Medical Laboratory Technology Journal
11(2), 2025, 220-234
Received 2025-12-09; Revised 2025-07-10; Accepted 2025-07-11
Available online at : http://ejurnal-analiskesehatan.web.id

# Hematological Effects of *Vigna unguiculata* subsp. *cylindrica* in Anemic Mice: An Experimental Study

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DOI: 10.31964/mltj.v11i2.687

**Abstract:** Anemia is a global health problem that impairs cognitive function, physical performance, and maternal–child health. This study aimed to evaluate the hematological effects of *Vigna unguiculata* subsp. *cylindrica* (Nagara Bean) as a potential functional food in sodium nitrite ($NaNO_2$)-induced anemic mice. Thirty-three male BALB/c mice (8–12 weeks, 25–30 g) were randomly assigned into six groups: normal control (K), negative control (K−, anemia-induced), positive control (K+, iron-supplemented), and three treatment groups (P1, P2, P3) that received Nagara Bean-based feed for 1, 2, and 3 weeks, respectively. Hemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) levels were measured using Point-of-Care Testing (POCT) and manual cell counting. The results showed increases in Hb, Hct, and RBC across the treatment groups; however, statistical analysis indicated that these improvements were primarily associated with the duration of feed administration rather than treatment type. The greatest hematological improvement was observed after two weeks of feeding (P2). These findings indicate that Nagara Bean supplementation supports hematological recovery in anemic mice. The results suggest that *Vigna unguiculata* subsp. *cylindrica* has potential as a natural, affordable, and locally available source of bioavailable iron for anemia prevention and dietary intervention programs.
**Keywords:** Anemia*; Vigna unguiculata* subsp. *cylindrica*; hemoglobin; hematocrit; Nagara Bean.

## INTRODUCTION

Anemia remains a major global health concern that contributes to impaired cognitive and motor development in children, reduced work capacity among adults, and lower national economic productivity (Black et al., 2021; Marcus et al., 2021). Globally, anemia affects approximately half a billion women aged 15–49 years and 269 million children aged 6–59 months, with an overall prevalence of 30% (539 million) in non-pregnant women and 37% (32 million) in pregnant women. In Indonesia, the 2023 Survei Kesehatan Indonesia (SKI) reported anemia prevalence rates of 15.5% among adolescents aged 15–24 years, 18% among adolescent girls, and 27.8% among pregnant women (SKI, 2023). According to the World Health Organization (WHO) classification (2008), these figures fall within the moderate category (20–39%) (Augustin et al., 2015). Anemia during pregnancy increases the risk of preterm delivery, low birth weight, and depleted iron stores in newborns (OMS, 2023). The primary causes of anemia include iron deficiency due to inadequate dietary intake, impaired intestinal absorption, or excessive blood loss (Fertrin, 2020; Kumar et al., 2022; Montoro-Huguet et al., 2021).

Although synthetic iron supplementation remains the standard intervention, common side effects such as nausea and constipation, as well as issues of accessibility and cost, often reduce treatment compliance (Njapndounke et al., 2021).

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Consequently, natural iron-rich food sources have emerged as promising alternatives. One such source is *Vigna unguiculata* (cowpea), a legume widely cultivated in Asia and Africa and often referred to as the *"poor man's meat"* due to its high nutritional value and affordability (Sardar et al., 2024; Thangadurai, 2005). Its subspecies, *Vigna unguiculata* subsp*. cylindrica* (commonly known as Nagara Bean), is characterized by a high protein content (23–32%), resistant starch, dietary fiber, phytochemicals, and low fat (Mwangwela, 2008; Sardar et al., 2024).

