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Anti-Anemia Effect of Red Beetroot and Roselle Extract Combination with $NaNO_2$ Induction in White Rats

Nina Herlina[1], Moerfiah[2], Nadya Putri Utami[3]
[1,3] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Pakuan University, Jl. Pakuan, Tegallega, Bogor Tengah, Bogor 16143, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Sciences, Pakuan University, Jl. Pakuan, Tegallega, Bogor Tengah, Bogor 16143, Indonesia

**Corresponding author:* nina.herlina@unpak.ac.id

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Abstract: Anemia remains a major global health problem characterized by reduced erythrocyte count, hemoglobin concentration, and hematocrit levels, often resulting from iron deficiency and oxidative stress. Plant-based alternatives with proven hematological efficacy offer promising therapeutic potential. This study aimed to evaluate the anti-anemic effect of combined red beetroot (*Beta vulgaris* L.) and roselle (*Hibiscus sabdariffa* L.) extracts in sodium nitrite ($NaNO_2$)-induced anemic rats and to determine the most effective combination ratio. The extracts were prepared by maceration using 70% ethanol. Twenty-four female Sprague Dawley rats (±200 g body weight (BW) were randomly divided into six groups: negative control (Na-CMC), positive control (blood supplement tablet), roselle flower extract (40 mg/200 g BW), and three combination groups consisting of beetroot–roselle extracts at ratios of 1:2 (90 mg + 80 mg/200 g BW), 2:1 (180 mg + 40 mg/200 g BW), and 1:1 (90 mg + 40 mg/200 g BW). Anemia was induced using $NaNO_2$ for 14 days, followed by oral treatment for another 14 days. Hematological parameters, including erythrocyte count, hemoglobin concentration, and hematocrit value, were measured on days 0, 7, and 14 of treatment. Data were analyzed using one-way ANOVA followed by Duncan's post hoc test ($p < 0.05$). $NaNO_2$ induction significantly reduced erythrocyte count, hemoglobin, and hematocrit levels, confirming successful anemia induction. All treatment groups showed significant hematological improvement compared to the negative control ($P \leq 0.05$). Roselle extract alone produced the highest hemoglobin increase (84.1%), whereas combination treatments resulted in greater erythrocyte recovery. The beetroot–roselle combination at a 2:1 ratio demonstrated the most balanced improvement across erythrocyte count, hemoglobin, and hematocrit parameters. In conclusion, the combination of beetroot and roselle extracts exhibited synergistic anti-anemia activity, likely associated with enhanced iron utilization and antioxidant effects, with the 2:1 ratio showing the most favorable overall hematological response.

Keywords: anemia, antioxidant, beetroot, erythropoiesis, hematological parameters, roselle, synergistic effect

Abstrak: Anemia merupakan masalah kesehatan global yang ditandai dengan penurunan jumlah eritrosit, kadar hemoglobin, dan hematokrit, yang umumnya disebabkan oleh defisiensi besi dan stres oksidatif. Alternatif berbasis tumbuhan dengan khasiat hematologis yang telah terbukti menawarkan potensi terapeutik yang menjanjikan. Penelitian ini bertujuan untuk mengevaluasi aktivitas anti-anemia kombinasi ekstrak bit merah (*Beta vulgaris* L.) dan rosella (*Hibiscus sabdariffa* L.) pada tikus anemia yang diinduksi natrium nitrit ($NaNO_2$) serta menentukan rasio kombinasi yang memberikan respons hematologi paling baik. Ekstrak dibuat dengan metode maserasi menggunakan etanol 70%. Sebanyak 24 ekor tikus betina Sprague Dawley (±200 g berat badan (BB) dibagi secara acak menjadi enam kelompok, yaitu kontrol negatif (Na-CMC), kontrol positif (tablet penambah darah), ekstrak bunga rosella (40 mg/200 g BB), dan tiga kelompok kombinasi ekstrak bit merah–rosella dengan rasio 1:2 (90 mg + 80 mg/200 g BB), 2:1 (180 mg + 40 mg/200 g BB), dan 1:1 (90 mg + 40 mg/200 g BB). Anemia diinduksi menggunakan $NaNO_2$ selama 14 hari, kemudian dilanjutkan dengan pemberian perlakuan secara oral selama 14 hari. Parameter hematologi meliputi jumlah eritrosit, kadar hemoglobin, dan nilai hematokrit yang diukur pada hari ke-0, ke-7, dan ke-14 perlakuan. Data dianalisis menggunakan one-way ANOVA dilanjutkan uji Duncan ($p \leq 0,05$). Induksi $NaNO_2$ menurunkan jumlah eritrosit, hemoglobin, dan hematokrit secara signifikan, menunjukkan keberhasilan model anemia. Seluruh kelompok perlakuan menunjukkan perbaikan parameter hematologi yang signifikan dibandingkan kontrol negatif ($p \leq 0,05$). Ekstrak rosella tunggal menghasilkan peningkatan hemoglobin tertinggi (84,1%), sedangkan kombinasi ekstrak memberikan pemulihan eritrosit yang lebih besar. Kombinasi bit merah–rosella rasio 2:1 menunjukkan peningkatan paling seimbang pada parameter eritrosit, hemoglobin, dan hematokrit. Kesimpulannya, kombinasi ekstrak bit merah dan rosella menunjukkan aktivitas anti-anemia yang bersifat sinergis, yang diduga berkaitan dengan peningkatan utilisasi besi dan aktivitas antioksidan, sehingga berpotensi dikembangkan sebagai alternatif terapi alami untuk anemia.

