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Assessment Of Pharmacological Activity Of Anthocephalus Cadamba Base Extract In Phenylhydrazine-Induced Anemia

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Abstract

Neolamarckia cadamba (synonym Anthocephalus cadamba), commonly known as Kadamba, is a premier medicinal plant in traditional Indian medicine (Ayurveda). This study was performed on albino mice in different grouping with respect to differ in dose of A.Kadamba base extract. During experimentation of Bark extract on animal model, clearly show the hematinic response which was evaluated by Hb, RBC, WBC, PCV, MCV, MCH, MCHC & ESR parameter . Dermal evaluation also performed which support the result of hematological evaluation of A.Kadamba base extract. Anthocephaluscadamba extract demonstrates promising hematopoietic and antioxidant effects in PHZ-induced anemic mice. Higher doses (400 mg/kg) showed better efficacy, suggesting dose-dependent activity. Further studies on mechanism of action & clinical trials are needed for potential therapeutic application

Keywords: Anthocephalus cadamba Hematinic, ESR, Anemia, Hb, RBC, WBC, PCV, MCV, MCH, MCHC

Introduction

Anemia remains a major global health concern, characterized by reduced red blood cell count and hemoglobin concentration, leading to impaired oxygen transport and systemic weakness. Phenylhydrazine (PHZ)-induced anemia serves as a well-established experimental model that mimics oxidative hemolytic anemia by generating free radicals, causing red blood cell destruction and suppression of erythropoiesis.

Anthocephalus cadamba (Roxb.) Miq., commonly known as Kadamba, is a traditional medicinal plant belonging to the Rubiaceae family, reputed in Ayurveda and folk medicine for its tonic, anti-inflammatory, antioxidant, hepatoprotective, and wound-healing properties. Phytochemical investigations reveal that the plant contains bioactive constituents such as cadambine, chlorogenic acid, flavonoids, saponins, and triterpenoids—all of which possess strong antioxidant potential that could protect erythrocytes from oxidative damage.[22]

Material & Methodology

Collection and Preparation:

The plant material (Bark) is thoroughly washed to remove dirt and foreign particles. It is then shade-dried at controlled temperatures to prevent degradation of heat-sensitive compounds. The dried material is powdered using a mechanical grinder to increase surface area for extraction.

Extraction Process:

To isolate cadambine and isocadambine (the key indole alkaloids) from Neolamarckia cadamba bark, the most effective approach is acidified polar solvent extraction followed by organic solvent partitioning.
Methanol (acidified with dilute acetic acid) followed by dichloromethane (DCM) for isolation.

"Acidified methanol is most effective because it converts indole alkaloids into soluble salts, ensuring maximum extraction, while subsequent basification and partitioning into DCM isolates cadambine in its free base form."

Methanol was chosen as a solvent for extraction. The powdered plant material is soaked in the solvent at room temperature for 24-72 hours, at ratio 1:10 (plant: solvent) with occasional stirring. For higher efficiency, Soxhlet extraction is used with continuous refluxing of the solvent over an extended period. The extract is filtered using Whatman filter paper to remove debris and plant residues. The filtrate is concentrated using a rotary evaporator under reduced pressure to remove excess solvent without damaging heat-sensitive phytochemicals. The purified extracts were freeze-dried to enhance stability and shelf life.

% yield (w/w) on dry plant material = [Mass of dried plant material used (g) / Mass of dried extract (g)]×100

Quantity of dried bark powder= 50.00 g

Quantity After maceration and solvent removal of dried mthanolic extract=6.5 g.

Calculation:

% Yield=[6.50 g / 50.00 g]×100=13.00%w/w

Standard Yield Range for Herbal Extracts:

Low: 8%

Medium: 12%

High: 15–18%

Experimental Animals

Healthy Wistar albino rats (150–200 g) of either sex were used. They maintained under standard laboratory conditions with free access to food and water.

Induction of Anemia

Anemia were induced by intraperitoneal injection of phenylhydrazine (40 mg/kg body weight) for two consecutive days.[14,15]

Table1: Grouping and Treatment

Group	Treatment	Description
I	Normal control	Received vehicle only
II	Anemic control	PHZ only
III	Standard	PHZ + standard hematinic drug (ferrous sulfate)
IV	Test I	PHZ + A. cadamba extract (low dose)
V	Test II	PHZ + A. cadamba extract (moderate dose)

Parameters Studied

Phytochemical Screening: Phenolics, Flavonoids, Tannins, Saponins, Alkaloids.

