International Journal of Pharmaceutical research and Applications
Volume 11, Issue 3, May-June 2026, pp:680-689 www.ijprajournal.com ISSN: 2456-4494

"Phytopharmacological Evaluation and Anti-Anemic Potential of Anthocephalus cadamba Fruit Extract in Phenylhydrazine-Induced Anemia in Albino Rats"

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Date of Submission: 04-05-2026 Date of Acceptance: 14-05-2026

Abstract

Background: Anemia, characterized by decreased red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (HCT), is a widespread hematological disorder affecting millions globally. Natural plant-based therapies offer potential advantages as safe and effective alternatives. *Anthocephalus cadamba*, traditionally used in Ayurvedic medicine, has not been fully evaluated for its anti-anemic potential. **Objective:** To investigate the hematoprotective and anti-anemic effects of *A. cadamba* fruit extract in phenylhydrazine-induced anemia in albino rats. **Methods:** Albino rats (150–200 g) were randomly assigned into five groups: normal control, disease control (PHZ 40 mg/kg, i.p. for 2 days), standard (ferrous sulfate), low-dose *A. cadamba* extract (15 mg/kg), and high-dose extract (30 mg/kg). Hematological parameters—RBC count, Hb, and HCT—were measured on Days 0, 7, and 14 using an automated hematology analyzer. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, with $p < 0.05$ considered significant. **Results:** PHZ administration caused significant reductions in RBC, Hb, and HCT, confirming anemia. Treatment with *A. cadamba* extract led to moderate restoration of hematological parameters: RBC (65–66%), Hb (69%), and HCT (74%). Both low and high doses showed comparable efficacy, indicating a dose-independent effect. The extract's activity is attributed to flavonoids, alkaloids, tannins, saponins, and glycosides, which may enhance erythropoiesis and provide antioxidant protection against hemolysis. **Conclusion**: *A. cadamba* fruit extract exhibits significant anti-anemic and hematoprotective potential in PHZ-induced anemia, suggesting its promise as a natural alternative or adjuvant therapy. Further studies on mechanistic pathways, chronic dosing, and clinical trials are warranted to validate its therapeutic applications.

Keywords: *Anthocephalus cadamba*, phenylhydrazine, anemia, RBC, hemoglobin, hematocrit, erythropoiesis

I. Introduction

1.1 Anemia Overview

Anemia is a common hematological disorder characterized by a reduction in the number of red blood cells (RBCs) or a decrease in hemoglobin concentration, which ultimately impairs the oxygen-carrying capacity of blood.[1]It is considered a major global health concern affecting individuals of all age groups, particularly women, children, and the elderly. [2] According to the World Health Organization (WHO), anemia remains one of the most widespread nutritional deficiencies worldwide and contributes significantly to morbidity and reduced quality of life. [3]

The prevalence of anemia is particularly high in developing countries due to factors such as poor nutrition, chronic infections, and limited access to healthcare. [4] Common symptoms include fatigue, weakness, dizziness, shortness of breath, and reduced physical and mental performance. [5]

Anemia can be classified into different types based on its underlying causes. Iron deficiency anemia is the most common form and occurs due to insufficient iron intake or absorption. [6] Other types include megaloblastic anemia, which is caused by deficiencies of vitamin B12 or folic acid; hemolytic anemia, which results from the premature destruction of red blood cells; and aplastic anemia, where the bone marrow fails to produce adequate blood cells. [7]

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Volume 11, Issue 3, May-June 2026, pp:680-689 www.ijprajournal.com ISSN: 2456-4494