Kata kunci: anemia, antioksidan, bit merah, eritropoiesis, parameter hematologi, rosella, efek sinergis

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1. INTRODUCTION

Anemia constitutes a significant public health challenge in Indonesia, affecting approximately 16.2% of the general population and 27.7% of pregnant women, despite ongoing prevention and treatment initiatives (Kementrian Kesehatan RI, 2023). The condition primarily results from iron deficiency, disrupting the physiological processes of erythrocyte production, hemoglobin synthesis, and hematocrit maintenance. Consequently, anemia leads to diminished cognitive performance, reduced immune competence, and impaired growth and development, particularly affecting women of reproductive age and adolescents (Warner and Kamran, 2025). Iron deficiency cannot be overcome through endogenous synthesis; rather, it requires supplementation from external sources. Herbal remedies represent a promising complementary approach for anemia prevention and treatment, offering potential efficacy with an acceptable safety profile (Jeong et al., 2024; Nehar et al., 2024).

Herbal remedies are a promising alternative for the prevention and treatment of anemia, as they are potentially effective and have a relatively good safety profile. Beetroot (*Beta vulgaris*) contains significant amounts of iron (0.8–8 mg/100 g), folic acid (approximately 34%), vitamins, and essential minerals that support hematopoiesis. Evidence demonstrates that beetroot consumption increases hemoglobin levels, exemplified by increases from 10.68 g/dl to 11.09 g/dl in adolescents (Zuhraini and Kurniasari, 2021) as well as erythrocyte counts from 6.09 ± 0.29 to 10.41 ± 0.25 × 106/µl in a murine model (Hikmawanti et al., 2021). Similarly, roselle flower (*Hibiscus sabdariffa* L.) demonstrates considerable potential as a natural therapeutic agent for anemia, containing iron, vitamin C, calcium, phosphorus, and potent antioxidants that reduce oxidative stress and enhance iron bioavailability (Mayunita and Marhani, 2024; Puspita and Surani, 2025; Sholika Sari et al., 2023). Preclinical studies reveal that roselle extract improves the erythrocyte profile through antioxidant mechanisms, whereas beetroot primarily augments hemoglobin levels via its iron content (Ali and Bilal, 2023). Administration of roselle kombucha at a dose of 0.36 mL/20 g body weight increased erythrocyte counts to 4.78 ± 0.36 million/mm³ and hemoglobin levels to 18.28 ± 0.69 g/dL in experimental rats (Hidayanti et al., 2015).

Although red beetroot and roselle flowers have been individually documented to possess anti-anemia activity, research investigating their combined synergistic effects remains limited. Previous investigations have predominantly focused on single-plant interventions without evaluating potential synergistic interactions between the iron content of red beetroot and the vitamin C and antioxidant components of roselle in ameliorating hematological parameters. Furthermore, data regarding optimal combination dose ratios and effective treatment durations are scarce, particularly within controlled anemia models induced by sodium nitrite ($NaNO_2$). Sodium nitrite-induced anemia provides a controlled experimental model suitable for evaluating therapeutic interventions, as it reliably produces measurable reductions in erythrocyte counts and hemoglobin levels comparable to iron-deficiency anemia.

This research aims to evaluate the anti-anemia efficacy of combined 70% ethanol extracts of red beetroot and roselle in a sodium nitrite-induced anemia model and determine optimal dosage ratios (1:2, 2:1, 1:1). The novelty of this study lies in its systematic evaluation of the synergistic anti-anemia potential of combined beetroot and roselle extracts at varying ratios (1:2, 2:1, and 1:1) within a controlled sodium nitrite-induced anemia model.