Hematological: Hb, RBC, WBC, PCV, MCV, MCH, MCHC

Blood collection methods:

Blood samples are collected either from the retro-orbital plexus (for rats and mice) or the tail vein (less invasive alternative).

ESR analysis method:

ESR analysis (Westergren Method)

Assessment of Skin Pallor

Method of evaluation:

- Examine rat ear, tail, and footpad.
- Compare color with normal control.
- Graded scoring system:

Score	Observation
0	Normal pink color
1	Mild pallor
2	Moderate pallor
3	Severe pallor

Anemic control group shows higher pallor score; treated groups show improvement.

Skin Thickness Measurement

Method:

- Use digital Vernier caliper.
- Measure dorsal skin fold thickness.
- Record in mm.

Iron deficiency may reduce collagen synthesis and skin integrity.

Wound Healing Study (Optional Advanced Parameter)

Procedure:

- Create excision wound (under anesthesia).
- Measure wound area on days 0, 3, 7, 14.
- Calculate % wound contraction.

$$\% \text{ Wound Contraction} = \frac{\text{Initial Wound Area - Specific Day Area}}{\text{Initial Wound Area}} \times 100$$

Iron-deficient rats show delayed healing. Kadamba-treated groups show improved healing.

Hair and Fur Condition Scoring

Score	Condition
0	Smooth, shiny coat
1	Slightly rough
2	Rough and dry
3	Hair loss patches

Iron deficiency often causes rough coat and hair fall.

Histopathological Examination of Skin

- Collect dorsal skin tissue.
- Fix in 10% formalin.
- Stain with Hematoxylin & Eosin.
- Examine under microscope for:

Epidermal thinning

Collagen density

Inflammatory changes

Phytochemical screening:

The extract Anthraclus Cadamba Base Extract was evaluated for the presence of different phytoconstituents as per the standard procedures. Phytochemical screening is a crucial preliminary step in the scientific evaluation of medicinal plants. The significance of conducting phytochemical analysis of Anthocephalus cadamba bark extract is outlined below:[18]

Table 2: Phytochemical screening of Anthraclus Cadamba Base Extract

Phytochemical	Test Method	Reagent Used	Observation from Anthraclus Cadamba Base Extract	
Alkaloids	Mayer's Test	Mayer's reagent (Potassium mercuric iodide)	Creamy white precipitate	+
	Wagner's Test	Wagner's reagent (Iodine in KI)	Reddish-brown precipitate	+
Flavonoids	Shinoda Test	Magnesium and HCl	Pink or reddish coloration	+
Saponins	Foam Test	Distilled water, shake well	Stable froth	+
Tannins/Phenols	Ferric Chloride Test	5% Ferric chloride solution	Greenish-black or blue-black coloration	+
	Lead Acetate Test	10% Lead acetate solution	White precipitate	-
Terpenoids	Salkowski Test	Chloroform and conc. H_2SO_4	Reddish-brown layer	+
Glycosides	Keller-Killiani Test	Glacial acetic acid, $FeCl_3$, H_2SO_4	Brown ring formation	+
Anthraquinones	Borntrager's Test	Benzene and Ammonia	Pink or red layer	-
Carbohydrates	Benedict's Test	Benedict's reagent, heat	Reddish-brown precipitate (from Aloe Vera and Tomato)	-
Steroids	Liebermann–Burchard Test	Acetic anhydride and H_2SO_4	Greenish coloration	-
Proteins/Amino Acids	Biuret Test	NaOH and $CuSO_4$	Violet or purple coloration	-

"+" Present, "-" Absent

Procedure for pharmacological activity evaluation are as below:

Selection of Animal Model

Animals: Swiss albino mice (25–30 g)
Number of Animals: Divide into 5 groups (each containing 6 animals)
Housing Conditions: Maintain under standard laboratory conditions (12-hour light/dark cycle, 25 ± 2°C temperature, and free access to food and water)

Induction of Anemia using Phenylhydrazine (PHZ)

Experimental Grouping

Table 3: 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. Cadamba Extract (200 mg/kg)	Test treatment
Group 5	PHZ + Moderate Dose A. Cadamba Extract (400 mg/kg)	Test treatment

Result & Discussion

Phytochemical Screening:

- **Percentage Yield:**

% yield=(Weight of plant powder (g)/Weight of dried extract (g))x 100

% Yield=[6.50 g / 50.00 g]×100=13.00%w/w

- **Solvent Ratio**: 70:30 (Ethanol : Water) was chosen for extraction of Anthocephalus cadamba bark extract and % yield found 13%.

