 0.00% in the PHZ-only group to 10.50 ± 1.56% in the 50 mg/kg extract group. The 50 mg/kg and 100 mg/kg extract groups showed significantly higher values compared to the PHZ-only and folic acid groups, indicating increased platelet size variation during this phase. On Day 14, the PHZ-only group recorded the highest PDW value of 13.70 ± 2.40%, which was significantly different from all other groups.

Table 6: Effect of Polyherbal Aqueous Leaf Extract on Platelet Distribution Width (PDW)%, in Phenylhydrazine-induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	11.75 ± 5.44^{c}	8.85 ± 0.64^{a}	11.55 ± 3.04^{bc}
PHZ	40	9.95 ± 3.04^{b}	8.70 ± 0.00^{a}	13.70 ± 2.40^{d}
Normal Control	-	9.20 ± 2.12^{b}	9.70 ± 0.00^{b}	12.00 ± 0.00^{c}
Extract	25	8.70 ± 1.84^{a}	9.60 ± 1.13^{b}	10.75 ± 1.63^{b}
Extract	50	9.20 ± 1.13^{b}	10.50 ± 1.56^{c}	8.60 ± 0.85^{a}
Extract	100	8.95 ± 2.19^{a}	10.10 ± 0.14^{c}	9.50 ± 0.00^{ab}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

As shown in Table 7, the Plateletcrit values showed a mild decline across all groups over the 14 days. On Day 1, PCT ranged from 0.55 ± 0.10% in the PHZ-only group to 0.80 ± 0.20% in the folic acid group. The normal control recorded 0.70 ± 0.10%, while the extract-treated groups recorded 0.65 ± 0.10, 0.60 ± 0.10, and 0.75 ± 0.10% for 25, 50, and 100 mg/kg, respectively. No significant differences were observed among the groups at this time point. By

Day 7, PCT values decreased slightly in all groups. On Day 14, PCT further stabilized between 0.35 ± 0.10% and 0.45 ± 0.10%. No significant differences were observed among the groups

Table 7: Effect of Polyherbal Aqueous Leaf Extract on Plateletcrit in Phenylhydrazine-induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	0.80 ± 0.20[a]	0.55 ± 0.10[a]	0.45 ± 0.10[a]
PHZ	40	0.55 ± 0.10[a]	0.40 ± 0.10[a]	0.35 ± 0.10[a]
Normal Control	-	0.70 ± 0.10[a]	0.50 ± 0.10[a]	0.40 ± 0.10[a]
Extract	25	0.65 ± 0.10[a]	0.50 ± 0.10[a]	0.40 ± 0.10[a]
Extract	50	0.60 ± 0.10[a]	0.45 ± 0.10[a]	0.35 ± 0.10[a]
Extract	100	0.75 ± 0.10[a]	0.55 ± 0.10[a]	0.45 ± 0.10[a]

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

Table 8 shows that the Platelet Large Cell Ratio values varied significantly among treatment groups across the 14 days. On Day 1, the 100 mg/kg extract group recorded the highest P-LCR value of 19.80 ± 0.00%, followed closely by the folic acid group at 19.60 ± 7.07%. The PHZ-only group recorded 16.90 ± 5.30%, while the normal control recorded 9.80 ± 3.39%. The 25 mg/kg and 50 mg/kg extract groups recorded 15.50 ± 3.39% and 3.50 ± 4.95%, respectively. The 50 mg/kg group was significantly lower than all other groups at this time point. By Day 7, the 100 mg/kg extract group maintained the highest value at 20.60 ± 2.26%. On Day 14, the PHZ-only group recorded the highest P-LCR value of 18.40 ± 7.35%, indicating sustained platelet turnover. The normal control and 100 mg/kg extract groups recorded 16.90 ± 2.69% and 15.05 ± 2.33%, respectively. The 25 mg/kg and 50 mg/kg extract groups recorded 10.45 ± 0.21% and 1.80 ± 2.55%, respectively, with the 50 mg/kg group showing the lowest and most stable value.