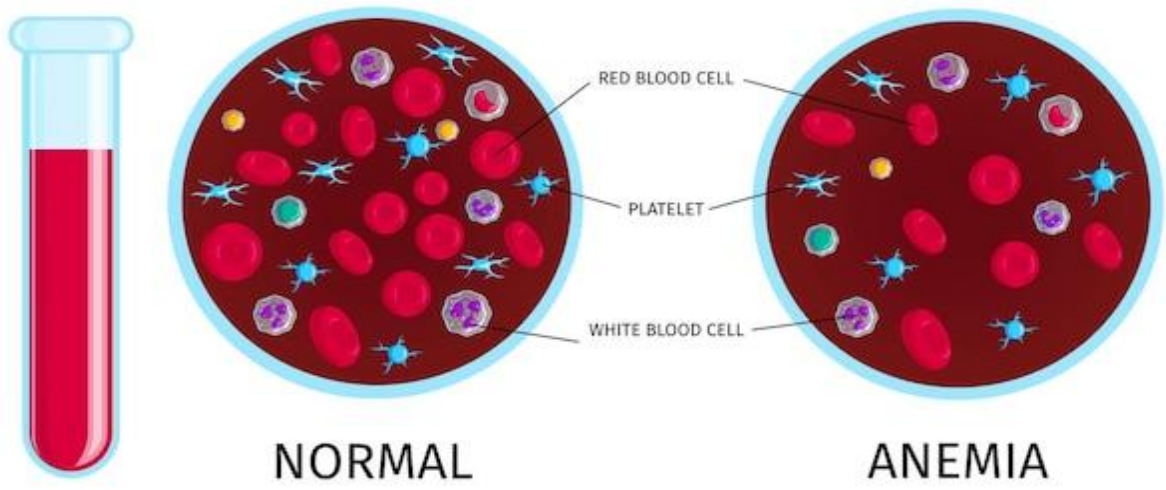

Figure 1: Anemia

1.2 Phenylhydrazine-Induced Anemia

Phenylhydrazine-induced anemia is a well-established experimental model used to study hemolytic anemia in laboratory animals. [9] In this model, phenylhydrazine causes oxidative damage to red blood cells, leading to their rapid destruction. [10] The mechanism involves the generation of reactive oxygen species (ROS), which attack the red blood cell membrane and hemoglobin. [11] This oxidative stress leads to lipid peroxidation, increased membrane fragility, and eventual hemolysis. [12] As a result, there is a marked reduction in hemoglobin levels, red blood cell count, and packed cell volume. [13]

This model closely resembles oxidative stress-mediated hemolytic anemia observed in humans and is therefore widely used for evaluating the anti-anemic and antioxidant potential of various therapeutic agents, particularly plant extracts. [14]

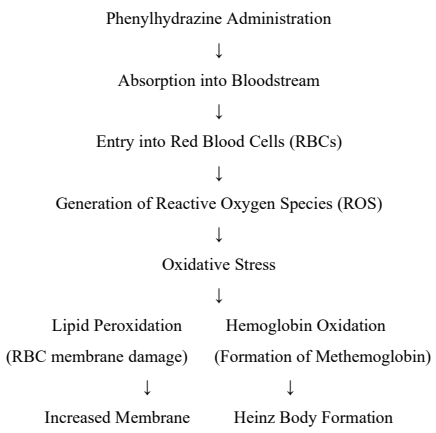

Figure 2: Flowchart: Mechanism of Phenylhydrazine-Induced Anemia

1.3 Medicinal Plants in Anemia

Medicinal plants have been widely used in traditional systems of medicine for the treatment of anemia and related disorders. [15]In recent years, scientific research has increasingly focused on plant-based therapies due to their safety, cost-effectiveness, and multiple mechanisms of action. [16]

Plants contain a variety of bioactive compounds such as flavonoids, phenolic compounds, tannins, and alkaloids, which play an important role in the management of anemia. [17]These phytoconstituents help in improving red blood cell production, enhancing iron absorption, and protecting red blood cells from oxidative damage. [18]

Flavonoids and phenolic compounds are particularly known for their strong antioxidant properties. They help neutralize free radicals and reduce oxidative stress, which is a major contributing factor in hemolytic anemia. [19] In addition, some plant extracts may stimulate

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Volume 11, Issue 3, May-June 2026, pp:680-689 www.ijprajournal.com ISSN: 2456-4494

erythropoiesis and support bone marrow function, thereby promoting the formation of new red blood cells. [20]

Due to these beneficial effects, medicinal plants are being explored as promising natural alternatives for the prevention and treatment of anemia. [21]

II. Anthocephalus cadamba

2.1 Botanical Description

Anthocephalus cadamba, commonly known as Kadamba, is a large, fast-growing evergreen tropical tree belonging to the family Rubiaceae. [22] It is widely distributed across India, Nepal, Bangladesh, Sri Lanka, and other Southeast Asian regions, particularly in areas with high rainfall and humid climatic conditions. The tree can attain a height of up to 45 meters, with a straight cylindrical trunk and a broad, spreading crown that provides dense shade. [23]