*Vigna unguiculata* contains 71.43 mg/kg iron, 39.29 mg/kg zinc, and 6.53 mg/kg copper, which are essential for hemoglobin synthesis and iron metabolism (Gerrano et al., 2022). It is also rich in vitamin C, vitamin A (*β-carotene*), and folate (vitamin B9) (Imungi & Potter, 1983; Sodedji et al., 2022), which act synergistically to enhance non-heme iron absorption, mobilize iron from body stores, and support DNA synthesis and erythrocyte formation (Daniel Ikechukwu et al., 2024). Moreover, *Vigna unguiculata* exhibits anti-sickling activity by preventing erythrocyte deformation in sickle-cell anemia, primarily through its anthocyanin content (Egba et al., 2012; Mpiana et al., 2009). The presence of essential amino acids such as glycine (9.5%) and histidine (4.5%) also supports porphyrin biosynthesis, a key step in heme formation (Egba et al., 2011; Zaheer et al., 2020). Additionally, its natural antioxidants including flavonoids and polyphenols protect erythrocyte membranes from oxidative damage (Siddhuraju & Becker, 2007; Zia-Ul-Haq et al., 2013).

Iron in *Vigna unguiculata* serves as a crucial substrate for erythropoiesis (red blood cell formation). Following ingestion, non-heme iron is absorbed in the small intestine through Divalent Metal Transporter 1 (DMT1) and subsequently transported to the bone marrow for heme synthesis (Correnti et al., 2024). Adequate iron availability promotes the production of healthy erythrocytes, reflected in optimal hematocrit (Hct) levels (Daniel Ikechukwu et al., 2024). Furthermore, the protein content of *Virgina unguiculata* contributes to globin synthesis and regulates iron metabolism through the formation of transferrin and ferritin (Dewi & Sajiman, 2023).

Several in vivo studies have demonstrated the strong anti-anemic potential of the genus Vigna. Administration of composite flour from *V. unguiculata* in diabetic Wistar rats significantly increased hemoglobin (Hb) and hematocrit (Hct) levels, approaching normal values (Njapndounke et al., 2021). Similar blood-boosting effects were observed in anemic rats, where the iron and protein contents of *V. unguiculata* increased Red Blood Cell (RBC) counts and hemoglobin concentration, thereby improving hematocrit values (Daniel Ikechukwu et al., 2024). Likewise, supplementation with cowpea-based snacks in iron-deficiency anemic rats elevated Hb levels by 3.35 g/dL and significantly increased serum ferritin, confirming the efficacy of V. unguiculata in stimulating erythropoiesis (Dewi & Sajiman, 2023).

These findings highlight the potential of *Vigna unguiculata* subsp*. cylindrica* as a functional food ingredient with anti-anemic properties, providing essential iron and protein to support erythrocyte and hemoglobin synthesis. However, studies specifically evaluating the hematological effects of *Vigna unguiculata* subsp*. cylindrica* extract on parameters such as Hb and Hct remain limited. Therefore, the present study aimed to analyze the effects of *Vigna unguiculata* subsp*. cylindrica* on hemoglobin concentration and hematocrit levels in anemic mice models as an initial step toward assessing its therapeutic potential prior to clinical application in humans.

## MATERIALS AND METHODS

### Materıals

The study utilized Kacang Nagara (*Vigna unguiculata* subsp*. cylindrica*) obtained from Nagara District, Hulu Sungai Selatan Regency, South Kalimantan, Indonesia. Anemia was experimentally induced in animal models using Sodium Nitrite ($NaNO_2$). A total of 33 male BALB/c mice, aged 8–12 weeks and weighing 25–30 g, were used as experimental subjects. The Kacang Nagara pellet formulation was administered as the intervention diet, while Standard Pars Comfeed served as the control and basal diet containing 10% binder material. Hematological parameters were assessed using a Point-of-Care Testing (POCT) device, and Red Blood Cell (RBC) counts were verified manually using Hayem's solution according to standard hematological procedures.

### Research Design

This study employed an experimental post-test-only control group design to evaluate the efficacy of Kacang Nagara (*Vigna unguiculata* subsp*. cylindrica*) as a dietary intervention. A total of 33 male BALB/c mice (aged 8–12 weeks, weighing 25–30 g) were randomly assigned into five groups: Normal Control (K) (n = 5, non-anemic, fed a standard diet), Negative Control (K−) (n = 5, anemia-induced, fed a standard diet), Positive Control (K+) (n = 5, anemia-induced, fed a standard diet supplemented with iron), and three treatment groups (P1, P2, P3) (each n = 6, anemia-induced, fed a Kacang Nagara-based diet for 1 week, 2 weeks, and 3 weeks, respectively). The negative control group served as a reference to distinguish hematological recovery effects due to the dietary intervention from natural physiological recovery under anemic conditions.