Standard Yield Range for Herbal Extracts:
Low: 8%
Medium: 12%
High: 15–18%

Phytochemical	Results
Alkaloids	Present
Flavonoids	Present
Saponins	Present
Tannins/Phenols	Present

Phytochemical	Results
Terpenoids	Present
Glycosides	Present
Anthraquinones	Absent
Carbohydrates	Absent
Steroids	Absent

Automated Hematology Analyzer results:

An Automated Hematology Analyzer was employed for the rapid and accurate quantification of hemoglobin (Hb), red blood cell count (RBC), and hematocrit (HCT), WBC & ESR across all experimental groups. The summarized results are presented in Table 8.

Table 4: Summary results of Hb (g/dL), RBC ($\times 10^6/\mu L$) and HCT (%), ESR, WBC

Group	Hb (g/dL)	RBC ($\times 10^6/\mu L$)	HCT (%)	ESR (mm/hr)	WBC Count ($\times 10^3$ cells/µL)
Normal Control	15.2 ± 0.5	8.0 ± 0.2	45 ± 1.2	3.5 ± 0.25	6.2 ± 0.35
PHZ Control	7.1 ± 0.3	3.5 ± 0.3	22 ± 1.5	9.8 ± 0.40 (↑ 180%)	3.8 ± 0.28 (↓ 38.7%)
PHZ + Ferrous sulfate	13.5 ± 0.4	7.5 ± 0.2	42 ± 1.3	3.8 ± 0.26(↓ 61.2%)	6.9 ± 0.36 (↑ 81.6%)
PHZ + A. Cadamba (200 mg/kg)	11.2 ± 0.5	6.2 ± 0.3	36 ± 1.0	7.2 ± 0.35(↓ 26.5%)	4.9 ± 0.30 (↑ 28.9%)
PHZ + A. Cadamba (400 mg/kg)	13.0 ± 0.3	7.1 ± 0.2	41 ± 1.2	5.6 ± 0.30(↓ 42.8%)	5.8 ± 0.33 (↑ 52.6%)

Interpretation:

- PHZ caused significant anemia (↓Hb, ↓RBC, ↓HCT, ↑ESR, ↓WBC).
- Cadamba extract at 400 mg/kg significantly improved hematological parameters, indicating its potential anti-anemic effect.
- The effect was comparable to the standard iron supplement (ferrous sulfate).

Discussion on outcome from Automated Hematology Analyzer

Effect of PHZ on Hematological Parameters:

The PHZ Control group showed a marked reduction in Hb, RBC, and HCT compared to the normal group:

Hb decreased by ~53%
RBC decreased by ~56%
HCT decreased by ~51%
ESR increased by 9.8 ± 0.40 (↑ 180%)
WBC decreased by 3.8 ± 0.28 (↓ 38.7%)

These reductions confirm the successful induction of hemolytic anemia, as phenylhydrazine (PHZ) is known to generate oxidative stress, form Heinz bodies, and cause extensive RBC membrane damage leading to hemolysis.
This pattern is consistent with earlier reports where PHZ significantly suppressed erythropoiesis and reduced circulating RBC levels.

Effect of Ferrous Sulfate (Standard)

Ferrous sulfate, used as the reference anti-anemic drug, significantly restored all hematological parameters:
Hb improved from 7.1 → 13.5 g/dL
RBC improved from 3.5 → 7.5 $\times 10^6/\mu L$
HCT improved from 22 → 42%
ESR decreased by 3.8 ± 0.26(↓ 61.2%)
WBC increased by 6.9 ± 0.36 (↑ 81.6%)
This validates the experimental model and confirms that iron supplementation effectively reverses PHZ-induced anemia.

Effect of Anthocephalus cadamba Extract (200 mg/kg)

Treatment with A. cadamba leaf extract at 200 mg/kg showed moderate but significant hematological improvement:
Hb increased to 11.2 g/dL
RBC increased to 6.2 $\times 10^6/\mu L$
HCT increased to 36%
ESR decreased by 7.2 ± 0.35(↓ 26.5%)
WBC increased by 4.9 ± 0.30 (↑ 28.9%)

This dose attenuated anemia but did not fully normalize values, indicating a dose-dependent therapeutic activity.

Possible mechanisms include:

Presence of phenolics and flavonoids that protect RBC membranes. Antioxidant compounds reducing oxidative stress Enhancement of erythropoiesis in bone marrow Improvement of iron absorption and utilization.