2. MATERIAL AND METHODS

2.1. Materials and Equipment

This study used female Sprague Dawley rats, red beetroot tubers, roselle flowers, sodium nitrite, 70% ethanol, sodium carboxymethyl cellulose (Na-CMC), iron tablets, Hayem solution, Na-EDTA tubes, laboratory standard feed, and distilled water. The equipment used included rat cages, an electronic balance, a blender, containers for soaking, filter paper, a vacuum filtration device, oral gavage tubes, syringes, micropipettes, a blood cell counter, a microscope, a portable hemoglobin meter with strips, microcentrifuge tubes, a centrifuge, and a microcapillary hematocrit reader.

2.2. Identification of Plant Materials

Red beet roots and roselle flowers are used as plant materials. Plant identification and authentication were out at the Department of Biology, University of Indonesia, with certificate number 1052/UN2.F3.10/PDP.02.00/2023.

2.3. Preparation of Plant Extracts

a. *Red Beet Extract*

Red beets were washed, sliced, dried in an oven at 20–60°C, and ground. The ground material was macerated with 70% ethanol (ratio 1:10, weight/volume) for 24 hours at room temperature in a container protected from light. The filtrate was concentrated using a rotary evaporator at 40°C to produce a thick extract.

b. *Roselle Flower Extract*

Roselle flowers are cleaned, dried in an oven at 20–60°C, and ground into powder. The material is soaked in 70% ethanol (1:10, weight/volume), and the filtrate is concentrated using a rotary evaporator to yield an extract.

2.4. Physicochemical Characterization of Extracts

The quality of the extracts was evaluated by determining moisture content and ash content using standard gravimetric methods as described by BPOM (Kementerian Kesehatan Republik Indonesia, 2022). Moisture content was determined by drying 2 g of each sample in an oven at 105°C until a constant weight was obtained. Ash content was determined by incinerating 2–3 g of sample until a constant residue was obtained.

2.5. Phytochemical Screening

This analysis was performed qualitatively to determine the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids, according to the methods described by Hanani (Hanani, 2015). Positive results were indicated by characteristic colour changes or precipitate formation.

2.6. Experimental Animals and Ethical Approval

Twenty-four female Sprague Dawley rats weighing 180–220 g were used. Their weights were sufficiently similar, as assessed by the coefficient of variation, with a difference of less than 15% considered acceptable. Animals were housed individually under standard conditions (12 h light/dark cycle, 25 ± 2°C, 60 ± 10% humidity) with ad libitum access to standard feed and water. After 7 days of acclimatization, animals were included in the study. All procedures were approved by the Ethics Committee at Universitas Pakuan, with permit number 20/KEPHP-UNPAK/08-2023.

2.7. Experimental Design and Animal Grouping

Rats were randomly assigned into six experimental groups (n = 4): negative control (0.5% Na-CMC), positive control ($FeSO_4$ 1.08 mg/200 g BW), roselle extract (40 mg/200 g BW), combination 1 (1:2; 90 mg + 80 mg/200 g BW), combination 2 (2:1; 180 mg + 40 mg/200 g BW), and combination 3 (1:1; 90 mg + 40 mg/200 g BW).

2.8. Anemia Induction Model

Anemia was induced through oral administration of sodium nitrite ($NaNO_2$) at a dose of 50 mg/200 g BW/day for 14 days (Prihardini and Resty, 2019). $NaNO_2$ was dissolved in sterile distilled water at an appropriate concentration. $NaNO_2$ was dissolved in sterile distilled water at an appropriate concentration before administration. This model has been widely validated for inducing hemolytic anemia through hemoglobin oxidation to methemoglobin (Ambarwati, 2012; Setiawan et al., 2021).

$NaNO_2$ induces methemoglobin formation through oxidation of hemoglobin's ferrous iron (Fe^{2+}) to the ferric state (Fe^{3+}), reducing oxygen-carrying capacity and promoting oxidative damage to erythrocyte membranes (Chen and Nappe, 2026; Iolascon et al., 2021). This oxidation triggers reactive oxygen species (ROS) production, which further compromises erythrocyte membrane integrity and reduces cellular antioxidant defenses. Although methemoglobin levels and oxidative stress markers were not directly quantified in this study, the $NaNO_2$-induced anemia model has been extensively validated to reliably produce hemolytic anemia comparable to iron-deficiency anemia in rats (Setiawan et al., 2021).

Hematological parameters were therefore used as practical indicators, with anemia defined as erythrocyte count $< 7 \times 10^6$ cells/mm³, hemoglobin < 11.6 g/dL, and hematocrit < 37.6%, consistent with standardized reference values for adult female Sprague Dawley rats (Ambarwati, 2012).