### Nagara Bean Content Analysis

A laboratory-based analytical method was employed to determine the nutritional composition and iron (Fe) content of Nagara Bean (*Vigna unguiculata* subsp*. cylindrica*). Selected seeds were soaked in water for 6 hours, peeled, and oven-dried at 60°C for 8 hours. The dried seeds were then ground and sieved through an 80-mesh screen. The resulting dry powder was milled into a fine flour with an approximate particle size of 500 µm and stored in airtight containers at 9 ± 2°C until further analysis (Adekunle, 2014; Iswahyudi & Putri, 2022).

### Proximate Analysis

Proximate analysis was conducted to determine the contents of moisture, ash, protein, fat, crude fiber, and carbohydrates. Moisture content was measured using the oven-drying method at 105 °C for 3 hours until a constant weight was obtained (AACC 44-15A) (AACC, 2000). Ash content was determined following the AOAC 942.05 method by incineration at 550 °C for 12 hours. Protein content was analyzed using the Kjeldahl method (AOAC 984.13) by determining the total nitrogen content and multiplying it by a conversion factor of 6.25. Crude fat was analyzed using the Soxhlet extraction method (AOAC 920.39) with petroleum ether as the solvent for approximately 6 hours. Crude fiber was determined through sequential refluxing with 1.25% $H_2SO_4$ and 1.25% NaOH, followed by drying and ashing (AOAC 6865) (AOAC, 2007). Carbohydrate content was calculated by the by-difference method, subtracting the sum of moisture, ash, fat, and protein contents from 100% (Almatsier, 2001). All analyses were performed in duplicate, and results were expressed on a dry weight basis (% dw).

### Iron (Fe) Content Analysis

Iron content was determined using the wet digestion method, followed by measurement with an *Atomic Absorption Spectrophotometer* (AAS) according to

AOAC 985.01 (AOAC, 2007). Approximately 1 g of sample was placed in a Kjeldahl flask, mixed with 5 mL of concentrated $HNO_3$ and 1 mL of $HClO_4$, and left overnight for pre-digestion. The mixture was then gradually heated at 100–150 °C until the solution became clear, cooled, and diluted with distilled water to a final volume of 50 mL. The digested solution was filtered through Whatman No. 42 filter paper and analyzed using AAS at a wavelength of 248.3 nm. Calibration was performed using standard Fe solutions ranging from 0.5 to 5.0 mg/L (AOAC, 2007).

**Preparation of Nagara Bean Pellets**

The preparation of feed pellets began with the pre-processing of Nagara Beans (*Vigna unguiculata subsp. cylindrica*), which involved washing, brief soaking for 30 minutes, and boiling for 30 minutes to reduce antinutritional compounds and improve digestibility (Kapravelou et al., 2020). The boiled seed coats were removed, and the beans were ground into a fine paste. Subsequently, 450 g of ground Nagara Bean was mixed and homogenized with 50 g of standard Pars Comfeed powder to achieve a 90:10 ratio. The inclusion of 10% standard feed served not only as a binder but also to maintain nutritional consistency and caloric equivalence between the treatment and control groups, ensuring that observed hematological variations were attributable solely to the Nagara Bean intervention rather than differences in total energy intake (Lewis & Southern, 2000). The mixture was then shaped into pellets using a pellet press (3–5 mm diameter) and oven-dried at a controlled temperature of 60°C for 8 hours to reduce moisture content and ensure the physical stability of the pellets (Kapravelou et al., 2020).