Table 8: Effect of Polyherbal Aqueous Leaf Extract on Platelet Large Cell (P-LCR%) Ratio in Phenylhydrazine-induced anemic Wistar rats.

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	19.60 ± 7.07^{d}	14.10 ± 0.14^{c}	8.40 ± 6.79^{b}
PHZ	40	16.90 ± 5.30^{cd}	16.70 ± 0.00^{cd}	18.40 ± 7.35^{d}
Normal Control	-	9.80 ± 3.39^{b}	14.10 ± 0.00^{c}	16.90 ± 2.69^{cd}
Extract	25	15.50 ± 3.39^{c}	10.40 ± 0.85^{a}	10.45 ± 0.21^{bc}
Extract	50	3.50 ± 4.95^{a}	11.55 ± 5.30^{b}	1.80 ± 2.55^{a}
Extract	100	19.80 ± 0.00^{d}	20.60 ± 2.26^{d}	15.05 ± 2.33^{c}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

In Table 9, Mean Platelet Volume values remained stable across all groups throughout the 14-day study period. On Day 1, MPV ranged from 8.38 ± 0.92 fL in the normal control group to 8.48 ± 0.81 fL in the 50 mg/kg extract group. The PHZ-only group recorded 8.45 ± 0.88 fL, while the folic acid group recorded 8.43 ± 0.85 fL. The 25 mg/kg and 100 mg/kg extract groups recorded 8.40 ± 0.90 fL and 8.44 ± 0.86 fL, respectively. By Day 7, a slight increase was observed in all groups. On Day 14, MPV values stabilized between 8.45 ± 0.89 fL and 8.55 ± 0.80 fL across all groups. No significant differences were observed among the groups.

Table 9: Effect of Polyherbal Aqueous Leaf Extract on Mean Platelet Volume in Phenylhydrazine-induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	8.43 ± 0.85^{a}	8.70 ± 0.67^{a}	8.48 ± 0.79^{a}
PHZ	40	8.45 ± 0.88^{a}	8.73 ± 0.71^{a}	8.53 ± 0.82^{a}
Normal Control	-	8.38 ± 0.92^{a}	8.68 ± 0.63^{a}	8.45 ± 0.89^{a}
Extract	25	8.40 ± 0.90^{a}	8.65 ± 0.69^{a}	8.50 ± 0.85^{a}
Extract	50	8.48 ± 0.81^{a}	8.75 ± 0.65^{a}	8.55 ± 0.80^{a}
Extract	100	8.44 ± 0.86^{a}	8.70 ± 0.67^{a}	8.53 ± 0.83^{a}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

In Table 10, the results showed that lymphocyte percentages showed a progressive increase across all groups over the 14 days. On Day 1, values ranged from 65.0 ± 2.0% in the PHZ-only group to 70.0 ± 2.0% in the normal control group. The folic acid, 25, 50, and 100 mg/kg extract groups recorded 68.0 ± 2.0%, 67.0 ± 2.0%, 66.0 ± 2.0%, and 69.0 ± 2.0%, respectively. The normal control and 100 mg/kg extract groups were significantly higher than the PHZ-only and lower dose extract groups. By Day 7, lymphocyte percentages increased slightly in all groups. On Day 14, all groups showed a marked increase; the 100 mg/kg extract group (84.0 ± 2.0%) was not significantly different from the normal control (85.0 ± 2.0%), while the PHZ-only and 50 mg/kg groups remained significantly lower.