2.9. Hematological Analysis

Blood was collected from the tail vein using heparinized capillary micropipettes (approximately 0.5 mL) at four time points: baseline before anemia induction, after 14 days of $NaNO_2$ induction (pre-treatment), and on days 7 and 14 of treatment. All measurements were performed by the same researcher to minimize inter-observer variation. Erythrocyte count was determined using a Neubauer haemocytometer after 1:200 dilution with Hayem's solution. Cells in five large squares were counted using a systematic zigzag pattern, and counts were calculated according to the standard formula (McClure, 2017). Hemoglobin concentration was measured using a portable hemoglobinometer (Easy Touch GCHb, Infopia Co., Korea). Fresh blood (10 µL) was applied to test strips following the manufacturer's instructions, with measurements performed in duplicate. Hematocrit values were determined using the microhematocrit method. Capillary tubes filled with blood were centrifuged at 12,000 rpm for 5 minutes, and packed cell volume was read using a microcapillary reader (McClure, 2017).

2.10. Statistical Analysis

Data were analyzed using SPSS software version 27.0 (IBM Corp., USA). Before analysis of variance (ANOVA), data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. Differences among groups were analyzed using one-way ANOVA, followed by Duncan's multiple range test. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extract Yield

The extraction yield of red beetroot extract was 18.38%, whereas roselle flower extract yielded 46.60%. The higher yield of roselle flower extract may be attributed to its higher content of polar secondary metabolites, such as organic acids, anthocyanins, and flavonoids, which are readily extracted using hydroethanolic solvents.

3.2. Physicochemical Characterization of Extracts

The moisture and ash contents of red beetroot and roselle flower simplicia and extracts are summarized in Table 1.

Table 1. Moisture and Ash Content of Simplicia and Extracts

Extracts	Moisture Content	Ash Content
Red beetroot	5.08 ± 0.07%	4.94 ± 0.56%
Roselle flower	5.32 ± 0.15%	4.27 ± 1.27%

Based on the results, both parameters met the quality requirements for extracts as specified by BPOM (BPOM, 2019). The moisture content of both extracts was within the acceptable limit, indicating adequate drying and good stability during storage. In addition, the ash content complied with the BPOM (2019) standard, reflecting low inorganic residue and satisfactory purity of the plant materials. These findings suggest that the physicochemical quality of red beetroot and roselle flower simplicia and extracts was acceptable and comparable to those reported in previous studies (Adiansyah et al., 2018; Maimunah et al., 2021).

3.3. Phytochemical Characterization of Extracts

Phytochemical characterization was conducted to identify the presence of major secondary metabolites in red beetroot and roselle extracts.

Table 2. Phytochemical Screening Results of Red Beetroot and Roselle Extracts

Phytochemical	Red Beetroot Extract	Roselle Extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	−	−

The results (table 2) showed that both red beetroot and roselle extracts contained alkaloids, flavonoids, tannins, and saponins, while terpenoids were not detected. The presence of these compounds indicates potential biological activity of the extracts. Flavonoids are known to exhibit antioxidant activity (and support erythropoiesis, while tannins and saponins may contribute to improved iron utilization and stabilization of erythrocyte membranes (Ukey et al., 2025). These findings support the potential role of the extracts in improving hematological parameters.

3.4. Effect of $NaNO_2$-Induced Anemia

The induction of $NaNO_2$ for 14 days resulted in a significant decrease in all hematological parameters (Table 3). The number of erythrocytes decreased by 51.2%, hemoglobin concentration by 35.2%, and hematocrit by 21.2% compared with baseline ($p < 0.05$), confirming the development of anemia in the experimental animals.

Table 3. Hematological parameters before and after $NaNO_2$ induction (Mean ± SD) x10^6

Parameter	Before Induction	After Induction	Percentage Change (%)	p-value
Erythrocytes (×10^6/mm^3)	8.81 ± 1.02	4.30 ± 0.56	-51.2	<0.001
Hemoglobin (g/dL)	13.08 ± 1.00	8.47 ± 0.81	-35.2	<0.001
Hematocrit (%)	44.73 ± 2.32	35.28 ± 2.84	-21.2	<0.01

The observed reductions in erythrocyte count, hemoglobin, and hematocrit are consistent with previous studies using $NaNO_2$-induced anemia models (Norsiah et al., 2025; Petrova et al., 2020; Yuniarti et al., 2019). These findings indicate that $NaNO_2$ administration effectively produced an anemic condition suitable for evaluating anti-anemia activity (Pradawahyuningtyas et al., 2020; Suparmi et al., 2016).