**Animal Treatment**

A total of 33 male BALB/c mice (*Mus musculus*), aged 8–12 weeks and weighing 25–30 g, were used in this study. Prior to treatment, the animals underwent a one-week acclimatization period in individual cages under controlled environmental conditions (temperature 25°C, adequate ventilation, and a 12-hour light/dark cycle). Following acclimatization, all mice except those in the Normal Control (K) group were induced with anemia using Sodium Nitrite ($NaNO_2$), a hemolytic agent that oxidizes $Fe^{2+}$ to $Fe^{3+}$, forming methemoglobin (MetHb) incapable of binding oxygen, thereby inducing acute hemolytic anemia (Gluhcheva et al., 2012; Suparmi et al., 2025).
$NaNO_2$ was administered orally at a dose of 1.5 mg per mouse (50 mg/kg body weight) for 3–7 consecutive days, based on the established $LD_{50}$ value for experimental mice (Ambarwati, 2012). Successful anemia induction was confirmed through hemoglobin (Hb) and hematocrit (Hct) measurements using *Point-of-Care Testing* (POCT) from tail vein blood samples. Measurements were performed with an EasyTouch® GCHb analyzer (Taiwan), and mice were classified as anemic when Hb < 10 g/dL (Ambarwati, 2012).

After anemia was confirmed, the treatment groups (P1, P2, and P3) received Nagara Bean-based pellet feed at a dose of 20 g per day. The treatment duration differed among groups: P1 received the intervention for 1 week, P2 for 2 weeks, and P3 for 3 weeks. Hematological parameters (hemoglobin and hematocrit) were subsequently measured at the end of each treatment period (week 1, week 2, and week 3, respectively) to evaluate the efficacy and rate of hematological recovery as a function of intervention duration

**Hematological Testing Procedure**

At designated time points before induction, after anemia induction, and at the end of weeks 1, 2, and 3 of treatment mice were fasted for 4–6 hours to minimize biochemical variability. For terminal blood collection with sufficient sample volume, animals were anesthetized using ketamine (80–100 mg/kg body weight) administered

intraperitoneally (Silva-Santana et al., 2020). Blood was collected via cardiac puncture using a sterile syringe, and the whole blood was gently aspirated and transferred into K3EDTA coated tubes to prevent coagulation. Samples were immediately analyzed for hematological parameters.

Hemoglobin (Hb) and hematocrit (Hct) levels were measured promptly using a Point-of-Care Testing (POCT) device, *EasyTouch® GCHb* (Taiwan), which operates based on electrical potential change for Hb detection and conductivity based calculation for Hct (Riswari et al., 2022; Whitehead et al., 2019). Red blood cell (RBC) counts were determined manually: blood samples were diluted with Hayem's solution, loaded into a hemocytometer, and counted under a microscope. The results were then converted to express the total RBC count per unit volume of blood (Bolliger & Everds, 2012; Math et al., 2016).

**Ethical Approval**

Ethical clearance for this study was obtained from the Ethics Committee of the Banjarmasin Ministry of Health Polytechnic, Indonesia (Approval No. 100/KEPK-PKB/2025). All experimental procedures were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals.

## RESULTS AND DISCUSSION

### Proximate Composition and Iron Content of *Vigna unguiculata* subsp*. cylindrica*

Proximate analysis of *Vigna unguiculata* subsp*. cylindrica* (Nagara Bean) was conducted in triplicate for each parameter. The results indicated that Nagara Bean possesses a balanced nutritional composition, dominated by carbohydrates and protein, with a notably high iron (Fe) content. The mean values of the proximate composition and iron content are presented in Table 1.

Table 1. Proximate composition and iron (Fe) content of *Vigna unguiculata* subsp. *cylindrica* (Nagara Bean).

| Parameter | Average Value (per 100 g) |
|---|---|
| Iron (Fe) Content | 1.044 ppm |
| Protein Content | 26.59% |
| Carbohydrate Content | 57.73% |
| Fat Content | 3.67% |
| Moisture Content | 8.96% |
| Ash Content | 3.05% |

The protein content (26.59%), carbohydrate content (57.73%), fat (3.67%), ash (3.05%), moisture (8.96%), and iron (Fe) concentration (1.044 ppm) indicate that *Vigna unguiculata* subsp*. cylindrica* (Nagara Bean) possesses a high potential as a plant-based iron source capable of supporting hemoglobin synthesis and enhancing the erythropoiesis process.

### Hematological Profile Improvement After Nagara Bean Treatment

Evaluation of hemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) counts was performed to assess the hematological effects of diets supplemented with *Vigna unguiculata* subsp. *cylindrica* (Nagara Bean). The mean hematological values for the treatment groups are presented in Table 2, while those for the control groups are shown in Table 3.