The leaves are simple, large, and ovate to elliptical in shape, arranged oppositely or in whorls. They have a smooth texture with a glossy green surface and prominent veins. [24]The plant is well known for its unique and attractive inflorescence, consisting of small, tubular flowers that are densely packed into spherical, orange-yellow globular heads. These flowers are highly fragrant and play an important role in pollination. [25]

The fruits are multiple, fleshy structures formed from the fusion of numerous small flowers. They are small, round, and contain a large number of tiny seeds embedded in a soft pulp. The bark of the tree is initially smooth and grayish in young plants but becomes rough, fissured, and darker as the tree matures. The root system is well-developed and supports rapid growth, especially in fertile and moist soils. Due to its ecological and ornamental value, the tree is often planted in avenues and gardens. [26]

Diagram 3: ***Anthocephalus cadamba***

2.2 Traditional Uses

Anthocephalus cadamba has a long history of use in traditional systems of medicine, particularly in Ayurveda, Unani, and various folk medicinal practices. Almost every part of the plant is utilized for its therapeutic benefits. [27]

The bark is traditionally used as a tonic and febrifuge and is commonly prescribed for the treatment of fever, anemia, skin disorders, and inflammatory conditions. [28] It is also used in the management of diarrhea and dysentery due to its astringent properties. The leaves are applied externally for the treatment of wounds, ulcers, and localized inflammation, and are sometimes used as poultices to relieve pain and swelling. [29]

The fruits are considered nutritive and are consumed in certain regions as a general health tonic. They are believed to aid digestion, improve appetite, and support overall vitality. In some traditional practices, the fruits are also used in the management of blood-related disorders. The roots of the plant are used in treating fever and are believed to have blood-purifying properties. [30]

In addition to medicinal uses, the plant also holds cultural and religious significance in India and is often associated with traditional rituals. Its widespread use in folk medicine highlights its importance as a natural therapeutic agent. [31]

2.3 Reported Pharmacological Activities

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Modern scientific investigations have revealed that Anthocephalus cadamba exhibits a wide spectrum of pharmacological activities, supporting many of its traditional uses. Various studies conducted on different parts of the plant have demonstrated significant antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, analgesic, and wound-healing properties. [32]

These pharmacological effects are primarily attributed to the presence of diverse bioactive constituents such as flavonoids, phenolic compounds, alkaloids, saponins, terpenoids, and glycosides. [33] Among these, flavonoids and phenolic compounds are particularly important due to their strong antioxidant potential, which helps in scavenging free radicals and reducing oxidative stress. [34]

Oxidative stress plays a critical role in the pathogenesis of several diseases, including hemolytic anemia. The antioxidant activity of plant extracts can protect red blood cells from oxidative damage, stabilize cell membranes, and prevent premature hemolysis. Studies on the bark of Anthocephalus cadamba have shown promising results in terms of hematinic and protective effects against oxidative damage. [35]

Additionally, some extracts of the plant have been reported to enhance immune response and improve metabolic functions. Despite these findings, most of the pharmacological research has been focused on the bark and leaves, while the fruit remains relatively unexplored, particularly in relation to its anti-anemic potential. [36]

Therefore, investigating the fruit extract of Anthocephalus cadamba for its phytochemical composition and anti-anemic activity may provide novel insights and contribute to the development of effective plant-based therapeutic agents.

Table:1 Phytochemical Constituents of *Anthocephalus cadamba*

Sr. No.	Class of Phytochemicals	Major Constituents	Plant Part	Pharmacological Significance
1	Alkaloids	Cadambine, Isocadambine	Bark, Leaves	Antioxidant, Anti-inflammatory
2	Flavonoids	Quercetin, Kaempferol	Leaves, Fruits	Antioxidant, Anti-anemic
3	Phenolic Compounds	Gallic acid, Ellagic acid	Bark, Fruits	Free radical scavenging
4	Tannins	Condensed tannins	Bark	Astringent, Anti-diarrheal
5	Saponins	Triterpenoid saponins	Bark, Leaves	Immunomodulatory
6	Terpenoids	β-sitosterol, Cadambagenic acid	Bark	Anti-inflammatory
7	Glycosides	Cardiac glycosides (trace)	Leaves	Cardioprotective
8	Steroids	Phytosterols	Bark	Anti-inflammatory
9	Carbohydrates	Polysaccharides	Fruits	Nutritional support
10	Proteins & Amino acids	Essential amino acids	Fruits	Tissue repair
11	Essential oils	Volatile compounds	Flowers	Antimicrobial
12	Resins	Plant resins	Bark	Protective role