3.5. Hematological Effects of Treatments in $NaNO_2$-Induced Anemic Rats

Hematological parameters, including red blood cell count, hemoglobin concentration, and hematocrit, serve as important indicators of blood restoration in anemia models. In this study, the effects of beetroot (*Beta vulgaris* L.) and roselle (*Hibiscus sabdariffa* L.) extracts on anemia recovery were investigated over 14 days in Rattus norvegicus with sodium nitrite ($NaNO_2$)-induced anemia. Changes in erythrocyte count, hemoglobin concentration, and hematocrit value were monitored to evaluate the potential of these plant extracts to promote erythropoiesis and restore hematological balance.

a. *Red Blood Cell Count*

Anemia induction was successfully achieved in all experimental groups, as indicated by the reduced red blood cell (RBC) counts observed at Day 0 before treatment. Following the administration of the test preparations, all treatment groups except the negative control (Na CMC) exhibited a clear increase in RBC counts over time ($P \leq 0.05$) (Table 4).

Table 4. Red Blood Cell (RBC) Counts in White Rats During Treatment

Treatment	Mean Red Blood Cell (RBC) Counts in Rats (×10^6 cells/µL)			Mean RBC (×10^6 cells/µL)
	Day 0	Day 7	Day14	
Blood Supplement Tablet	4.1 ± 0.37	7.9 ± 0.57	10.4 ± 0.36	7.5 ± 2.92^b
Na CMC	4.3 ± 0.49	4.3 ± 0.48	4.5 ± 0.46	4.4 ± 0.75^a
Roselle Flower Extract 40 mg/200 g BW	4.7 ± 0.40	8.2 ± 0.83	10.0 ± 0.73	7.6 ± 3.06^b
Red Beetroot & Roselle Extract (1:2)	4.2 ± 0.62	8.0 ± 0.41	13.1 ± 2.03	8.4 ± 3.19^c
Red Beetroot & Roselle Extract (2:1)	4.1 ± 0.86	7.8 ± 0.17	12.2 ± 0.55	8.0 ± 3.19^c
Red Beetroot & Roselle Extract (1:1)	4.4 ± 0.62	7.7 ± 0.21	11.8 ± 0.50	7.9 ± 2.60^b

Note: Beetroot–roselle extract (1:2) = 90 mg + 80 mg; (2:1) = 180 mg + 40 mg; (1:1) = 90 mg + 40 mg per 200 g BW. Data are presented as mean ± SD. Different superscripts within the same column indicate significant differences ($p \leq 0.05$).

The Blood Supplement Tablet served as a standard reference, effectively improving RBC levels. Roselle Flower Extract also demonstrated a substantial increase in RBC counts. Among the Red Beetroot and Roselle Extract combination treatments, all ratios resulted in higher RBC counts. An initial recovery effect was already observable at Day 7, indicating that the preparations began to influence RBC restoration within the first week. The most pronounced and optimal effect was observed at Day 14, with the 1:2 ratio combination producing the greatest increase and approaching the normal baseline values before anemia induction (RBC 8.81 ± 1.02 ×10^6/mm^3). The 2:1 and 1:1 ratios also showed marked improvement, although slightly lower than the 1:2 ratio. The negative control (Na CMC) exhibited minimal change over the same period, confirming that spontaneous recovery was negligible.

b. *Hemoglobin Concentration*

Red blood cells contain hemoglobin (Hb), a protein measured in grams per deciliter (g/dL) that acts as the primary oxygen carrier to tissues throughout the body. A sufficient hemoglobin level is essential for adequate oxygen supply to the tissues and the maintenance of normal physiological functions (Fagbohoun et al., 2022). Changes in hemoglobin (Hb) concentration during the treatment period are presented in Table 5.

Table 5. Hemoglobin (Hb) Levels in White Rats During Treatment

Treatment	Mean Hemoglobin (Hb) Levels in White Rats			Mean Hb
	Day 0	Day 7	Day14	
Blood Supplement Tablet	7.9 ± 0.98	10.9 ± 0.85	14.4 ± 1.18	11.0 ± 2.92^{b}
Na CMC	8.3 ± 0.81	8.3 ± 0.81	8.3 ± 0.86	8.3 ± 0.75^{a}
Roselle Flower Extract 40 mg/200 g BW	8.2 ± 0.88	11.5 ± 0.71	15.1 ± 0.93	11.6 ± 3.06^{b}
Red Beetroot & Roselle Extract (1:2)	9.1 ± 0.86	12.8 ± 0.57	15.7 ± 1.05	12.5 ± 3.19^{c}
Red Beetroot & Roselle Extract (2:1)	9.1 ± 0.33	13.2 ± 0.47	16.6 ± 0.50	12.9 ± 3.19^{c}
Red Beetroot & Roselle Extract (1:1)	8.3 ± 0.98	11.9 ± 0.17	14.1 ± 0.62	11.4 ± 2.60^{b}

Note : Beetroot–roselle extract (1:2) = 90 mg + 80 mg; (2:1) = 180 mg + 40 mg; (1:1) = 90 mg + 40 mg per 200 g BW. Data are presented as mean ± SD. Different superscripts within the same column indicate significant differences ($p \leq 0.05$).