Tabel 2. Hematological Profile Improvement in Anemic Mice Fed with Nagara Bean Diet for Different Durations.

| Group (n) | Parameter | Mean Week 0 | Mean Final Week | Percentage Change (%) | Mean RBC |
|---|---|---|---|---|---|
| P1 (n=6) (1 Week) | Hemoglobin (g/dL) | 9.98 | 11.43 (Week 1) | +14.53% | 5.705.000 /uL |
| | Hematocrit (%) | 31.8 | 36.7 (Week 1) | +15.41% | (Week 1) |
| P2 (n=6) (2 Weeks) | Hemoglobin (g/dL) | 9.92 | 12.63 (Week 2) | +27.32% | 6.612.500 /uL |
| | Hematocrit (%) | 31.7 | 38.8 (Week 2) | +22.40% | (Week 2) |
| P3 (n=6) (3 Weeks) | Hemoglobin (g/dL) | 9.92 | 12.23 (Week 3) | +23.29% | 7.075.000 /uL |
| | Hematocrit (%) | 31.7 | 38.0 (Week 3) | +19.87% | (Week 3) |

Hemoglobin and hematocrit levels increased across all treatment groups following the administration of Negara beans. P1 showed rises of 14.53% (Hb) and 15.41% (Hct), while P2 exhibited the greatest improvements, with Hb increasing by 27.32% and Hct by 22.40%. In P3, Hb and Hct increased by 23.29% and 19.87%, respectively. Correspondingly, the final RBC counts were 5.71 × $10^6$/µL in P1, 6.61 × $10^6$/µL in P2, and 7.08 × $10^6$/µL in P3 (Table 2)

Table 3. Hematological Profile of Control Groups

| Group (n) | Parameter | Wk 0 | Wk 1 | % Change (Wk 1 vs 0) | Wk 2 | % Change (Wk 2 vs 1) | Wk 3 | % Change (Wk 3 vs 2) | Mean RBC (Wk 3) |
|---|---|---|---|---|---|---|---|---|---|
| Normal Control (K)(n=5) | Hb (g/dL) | 11.2 | 11.44 | +2.14% | 11.56 | +1.05% | 11.02 | -4.67% | 6.775.000/uL |
| | Hct (%) | 35.8 | 36.4 | +1.68% | 38 | +4.39% | 34.25 | -9.87% | |
| Negative Control (K-)(n=5) | Hb (g/dL) | 7.86 | 9.68 | +23.16% | 9.64 | -0.41% | 11.42 | +18.46% | 6.431.000/uL |
| | Hct (%) | 25.6 | 31.6 | +23.44% | 30.8 | -2.53% | 34 | +10.39% | |
| Positive Control (K+)(n=5) | Hb (g/dL) | 8.94 | 11.7 | +30.87% | 11.77 | +0.60% | 11.86 | +0.76% | 6.808.000/uL |
| | Hct (%) | 27.6 | 37 | +34.06% | 37 | +0.00% | 37.4 | +1.08% | |

Hemoglobin levels in the normal control group (K) remained relatively stable, ranging from 11.20 to 11.56 g/dL before slightly declining in Week 3. In contrast, the anemic control group ($K^-$) showed substantial improvement, with Hb increasing from 7.86 to 11.42 g/dL by Week 3. The Fe-supplemented control ($K^+$) also demonstrated a marked rise, from 8.94 to 11.86 g/dL. Similar patterns were observed for hematocrit: minimal fluctuation in K, notable increases in $K^-$ and $K^+$, and final RBC counts of 6.78 × $10^6$/µL (K), 6.43 × $10^6$/µL ($K^-$), and 6.81 × $10^6$/µL ($K^+$) (Table 3).