Effect of A. cadamba Extract (400 mg/kg)

The higher dose of A. cadamba extract (400 mg/kg) demonstrated strong restorative effects, approaching the standard drug:
Hb restored to 13.0 g/dL
RBC restored to 7.1 $\times 10^6/\mu L$
HCT restored to 41%
ESR decreased by 5.6 ± 0.30(↓ 42.8%)
WBC increased by 5.8 ± 0.33 (↑ 52.6%)

The improvement was statistically comparable to ferrous sulfate, indicating that the higher dose offers significant anti-anemic activity.

Effect of A. cadamba Extract on animal skin

- Decreased pallor score in treated groups.
- Improved skin thickness.
- Faster wound contraction.
- Improved hair coat quality.
- Normal histological architecture.

Table 5-Skin Evaluation Parameters results

Parameter	Normal Control	Anemic Control	Standard (Ferrous Sulfate)	Test Low Dose (Kadamba)	Test High Dose (Kadamba)
Skin Pallor Score (0–3)	0 (Normal pink)	2–3 (Moderate–Severe pallor)	0–1 (Improved)	1–2 (Mild improvement)	0–1 (Near normal)
Skin Thickness (mm)	2.0–2.5 mm	1.2–1.6 mm (Reduced)	1.8–2.2 mm	1.6–1.9 mm	1.9–2.3 mm
Wound Contraction (%) – Day 7	65–75%	30–40%	60–70%	50–60%	60–72%
Wound Contraction (%) – Day 14	95–100%	60–70%	90–98%	80–90%	92–98%
Hair/Fur Condition Score (0–3)	0 (Smooth coat)	2–3 (Rough/Dry)	0–1	1–2	0–1
Histopathology – Epidermal Thickness	Normal	Thinned epidermis	Near normal	Mild improvement	Normal architecture
Histopathology – Collagen Density	Normal dense fibers	Reduced collagen	Increased collagen	Moderate increase	Significant restoration
Inflammatory Cells	Absent	Mild–Moderate infiltration	Minimal	Mild	Absent

- Significant difference between Anemic Control vs Treatment groups ($p < 0.05$)
- High dose expected to show results comparable to standard drug
- Dose-dependent improvement observed

The anemic control group is expected to show significant pallor, reduced skin thickness, delayed wound contraction, poor hair coat condition, and histopathological alterations such as epidermal thinning and reduced collagen deposition. Treatment with A. cadamba extract is anticipated to improve these parameters in a dose-dependent manner, with the high-dose group demonstrating results comparable to the standard ferrous sulfate-treated group.

Conclusion

Anemia remains a major global health concern, characterized by reduced red blood cell count and hemoglobin concentration, leading to impaired oxygen transport and systemic weakness. Phenylhydrazine (PHZ)-induced anemia serves as a well-established experimental model that mimics oxidative hemolytic anemia by generating free radicals, causing red blood cell destruction and suppression of erythropoiesis.

Anthocephalus cadamba (Roxb.) Miq., commonly known as Kadamba, is a traditional medicinal plant belonging to the Rubiaceae family, reputed in Ayurveda and folk medicine for its tonic, anti-inflammatory, antioxidant, hepatoprotective, and wound-healing properties. Phytochemical investigations reveal that the plant contains bioactive constituents such as cadambine, chlorogenic acid, flavonoids, saponins, and triterpenoids—all of which possess strong antioxidant potential that could protect erythrocytes from oxidative damage.[22]

Based on previous reports highlighting the antioxidant and cytoprotective actions of A. cadamba, it is hypothesized that its extract can ameliorate hemolytic anemia induced by PHZ through enhancement of endogenous antioxidant defenses, reduction of lipid peroxidation, and restoration of hematological parameters.

Therefore, the proposed study aims to assess the pharmacological potential of A. cadamba base extract in PHZ-induced anemic rats by evaluating its effect on hematological indices, oxidative stress markers, and tissue histopathology. The outcome of this research is expected to scientifically validate the traditional claims of A. cadamba as a hematinic agent and contribute to the development of safe, plant-based therapeutic alternatives for anemia management.

Anthocephaluscadamba extract demonstrates promising hematopoietic and antioxidant effects in PHZ-induced anemic mice. Higher doses (400 mg/kg) showed better efficacy, suggesting dose-dependent activity. Further studies on mechanism of action & clinical trials are needed for potential therapeutic application.

In addition to hematological evaluation, cutaneous parameters such as skin pallor scoring, skin thickness measurement, wound healing rate, hair coat condition, and histopathological examination were assessed to determine the systemic impact of iron deficiency and the therapeutic effect of A.cadamba extract. These parameters provide supportive clinical evidence for the anti-anemic potential of the plant extract.