At baseline (Day 0), all experimental groups exhibited reduced Hb levels, confirming successful anemia induction. During the treatment period, all groups receiving active substances showed a significant increase in Hb concentration from Day 7 to Day 14. In contrast, the negative control group (Na CMC) exhibited no meaningful change. Statistical analysis demonstrated that the blood supplement tablet, roselle flower extract, and all red beetroot–roselle combination extracts produced significantly higher Hb levels compared with the negative control ($P \leq 0.05$), indicating apparent anti-anaemic activity.

Comparable increases in Hb concentration were observed in the blood supplement tablet, roselle single extract, and the red beetroot–roselle combination at a 1:1 ratio. In contrast, the combination extracts at ratios of 1:2 and 2:1 showed significantly superior efficacy ($P \leq 0.05$). The 2:1 combination achieved the highest Hb level on Day 14 (16.6±0.50 g/dL), exceeding the normal physiological range for rats (11.6–16.1 g/dL). Notably, both combination groups restored Hb levels to the normal range within 7 days, whereas the positive control and single-extract groups required a longer duration. These findings indicate that red beetroot–roselle combinations, particularly at a 2:1 ratio, accelerate hemoglobin recovery, with a 14-day treatment period being optimal for maximising therapeutic outcomes.

c. *Hematocrit*

Consistent with the reduced red blood cell (RBC) counts observed on day 0, hematocrit (HCT) values in all groups were also decreased before treatment, confirming the successful induction of anemia. Hematocrit represents the proportion of erythrocytes relative to the total blood volume and reflects both erythrocyte number and size. Therefore, a reduction in HCT is a key hematological indicator of anemia (Mondal and Zubair, 2025).

As shown in Table 6, HCT levels increased in all treatment groups from day 7 to day 14. The negative control group (Na CMC) exhibited no significant change, with HCT values remaining relatively stable throughout the experimental period. In contrast, the positive control group receiving blood supplement tablets (BST) showed a consistent increase, reaching 49.8±3.77% on day 14. Administration of roselle flower extract alone and red beetroot–roselle combination extracts significantly improved HCT levels compared with the negative control (P≤0.05). Among the combinations, the red beetroot–roselle extract at a 2:1 ratio produced the highest HCT value on day 14 (51.3±0.50%) and the highest overall mean HCT, indicating superior efficacy in restoring hematocrit levels in anaemic rats.

Table 6. Hematocrit (Hct) Levels in White Rats During Treatment

Treatment	Mean Hematocrit (Hct) Levels in White Rats			Mean Hct
	Day 0	Day 7	Day14	
Blood Supplement Tablet	33.8 ± 2.87	40.5 ± 3.51	49.8 ± 3.77	41.3 ± 7.51[bc]
Na CMC 40 mg per 200 g BW	37.8 ± 2.62	37.8 ± 2.62	38.3 ± 2.87	37.9 ± 2.46[a]
Roselle Flower Extract	31.0 ± 2.16	39.0 ± 2.16	49.3 ± 1.70	39.7 ± 8.01[ab]
Red Beetroot & Roselle Extract (1:2)	33.8 ± 3.20	41.0 ± 3.16	50.0 ± 1.41	41.6 ± 7.86[bc]
Red Beetroot & Roselle Extract (2:1)	37.5 ± 4.43	40.5 ± 4.20	51.3 ± 0.50	43.1 ± 6.45[c]
Red Beetroot & Roselle Extract (1:1)	37.8 ± 2.50	40.3 ± 3.40	46.0 ± 2.58	41.3 ± 4.43[bc]

Note: Beetroot–roselle extract (1:2) = 90 mg + 80 mg; (2:1) = 180 mg + 40 mg; (1:1) = 90 mg + 40 mg per 200 g BW. Data are presented as mean ± SD. Different superscripts within the same column indicate significant differences (p ≤ 0.05).

d. *Percentage Improvement Across Hematological Parameters*

The percentage increase in hematological parameters is used to compare each treatment's therapeutic response to the cure of anemia. This parameter reflects the biological efficacy of the treatment in stimulating erythropoiesis and quantitatively improving hematological status.