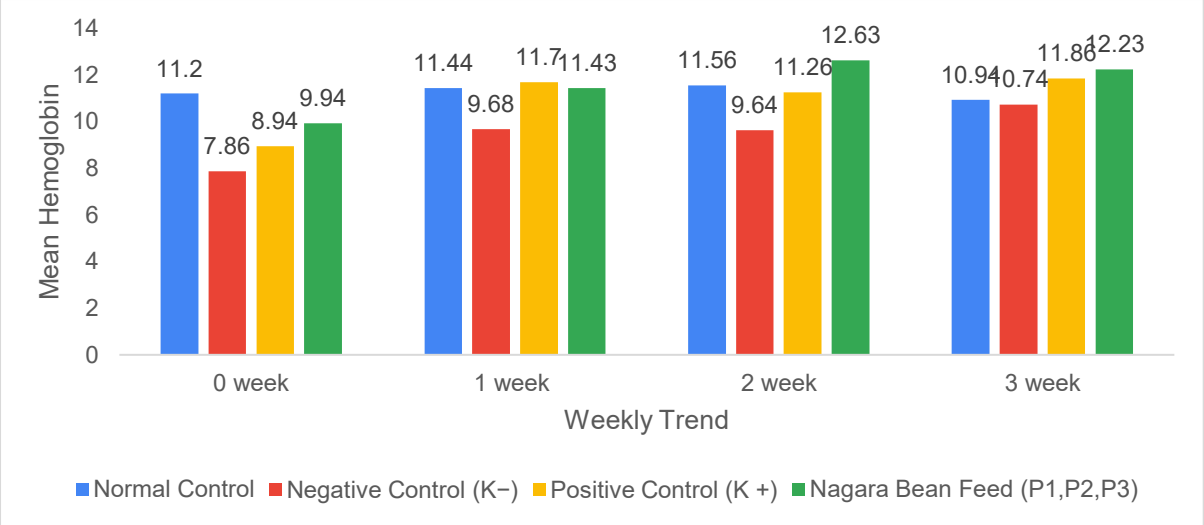


Figure 1. Weekly Trend of Mean Hemoglobin (Hb) Levels Across Experimental Groups

The weekly trend shows distinct patterns across groups. The normal control (K) maintained stable hemoglobin levels from Week 0 to Week 2, with a slight decline at Week 3. The negative control ($K^-$) exhibited a steady increase from 7.86 g/dL to 10.94 g/dL by Week 3. The positive control ($K^+$) showed a marked rise from 8.94 g/dL to 11.86 g/dL, maintaining consistently higher values after Week 1. Notably, the groups receiving Nagara bean feed (P1–P3) demonstrated progressive improvement, with hemoglobin increasing from 9.94 g/dL at baseline to 12.23 g/dL at Week 3, indicating a strong hematinic effect (Figure 1).

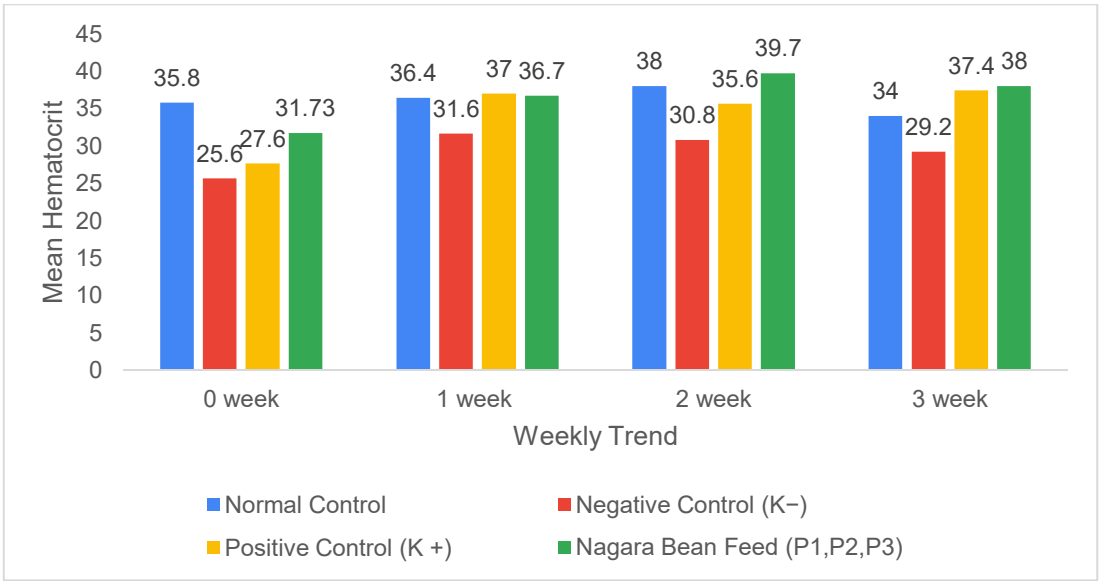


Figure 2. Weekly Trend of Mean Hematocrit (Hct) Levels Across Experimental Groups