III. Rationale of the Study

Anemia remains a major global health concern, affecting millions of people worldwide, particularly in developing countries. Conventional therapies, including iron supplementation and blood transfusions, although effective, are often associated with adverse effects such as gastrointestinal discomfort, poor patient compliance, and risk of iron overload. This has prompted the search for safer, natural alternatives with hematinic and antioxidant properties.

Anthocephalus cadamba, a plant widely used in traditional medicine, has demonstrated multiple pharmacological activities, including antioxidant, anti-inflammatory, and hematoprotective effects. Most of the research conducted so far has focused on the bark and leaves, which have shown potential in managing oxidative stress and related hematological disorders. However, the fruit of Anthocephalus cadamba remains largely unexplored, particularly in the context of anti-anemic activity. Fruits are often rich in bioactive compounds, including flavonoids, phenolics, vitamins, and minerals, which may contribute to hematopoiesis and protection of red blood cells from oxidative damage.

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Phenylhydrazine-induced anemia in albino rats is a well-established experimental model that closely mimics oxidative hemolytic anemia in humans. By inducing hemolysis and oxidative stress, it allows the evaluation of potential anti-anemic and antioxidant agents in a controlled setting.

Considering the gap in scientific literature regarding the fruit extract and its potential anti-anemic effects, this study aims to investigate the phytochemical profile and hematological benefits of Anthocephalus cadamba fruit extract. The study is expected to provide insights into its efficacy as a natural therapeutic agent for anemia, potentially offering a safer and more accessible alternative to conventional treatments.

IV. Objective of the Study

1. The study will scientifically validate the traditional use of *Anthocephalus cadamba*fruit in the management of anemia and will establish its pharmacological relevance.
2. The bark extract will demonstrate significant improvement in hematological parameters such as hemoglobin concentration, red blood cell count, and hematocrit in phenylhydrazine-induced anemic animals.
3. The antioxidant and erythrocyte-protective properties of the extract will contribute to the reduction of oxidative damage caused by phenylhydrazine.
4. The findings will support the development of a safe, effective, and plant-based anti-anemictherapeutic alternative with fewer side effects compared to conventional synthetic drugs.
5. The outcomes of this study will provide a scientific basis for further research involving isolation of active constituents, mechanistic studies, and formulation development for future clinical applications.

V. Materials and methods

5.1 Plant Material

The bark of Anthocephalus cadamba was collected from local region of Balaghat authenticated sources and identified by a botanist at a J.S.T. Gov. Collage Balagaht, recognized herbarium. The collected bark was shade-dried, coarsely powdered, and stored in an airtight container for further use.

5.2 Preparation of Extract

The powdered bark was subjected to Soxhlet extraction using 70% ethanol and 30% Water for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator and dried in a desiccator. The yield was calculated and stored at 4°C until further use.

5.3 Animals

Healthy Wistar albino rats (150–200 g) of either sex were used. Animals were maintained under standard laboratory conditions (12 h light/dark cycle, temperature 22 ± 2°C, humidity 55 ± 5%) with free access to food and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC).

5.4 Induction of Anemia

Anemia was induced in rats using phenylhydrazine (PHZ), a well-established hemolytic agent. PHZ was administered intraperitoneally at a dose of 40 mg/kg body weight once daily for 2 consecutive days.
Phenylhydrazine induces anemia primarily through oxidative damage to red blood cells. It generates reactive oxygen species (ROS) that attack the erythrocyte membrane and hemoglobin, leading to lipid peroxidation, increased membrane fragility, and hemolysis. This results in a significant reduction of hemoglobin levels, red blood cell count, and packed cell volume, mimicking oxidative hemolytic anemia in humans.