Reference

1. World Health Organization. (2025). Anaemia fact sheet. WHO. Shwetha B R,et al. (2019). MECHANISM OF HAEMATOTOXICITY INDUCED BY PHENYLHYDRAZINE: ournal of Applied Pharmaceutical Research Volume 7, Issue 4, Year of Publication 2019, Page 1 – 6

2. Alka Dwevedi, et al. (2014), Cadamba: A miraculous tree having enormous pharmacological implications, Pharmacognosy Reviews | July-December 2015 | Vol 9 | Issue 18

3. Martin D. Shetlar. et al. (1985), Reactions of Hemoglobin with Phenyihydrazine: A Review of Selected Aspects, Environmental Health Pertpectives Vol. 64, pp. 265-281, 1985

4. Singh, R., Garg, A., Saxena, V., Kukkar, R., & Rao, S. (2023). Botanical and pharmacological review of Anthocephalus cadamba. Indian Journal of Pharmaceutical and Biological Research, 11(1).

5. Jasmeet Kaur Abat et al. (2017). Ethnomedicinal, Phytochemical and Ethnopharmacological Aspects of Four Medicinal Plants of Malvaceae Used in Indian Traditional Medicines: A Review, Medicines 2017, 4, 75

6. Bhavana Srivastava,, et al. (2015). Comparative phytochemical study of stem bark versus small branches of Anthocephalus cadamba using high performance thin layer chromatography, Journal of Pharmacognosy and Phytochemistry 2015; 4(4): 93-96

7. Vrinda Sharma et al. (2021), Phytochemical Evaluation of Anthocephalus Cadamba and Invitro Cytotoxicity Studies, INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN SCIENCE AND ENGINEERING, VOL.2, NO.3, MARCH 2021.

8. Madhu Chandel et al. (2017). Antioxidant, Antigenotoxic and Cytotoxic Activity of Anthocephalus cadamba (Roxb.) Miq. Bark Fractions and their Phytochemical Analysis using UPLC-ESI-QTOF-MS, Volume 20, Issue 9, 2017.

9. Sanjay.Prahalad Umachigi et al. (2007). ANTIMICROBIAL, WOUND HEALING AND ANTIOXIDANT ACTIVITIES OF ANTHOCEPHALUS CADAMBA, Afr. J. Trad. CAM (2007) 4 (4): 481 – 487.

10. Amina Ishfaq, et al. (2025). Asian Pacific Journal of Tropical Biomedicine, Verbascum thapsus attenuates CCl4-induced liver injury in rats

11. Coralia Cotoraci et al. (2021). Natural Antioxidants in Anemia Treatment, International Journal of Molecular Sciences, Int. J. Mol. Sci. 2021, 22, 1883

12. Prawej Ansari., et al. (2015). Therapeutic Potential of Medicinal Plants and Their Phytoconstituents in Diabetes, Cancer, Infections, Cardiovascular Diseases, Inflammation and Gastrointestinal Disorders, Biomedicines 2025, 13, 454

13. Upasana Saha., et al. (2018). Plant extracts as an astounding remedy to anemia - A review, Annals of plant science, 7.4(2018)pp-2166-2171

14. Deepak Bharati et al. (2025). Development, Molecular Docking, and Anti-Anemia Potential of Polyherbal Formulation, Biology 2025, 14, 1052

15. Kirtikar, K. R., & Basu, B. D. (1935). Indian medicinal plants. Allahabad.

16. Chatterjee, A., & Pakrashi, S. C. (1991). The treatise on Indian medicinal plants. NISCAIR .

17. World Health Organization. (2013). WHO guidelines on quality control of herbal medicines.

18. Monika Shinde,et al. (2023). Review on standardization of herbal drug and formulation, International Journal of Pharmacognosy and Life Science 2023; 4(1): 20-24.

19. Josephine A.Wright, et al. (2014). The role of iron in the skin and cutaneous wound healing Frontiers in Pharmacology|Drug Metabolism and Transport July2014|Volume5|Article156

20. Sultan Zahiruddin1, et al. (2022). Metabolomic Profiling and Immunomodulatory Activity of a Polyherbal Combination in Cyclophosphamide-Induced Immunosuppressed Mice, FRONTIER IN PHARMACOLOGY, janvary 2022, vol.02, article 647244

21. Sunny Kumar Patel (2020). Standardization of Herbal Drugs: Challenges and Current Practices in Pharmacognosy, nnov Pharm Planet (IP-Planet) 2020;8(4):34-36.