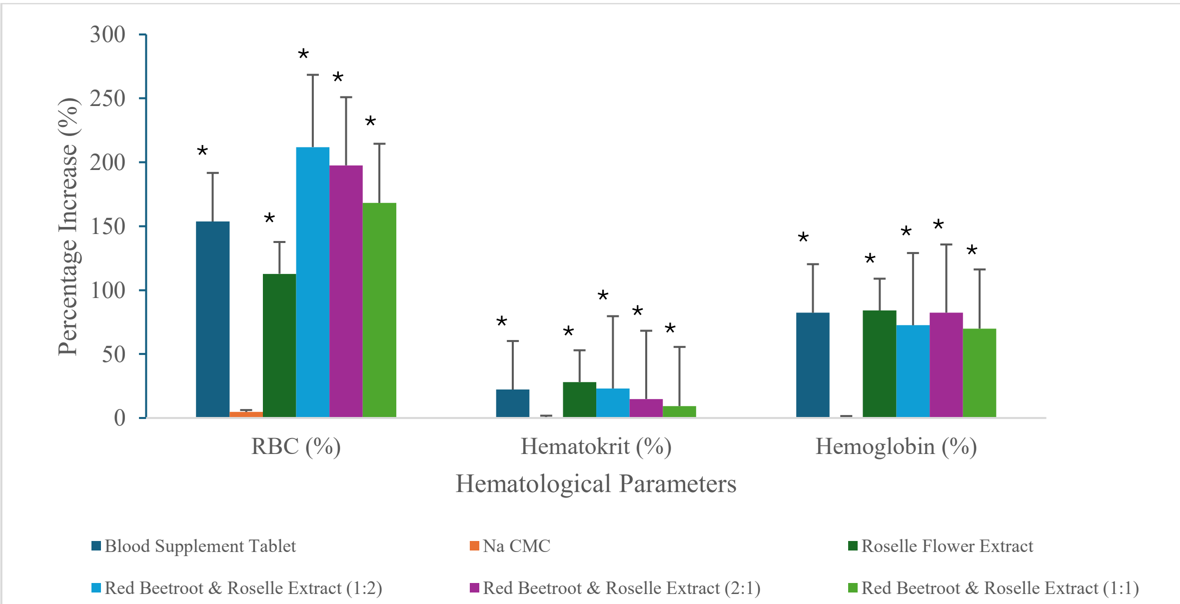

Figure 1. Percentage increase in erythrocyte (RBC), hematocrit, and hemoglobin levels following administration of red beetroot extract, roselle extract, and their combinations in $NaNO_2$-induced anemic rats. Data are presented as mean ± standard deviation (SD) (n = 4). * Indicates a statistically significant difference compared to the negative control group (p < 0.05)

Based on Figure 1, the negative control group (Na CMC) demonstrated minimal increases in all hematological parameters (RBC 2.0±1.5%, hemoglobin 5.8±3.2%, hematocrit 6.5±2.8%), confirming that anemia recovery did not occur spontaneously in the absence of pharmacological intervention. In contrast, the positive control group (blood supplement tablet) showed significantly higher increases in all hematological parameters (RBC 153.7±8.9%, hemoglobin 82.3±5.6%, hematocrit 65.4±7.1%, $p \leq 0.05$) compared to the negative control, thereby confirming the effectiveness of standard therapy in restoring hematological status.

All treatment groups, both single and combination extracts, demonstrated significantly higher percentage increases in hematological parameters compared to the negative control ($p \leq 0.05$), indicating a measurable anti-anemic effect. This confirms that red beetroot (*Beta vulgaris* L.), roselle (*Hibiscus sabdariffa* L.), and their combinations demonstrate measurable anti-anemic effects. A single treatment of roselle extract produced the highest hemoglobin response (84.1±6.3%), approaching the positive control. Combination treatments resulted in greater RBC increases than single extracts. The 1:2 beetroot-roselle combination achieved the maximum RBC response (211.9±12.4%), while the 2:1 combination demonstrated more balanced improvements across RBC (175.8±9.7%), hemoglobin (78.6±5.9%), and hematocrit (82.1±6.8%).