Table 10: Effect of Polyherbal Aqueous Leaf Extract on Lymphocytes in Phenylhydrazine induced anemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	68.0 ± 2.0^{a}	70.0 ± 2.0^{b}	82.0 ± 2.0^{b}
PHZ	40	65.0 ± 2.0^{a}	68.0 ± 2.0^{a}	80.0 ± 2.0^{a}
Normal Control	-	70.0 ± 2.0^{b}	70.0 ± 2.0^{b}	85.0 ± 2.0^{bc}
Extract	25	67.0 ± 2.0^{a}	69.0 ± 2.0^{a}	83.0 ± 2.0^{b}
Extract	50	66.0 ± 2.0^{a}	68.0 ± 2.0^{a}	81.0 ± 2.0^{a}
Extract	100	69.0 ± 2.0^{a}	71.0 ± 2.0^{b}	84.0 ± 2.0^{bc}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

Table 11 shows that Platelet counts varied significantly among the treatment groups over the 14 days. On Day 1, the normal control group recorded the highest platelet count of 900.00 ± 25.00 × $10^3/\mu L$, while the PHZ-only group recorded the lowest at 800.00 ± 25.00 × $10^3/\mu L$. The folic acid group recorded 850.00 ± 25.00 × $10^3/\mu L$. The 100 mg/kg (830.00 ± 25.00 × $10^3/\mu L$) extract group was not significantly different from the folic acid group (850.00 ± 25.00 × $10^3/\mu L$). By Day 7, the PHZ-only group showed a further decline to 750.00 ± 25.00 × $10^3/\mu L$. The 100 mg/kg extract group was significantly higher than the PHZ-only group and comparable to the folic acid group. On Day 14, the PHZ-only group remained lowest at 700.00 ± 25.00 × $10^3/\mu L$, while the normal control and folic acid groups stabilized at 860.00 ± 25.00 and 880.00 ± 25.00 × $10^3/\mu L$. The 100 mg/kg (840.00 ± 25.00 × $10^3/\mu L$) extract group-maintained platelet counts close to the normal control and folic acid groups.

Table 11: Effect of Polyherbal Extract on Platelet Counts in Phenylhydrazine induced anaemic Wistar rats

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14
Folic Acid	10	850.00 ± 25.00^{c}	870.00 ± 25.00^{d}	880.00 ± 25.00^{d}
PHZ	40	800.00 ± 25.00^{a}	750.00 ± 25.00^{a}	700.00 ± 25.00^{a}
Normal Control	-	900.00 ± 25.00^{d}	850.00 ± 25.00^{c}	860.00 ± 25.00^{c}
Extract	25	820.00 ± 25.00^{b}	800.00 ± 25.00^{b}	810.00 ± 25.00^{b}
Extract	50	810.00 ± 25.00^{ab}	780.00 ± 25.00^{ab}	790.00 ± 25.00^{ab}
Extract	100	830.00 ± 25.00^{bc}	820.00 ± 25.00^{bc}	840.00 ± 25.00^{bc}

Values are presented as mean ± SD; n=6. Mean values with similar superscripts within a column are not significantly different, P>0.05.

DISCUSSION

The cluster of differentiation 4 (CD_4) counts results obtained from this study showed that PHALE at doses of 50 and 100 mg/kg resulted in a gradual increase in CD_4 count across days 1, 7 and 14, with the 50 mg/kg (7.95 ± 0.12 cells/mm^3) and 100 mg/kg (7.94 ± 0.09 cells/mm^3) doses exhibiting a comparable effect to the normal control group (7.96 ± 0.10 cells/mm^3) by day 14, as shown in Table 1. PHALE treatment groups showed a significant increase in CD_4 count, suggesting an immunomodulatory effect. This study agrees with the findings of Okafor et al. (2018) who reported that *Ficus capensis* leaf extract increased CD_4^+/CD_8^+ ratios and enhanced the activity of the macrophage cells through the regulation of interleukin. Likewise, Uchegbu et al. (2021) stated that *Justicia carnea* enhances hematopoietic stem cells and leukocytes in anemia caused by phenylhydrazine. In addition to its hematologic impact, anemia has a great impact on the immune system. The immune system requires sufficient oxygen and nutrients and, in this case, the required nutrients are iron, folate, and vitamin B_{12}. Lack of these factors or their disproportion results in immunosuppression, predisposition to infections, and worsening of post-disease recovery. The possible mechanism that might have contributed to the rise in the counts of CD4 is that PHALE is either stimulating the production of CD4 and Thymus (T) cells or inhibiting their destruction, resulting in an increment of the count of CD4.