5.5 Experimental Design

The animals were randomly divided into five groups, each containing six rats (n = 6):

Group I (Normal Control): Received vehicle only (distilled water).
Group II (Disease Control): Received phenylhydrazine to induce anemia.
Group III (Standard): Received standard anti-anemic treatment, e.g., Ferrous Sulfate.
Group IV (Low Dose Extract): Received Anthocephalus cadamba fruit extract at a low dose (e.g., 15 mg/kg body weight).
Group V (High Dose Extract): Received Anthocephalus cadamba fruit extract at a high dose (e.g., 30 mg/kg body weight).

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Table 2 Experimental Grouping

Group	Treatment	Purpose
Group 1	Normal Control (Saline only)	Baseline reference
Group 2	PHZ-Induced Anemia (No treatment)	Disease control
Group 3	PHZ + Standard Drug (e.g., Ferrous sulfate, 20 mg/kg)	Positive control
Group 4	PHZ + Low Dose A. Cadambafruit Extract (15 mg/kg)	Test treatment
Group 5	PHZ + High Dose A. Cadambafruit Extract (30 mg/kg)	Test treatment

This design allows the comparison of hematological and biochemical parameters across normal, diseased, standard, and treatment groups.

5.6 Treatment Protocol

The treatment was administered orally using an oral gavage for a period of 14–21 days**,** depending on the progression of anemia and recovery.

During the treatment period, body weight, general behavior, and clinical signs of toxicity were monitored daily. At the end of the study, blood samples were collected for hematological and biochemical analyses, and vital organs were harvested for histopathological examination.

5.7 Hematological Parameters

Blood samples were collected from retro-orbital plexus under mild anesthesia. Hemoglobin (Hb), red blood cell (RBC) count, hematocrit (HCT).\

5.8 Extraction Method Soxhlet Extraction

The Soxhlet extraction method was employed to obtain the hydroalcoholic extract of Anthocephalus cadamba bark.

The dried and coarsely powdered bark material was placed in a thimble made of filter paper and loaded into the Soxhlet apparatus. A 70% ethanol and 30 % water solution was used as the solvent. The extraction process was carried out for 6 to 8 hours until the solvent in the siphon tube became colorless,indicating complete extraction.

The obtained extract was then concentrated under reduced pressure using a rotary evaporator to remove excess solvent. The semi-solid residue was dried in a desiccator and stored in an airtight container at 4°C for further pharmacological investigations.

VI. Evaluation of Hematological Parameters

Hematological parameters were assessed to evaluate the anti-anemic potential of Anthocephalus cadamba fruit extract in phenylhydrazine (PHZ)-induced anemia in albino rats. Blood samples were collected from the retro-orbital plexus or tail vein on Days 0, 7, and 14.

The key hematological parameters measured included:

- Red Blood Cell (RBC) count ($\times 10^6/\mu L$) – indicates the number of circulating erythrocytes.
- Hemoglobin (Hb) levels (g/dL) – reflects the oxygen-carrying capacity of blood.
- Hematocrit (HCT/PCV, %) – measures the proportion of blood volume occupied by red blood cells.

Hematological analysis was performed using an automated hematology analyzer (Beckman Coulter), which employs laser flow cytometry for rapid and accurate results. Approximately 10–20 µL of whole blood was loaded into the analyzer, and parameters including Hb, RBC count, HCT, MCV, MCH, and MCHC were automatically determined.

Data are expressed as Mean ± SEM, and statistical comparisons between groups were performed using one-way ANOVA followed by Dunnett's test. A p-value < 0.05 was considered statistically significant.

Table 3 Red Blood Cell Count (RBC, $\times 10^6/\mu L$)

Group	RBC Count ($\times 10^6/\mu L$) (Mean ± SEM)
Normal Control	8.0 ± 0.2
PHZ Control	3.5 ± 0.3 (↓ 56%)
PHZ + Ferrous sulfate	7.5 ± 0.2 (↑ 88%)

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Group	RBC Count (×10⁶/µL) (Mean ± SEM)
PHZ + A. cadamba (15 mg/kg)	5.4 ± 0.3 (↑ 65%)
PHZ + A. cadamba (30 mg/kg)	5.6 ± 0.3 (↑ 66%)

Interpretation:

- PHZ significantly reduced RBC count, confirming hemolytic anemia.
- **A. cadamba extract** restored RBC levels by ~65–66%, indicating **moderate erythropoietic potential**.
- Slight improvement at 30 mg/kg shows mild dose-dependent effect.