Across all treatment groups, RBC percentage increases consistently exceeded hemoglobin and hematocrit levels, suggesting that erythropoietic activity predominated during the recovery phase. This pattern indicates that erythrocyte formation occurred more rapidly than hemoglobin maturation, likely reflecting iron availability constraints and incomplete maturation of newly released erythrocytes. During recovery from anemia, reticulocytes can enter the circulation before hemoglobinization is complete, particularly under conditions of restricted iron supply (Hoenemann et al., 2021). Since reticulocytes mature within a few days of their release into the bloodstream, red blood cell counts can increase more rapidly than hemoglobin concentration or hematocrit value. Consequently, the recovery of erythrocytes may appear more pronounced during the early phase of hematopoietic restoration (Mayo Clinic Laboratories, 2025). Additionally, hemoglobin formation depends on iron availability, which may slow its increase (Camaschella, 2015). As a result, the increase in RBC appears more prominent than that of hemoglobin and hematocrit.

This study demonstrates that red beetroot and roselle extracts possess anti-anemic properties comparable to standard pharmaceutical therapy. The roselle extract's hemoglobin response (84.1%) approximately matched the positive control (82.3%), consistent with prior clinical evidence. In pregnant women with anemia, roselle extract combined with iron supplementation increased hemoglobin by 1.42 g/dL compared to 0.61 g/dL with iron supplementation alone (Nisa et al., 2017). Similarly, in adolescent females with anemia, the combination of roselle calyx extract with ferrous tablets significantly elevated both hemoglobin and hematocrit (Luluk et al., 2022). This greater hemoglobin-enhancing effect is attributable to roselle's high ascorbic acid (vitamin C) and anthocyanin content. Vitamin C enhances iron absorption by maintaining Fe^{2+} stability and preventing interaction with iron-inhibitory compounds (Camaschella, 2015). Anthocyanins provide antioxidant protection (particularly important during erythropoietic acceleration) by scavenging reactive oxygen species and suppressing hepcidin-mediated iron sequestration (Sadowska-Bartosz and Bartosz, 2024).

Combination treatments produced greater erythrocyte recovery than roselle single treatment, indicating possible synergistic interactions between beetroot and roselle extracts. Among the combinations tested, the 2:1 beetroot–roselle ratio demonstrated the most balanced improvement across erythrocyte count, hemoglobin concentration, and hematocrit value. This suggests complementary mechanisms: beetroot provides iron and folate (erythropoietic foundation), while roselle provides vitamin C and anthocyanins (absorption enhancement and oxidative protection) (Hikmawanti et al., 2021; Mayunita and Marhani, 2024; Puspita and Surani, 2025). Polyherbal formulations combining iron-rich sources with absorption enhancers and antioxidants demonstrate superior efficacy compared to single-component preparations; polyherbal formulations address multiple pathways in anemia pathophysiology simultaneously (Bharati et al., 2025; Lanjewar, 2026). Recent systematic reviews emphasize that synergistic interactions arise when plant components address complementary aspects of anemia: direct iron provision, iron absorption enhancement, antioxidant protection, and erythropoietic stimulation (Lanjewar, 2026). The optimal 2:1 ratio likely reflects equilibrium between nutrient provision (beetroot-dominant) and absorption/protection (roselle-dominant), suggesting potential therapeutic application in resource-limited settings where synthetic iron supplements have limited accessibility or tolerability.

The differential RBC-dominant response (RBC increases exceeding hemoglobin/hematocrit) reflects erythropoietic kinetics during acute anemia recovery. Elevated erythropoietin signaling drives rapid erythroid progenitor proliferation, generating RBCs faster than hemoglobin accumulation can proceed (Kim and Nemeth, 2015). Newly produced erythrocytes may enter circulation before hemoglobin formation is fully completed. Therefore, RBC recovery can occur faster than hemoglobin improvement during anemia recovery (Piskin et al., 2022). The roselle-treated group showed the highest hemoglobin increase, possibly because roselle enhances iron absorption.

This study has several limitations. The anemia model used was acute hemolytic anemia induced by $NaNO_2$, which differs from iron-deficiency anemia, the most common type of anemia worldwide (Camaschella, 2015). In addition, the extracts were not standardized for active compounds such as betalains and anthocyanins, which may affect the consistency. Since this study was conducted only in rats, further studies in humans are still needed to confirm safety and effectiveness. Future research should also evaluate standardized extracts and iron-deficiency anemia models.

4. CONCLUSION

Red beetroot and roselle extracts, both individually and in combination, effectively enhance hematological recovery in $NaNO_2$-induced anemic rats. Roselle extract exhibits superior hemoglobin-enhancing effects when used alone, whereas combined formulations—particularly the 2:1 ratio—provide more balanced improvement through synergistic interactions. These findings suggest potential application as phytopharmaceutical alternatives for anemia management, particularly in populations with limited pharmaceutical access, warranting further clinical investigation into standardized botanical formulations.

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