The cluster of differentiation 8 (CD_8) counts results obtained from this study showed that PHALE at all doses (25, 50, and 100 mg/kg) resulted in a gradual increase ($p<0.05$) in CD_8 count across days 1, 7, and 14. At day 14, the CD8 counts in all extract groups (3.13 ± 0.03 cells/mm^3, 3.11 ± 0.04 cells/mm^3, and 3.09 ± 0.05 cells/mm^3) were comparable to the normal control group (3.15 ± 0.03 cells/mm^3) as shown in Table 2, suggesting an immunomodulatory effect. This study concurred with present findings, indicated that the combination of plants indeed showed a synergistically high count of CD-cells and antioxidant enzymes, which gave better immune protection than single extracts. The given observation is consistent with the results of the current study, which shows that the polyherbal mixture is effective in alleviating oxidative stress and immunosuppression and boosting the adaptive immune responses due to complementary phytochemical interactions.

The White Blood Cell (WBC) result obtained from this study showed that PHALE at 25 mg/kg (5.00 ± 0.85b) resulted in a significant increase ($p< 0.05$) in WBC count, comparable to the normal control group (4.60 ± 0.42b) at day 14. The other doses of the extract (50 mg/kg and 100 mg/kg) showed varying effects on WBC count. The increment in extract 25 mg/kg indicates immunomodulatory action. This is consistent with the research by Njoku (2016), who established the immunostimulatory activity of *Ficus capensis* with the increased count of WBCs. Moreover, Onyeabo et al. (2017) found that *Justicia carnea* leaf extract was able to reverse the immunosuppressive anemia induced by phenylhydrazine in rats by returning the values of Hb, RBC, WBC, and HCT to almost normal. Potential processes that may have contributed to the rise in WBC include hematopoiesis stimulation, immune modulation, and a decrease in oxidative stress. This means that the extract can improve immune response.

In Table 4, the mid-sized cells result showed that the polyherbal aqueous leaf extract at 25 mg/kg (0.40 ± 0.00) and 100 mg/kg (0.35 ± 0.07) resulted in a significant increase ($p< 0.05$) in mid-sized cells, comparable to the normal control group (0.35 ± 0.07) by day 14. The 50 mg/kg dose of the extract showed a varying effect on mid-sized cells. The potential mechanism may be that the polyherbal aqueous leaf extract either increases the generation of mid-sized cells or decreases their destruction, resulting in the generation of mid-sized cells.

The outcome of the granulocytes result, as presented in Table 5, indicated that the 25 mg/kg (0.25 ± 0.05) and 100 mg/kg (0.20 ± 0.05) polyherbal aqueous leaf extract significantly increased ($p< 0.05$) the number of granulocytes, compared to the normal control group (0.20

± 0.05) at day 14. The 50 mg/kg extract dose demonstrated a varying effect on the granulocytes. The polyherbal extract treatment groups demonstrated a major impact on granulocytes, indicating a possible immunomodulatory effect.

The Platelet Distribution Width (PDW) outcome in Table 6 indicated that the Day 14 polyherbal aqueous leaf extract of 25 mg/kg and 100 mg/kg led to an overall significant reduction in the PDW% when compared to the normal control group. The effect of the 50 mg/kg dose of the extract on PDW was variable. The polyherbal extract treatment groups showed a significant effect on PDW%, suggesting a potential effect on platelet production or destruction. A possible mechanism could be that the polyherbal extract is affecting platelet production or destruction, leading to a decrease in PDW%.

The plateletcrit finding in this study in Table 7 indicated that the polyherbal aqueous leaf extract of 25 mg/kg (0.40 ± 0.10) and 100 mg/kg (0.45 ± 0.10) led to significant Plateletcrit increase and was similar to the normal control group (0.40 ± 0.10) on day 14. The 50 mg/kg dose of the extract was characterized by a different effect on Plateletcrit. The polyherbal extract treatment groups showed a significant effect on Plateletcrit, suggesting a potential effect on platelet production or destruction.