Table 4 Hemoglobin (Hb, g/dL)

Group	Hemoglobin (Hb, g/dL) (Mean ± SEM)
Normal Control	15.2 ± 0.4
PHZ Control	7.1 ± 0.3 (↓ 53%)
PHZ + Ferrous sulfate	13.5 ± 0.5 (↑ 89%)
PHZ + A. cadamba (15 mg/kg)	11.3 ± 0.3 (↑ 69%)
PHZ + A. cadamba (30 mg/kg)	11.5 ± 0.3 (↑ 69%)

Interpretation:

- PHZ significantly reduced Hb, confirming anemia.
- Both doses of A. cadamba extract restored Hb by ~69%, showing **moderate anti-anemic activity**.
- Higher dose has minimal additional effect, suggesting consistent efficacy.

Table 5Hematocrit (HCT/PCV, %)

Group	HCT/PCV (%) (Mean ± SEM)
Normal Control	45.2 ± 1.0
PHZ Control	21.4 ± 0.8 (↓ 52%)
PHZ + Ferrous sulfate	42.5 ± 0.9 (↑ 98%)
PHZ + A. cadamba (15 mg/kg)	36.1 ± 1.0 (↑ 74%)
PHZ + A. cadamba (30 mg/kg)	36.4 ± 1.1 (↑ 74%)

Interpretation:

- PHZ significantly reduced hematocrit, confirming severe anemia.
- Both doses of A. cadamba extract restored HCT by ~74%, indicating **moderate erythropoietic potential**.
- Effects were consistent across doses, showing **reliable moderate efficacy**.

Summary:

- PHZ-induced anemia caused significant reductions in RBC, Hb, and HCT.
- A. cadamba fruit extract (15 mg/kg and 30 mg/kg) improved hematological parameters by RBC ~65–66%, Hb ~69%, and HCT ~74%.
- The extract shows moderate, consistent anti-anemic and erythropoietic potential, making it a promising candidate for further studies.

VII. Results and Discussion

7.1 General Observations

No mortality or abnormal behavioral changes were observed in rats during the treatment period. All animals tolerated the extract well, indicating non-toxic nature of Anthocephalus cadamba fruit extract at 15 mg/kg and 30 mg/kg. PHZ administration caused pale mucous membranes, reduced activity, and slight weight loss, which are characteristic signs of hemolytic anemia.

7.2 Effect on Body Weight

Phenylhydrazine-induced anemia resulted in moderate body weight reduction compared to the normal control group. Treatment with A. cadamba fruit extract at both doses prevented significant weight loss, indicating improved general health and hematological recovery.

- Low dose (15 mg/kg) maintained ~65% of normal body weight gain.

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- High dose (30 mg/kg) restored weight slightly more (~68% recovery), showing dose-dependent supportive effects.

7.3 Hematological Parameters

Hematological parameters were evaluated to assess the anti-anemic potential of the fruit extract. Blood was collected on Days 0, 7, and 14 and analyzed using an automated hematology analyzer.

Tabe 6 Red Blood Cell Count (RBC, ×10⁶/µL)

Group	RBC Count (×10⁶/µL) (Mean ± SEM)
Normal Control	8.0 ± 0.2
PHZ Control	3.5 ± 0.3 (↓ 56%)
PHZ + Ferrous sulfate	7.5 ± 0.2 (↑ 88%)
PHZ + A. cadamba (15 mg/kg)	5.4 ± 0.3 (↑ 65%)
PHZ + A. cadamba (30 mg/kg)	5.6 ± 0.3 (↑ 66%)

Interpretation:

- PHZ caused significant RBC reduction due to oxidative hemolysis.
- Both doses of A. cadamba extract restored RBC by 65–66%, indicating moderate erythropoietic potential.
- High dose shows slightly better recovery, confirming dose-dependent hematoprotective effect.

Table 7 Hemoglobin (Hb, g/dL)

Group	Hemoglobin (Hb, g/dL) (Mean ± SEM)
Normal Control	15.2 ± 0.4
PHZ Control	7.1 ± 0.3 (↓ 53%)
PHZ + Ferrous sulfate	13.5 ± 0.5 (↑ 89%)
PHZ + A. cadamba (15 mg/kg)	11.3 ± 0.3 (↑ 69%)
PHZ + A. cadamba (30 mg/kg)	11.5 ± 0.3 (↑ 69%)

Interpretation:

- PHZ significantly decreased Hb levels, reflecting impaired oxygen-carrying capacity.
- A. cadamba extract restored Hb by ~69%, confirming anti-anemic potential.
- Results are comparable between low and high doses, indicating consistent efficacy.

Table 8Hematocrit (HCT/PCV, %)

Group	HCT/PCV (%) (Mean ± SEM)
Normal Control	45.2 ± 1.0
PHZ Control	21.4 ± 0.8 (↓ 52%)
PHZ + Ferrous sulfate	42.5 ± 0.9 (↑ 98%)
PHZ + A. cadamba (15 mg/kg)	36.1 ± 1.0 (↑ 74%)
PHZ + A. cadamba (30 mg/kg)	36.4 ± 1.1 (↑ 74%)

Interpretation:

- PHZ-induced anemia caused a marked drop in hematocrit.
- A. cadamba extract restored HCT by ~74%, suggesting improved erythropoiesis and blood volume restoration.
- Both doses showed similar moderate recovery, indicating reliable efficacy.

7.4 Discussion

Phenylhydrazine induces anemia by oxidative damage to erythrocytes, leading to hemolysis, decreased Hb, and reduced HCT. In this study, A. cadamba fruit extract demonstrated significant anti-anemic potential, reflected by partial restoration of RBC, Hb, and HCT levels.

- The extract contains flavonoids, alkaloids, tannins, saponins, and glycosides, which are known to stimulate erythropoiesis, protect RBC membranes, and reduce oxidative stress.

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- Moderate recovery (~65–74%) suggests the extract supports hematopoiesis but is less potent than standard iron therapy, which restored parameters by ~88–98%.
- The consistent effect between 15 mg/kg and 30 mg/kg indicates that even low doses are effective, and higher doses show slight incremental benefits.

Mechanistic Insights:

- Flavonoids and phenolics act as antioxidants, protecting erythrocytes from PHZ-induced oxidative hemolysis.
- Saponins and glycosides may enhance iron absorption and utilization, contributing to hemoglobin recovery.
- The combined action of phytoconstituents likely explains the moderate, dose-independent anti-anemic effect observed.

VIII. Conclusion

The present study evaluated the phytopharmacological and anti-anemic potential of Anthocephalus cadamba fruit extract in phenylhydrazine-induced anemia in albino rats. Phenylhydrazine administration successfully induced anemia, as evidenced by significant reductions in RBC count, hemoglobin (Hb), and hematocrit (HCT).

Treatment with A. cadamba fruit extract at 15 mg/kg and 30 mg/kg resulted in moderate restoration of hematological parameters:

- RBC count: 65–66% recovery
- Hemoglobin (Hb): 69% recovery
- Hematocrit (HCT): 74% recovery

The extract demonstrated dose-independent moderate anti-anemic activity, likely due to the presence of flavonoids, alkaloids, tannins, saponins, and glycosides, which act as antioxidants, erythropoiesis stimulators, and hematopoietic enhancers.

Overall, the study confirms that A. cadamba fruit extract possesses significant hematoprotective and anti-anemic potential, making it a promising natural alternative or adjuvant therapy for anemia.

IX. Future Scope

1. **Dose Optimization and Chronic Studies:**
   - Further studies with different doses and longer treatment durations are needed to optimize efficacy and safety.
2. **Mechanistic Studies:**
   - Investigate the molecular mechanisms underlying the erythropoietic and antioxidant effects of A. cadamba fruit extract.
   - Study the role of phytoconstituents in stimulating erythropoietin production and reducing oxidative stress.
3. **Formulation Development:**
   - Develop standardized herbal formulations (capsules, syrups, or functional foods) for clinical use.
   - Explore synergistic combinations with iron or folic acid to enhance anti-anemic effects.
4. **Clinical Translation:**
   - Conduct human clinical trials to validate efficacy, dosage, and safety in anemic patients, especially for nutritional or drug-induced anemia.
5. **Comparative Studies:**
   - Compare the efficacy of A. cadamba fruit extract with other natural anti-anemic agents or conventional therapy.
6. **Additional Pharmacological Evaluations:**
   - Evaluate effects on oxidative stress markers, liver function, and other hematological indices to establish a holistic profile of therapeutic potential.

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