The result of the Platelet Large Cell Ratio (P-LCR) in Table 8 revealed that the polyherbal aqueous leaf extract at 25 mg/kg (0.40 ± 0.10) and 100 mg/kg (0.45 ± 0.10) resulted in a significant increase in Plateletcrit, comparable to the normal control group (0.40 ± 0.10) by day 14. The 50 mg/kg dose of the extract showed a varying effect on Plateletcrit. The polyherbal extract treatment groups showed a significant effect on Plateletcrit, suggesting a potential effect on platelet production or destruction. The polyherbal extract treatment groups showed no significant changes in Mean Platelet Volume **(**MPV). The MPV values in all groups were comparable to each other. The polyherbal aqueous extract exhibits no significant effect on MPV, as shown in Table 9.

Lymphocyte**s** result showed that the polyherbal aqueous leaf extract at all doses 25 mg/kg (83.0 ± 2.0), 50 mg/kg (81.0 ± 2.0) and 100 mg/kg (84.0 ± 2.0) resulted in a significant increase ($p < 0.05$) in lymphocytes counts, comparable to the normal control group (85.0 ± 2.0) at day 14, as shown in Table 10. This finding is in agreement with the study of Elgabry *et al.* (2023), who found that the extract has the potential to regulate immune cell functions and cytokine synthesis, as hypothesized by the research on *Ipomoea batatas* polysaccharides. The possible mechanism through which the polyherbal aqueous leaf extract enhanced the number

of lymphocytes is by either promoting the production or inhibiting the destruction of lymphocytes. This immunomodulatory effect could enhance the body's ability to fight infections and restore immune function by promoting the proliferation and differentiation of lymphocytes and other leukocytes.

The platelet count result showed that the polyherbal aqueous leaf at 100 mg/kg (840.00 ± 25.00) resulted in a significant increase in platelet counts, comparable to the normal control group (860.00 ± 25.00) at day 14. The other doses of the extract (25 mg/kg and 50 mg/kg) showed varying effects on platelet counts, as shown in Table 11. The increase in platelet count was observed at 100mg/kg, suggesting that the extract support megakaryopoiesis. This is significant, as thrombocytopenia can be a complication in various immunosuppressive conditions, and its reversal contributes to overall hematological recovery. This result is correlated with the experiments of Sheth et al. (2021) who discovered that *Raktavardhak kadha* (RK) which is a polyherbal formulation exhibited a high antianemic effect on the rat in response to phenylhydrazine-induced anemia, where it was able to improve red blood cell counts, haemoglobin, and thrombin levels. In the same manner, a bi-herbal formulation, which consists of *Cymbopogon citratus* and *Picralima nitida* had hematological effects on phenylhydrazine-induced anemic Wistar rats that improved hematological parameters (Obazelu and Odionyenma, 2024). The potential explanation to the rise in platelet count is that the polyherbal extract could be promoting platelet production or preventing platelet destruction, and as a result causing the platelet count to rise.

CONCLUSION

This study demonstrated that polyherbal aqueous leaf extract (PHALE) attenuated selected immune and hematological alterations in phenylhydrazine-treated Wistar rats over 14 days. Treatment was associated with recovery of CD4 and CD8 counts and lymphocyte percentages toward normal-control values. CD4 recovery was most evident at 50–100 mg/kg, whereas improvements in total white blood cell and platelet counts were most apparent at 25 and 100 mg/kg, respectively. These findings indicate that the treatment response varied across parameters, supporting the immunoprotective and hematoprotective potential of PHALE within this experimental model.

The study contributes preliminary preclinical evidence supporting further investigation of polyherbal formulations for immune-cell and platelet alterations associated with

phenylhydrazine-induced anemia. However, the observed changes do not establish whether the extract acts by stimulating cell production, preventing cellular destruction, or influencing other biological pathways.

Given the small number of animals per group and the 14-day treatment period, further studies should include larger samples and longer observation periods to assess the reproducibility and persistence of these effects. Additional investigations should clarify the mechanisms of action, characterize the responses to different doses, and evaluate safety before potential therapeutic applications are considered.

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