Hematocrit levels in the normal control group (K) remained relatively stable from Week 0 to Week 2, followed by a slight decline in Week 3. The negative control group ($K^-$) showed a progressive increase from 25.6% to 31.6% in Week 1, rising further to 30.8% and 29.2% in subsequent weeks. The positive control group ($K^+$) exhibited consistent improvement, increasing from 27.6% at baseline to 39.7% in Week 2 and remaining elevated at Week 3. Meanwhile, the Nagara bean feed groups (P1–P3)

demonstrated steady hematocrit enhancement, rising from 31.7% at baseline to 38% by Week 3, indicating a clear hematinic effect (Figure 2).

**Statistical Analysis**

Table 4. Results of Linear Regression and ANOVA for Hemoglobin (Hb) and Hematocrit (Hct) Levels

| Test Statistics | Hemoglobin (Hb) | Hematocrit (Hct) |
|---|---|---|
| *Model Summary* (R2) | 0.141 | 0.129 |
| ANOVA (Sig) | 0.013 | 0.020 |
| *Coefficients* (Sig) (Treatment) | 0.492 | 0.447 |
| *Coefficients* (Sig) - (Week) | 0.004 | 0.007 |

The regression analysis showed that both hemoglobin (Hb) and hematocrit (Hct) models were statistically significant, indicating that the variables included in the analysis had a meaningful overall effect. However, only the duration of feeding had a significant impact on Hb ($p = 0.004$) and Hct ($p = 0.007$), while the type of treatment did not show a significant difference. The $R^2$ values—0.141 for Hb and 0.129 for Hct—indicate that the model explained a modest proportion of the variation in these hematological parameters. Overall, the results suggest that improvements in Hb and Hct were primarily driven by the length of the feeding period rather than the treatment type (Table 4).

The anemia model induced by Sodium Nitrite ($NaNO_2$) resulted in a significant decrease in hemoglobin (Hb) and hematocrit (Hct) levels in the anemic control group (Table 3). This finding is consistent with Gluhcheva et al. (2012), who reported that $NaNO_2$ acts as a hemolytic agent by oxidizing ferrous iron ($Fe^{2+}$) to ferric iron ($Fe^{3+}$), forming methemoglobin (MetHb)—a non-functional form of hemoglobin incapable of binding oxygen. The accumulation of MetHb reduces oxygen transport capacity and induces acute hemolytic anemia, characterized by decreased Hb and Hct levels and increased oxidative stress in erythrocytes. This condition accelerates hemolysis by damaging red blood cell membranes and impairing the activity of antioxidant enzymes such as catalase and superoxide dismutase (Albano et al., 2024; Ansari et al., 2015).

Proximate analysis (Table 1) revealed that Nagara Bean (*Vigna unguiculata* subsp*. cylindrica*) is a nutrient-dense food source, with high protein content (26.59%) and iron concentration (1.044 ppm), suggesting its strong potential as a dietary intervention for anemia. The significant increases in Hb and Hct levels among treatment groups confirm the efficacy of *Vigna unguiculata* subsp*. cylindrica* as a functional food in anemia recovery. Its composition, rich in non-heme iron, zinc, copper, protein, vitamin C, folate, and *β-carotene,* supports erythropoiesis through synergistic biochemical mechanisms (Thangadurai, 2005; Zaheer et al., 2020).

Non-heme iron is absorbed in the small intestine via *Divalent Metal Transporter 1* (DMT1) and transported to the bone marrow for heme synthesis, while the amino acids glycine and histidine act as precursors for porphyrin, the core component of hemoglobin (Chiabrando et al., 2014; Ginzburg et al., 2023). Vitamin C facilitates the reduction of $Fe^{3+}$ to $Fe^{2+}$, enhancing iron bioavailability (Lynch & Cook, 1980), whereas folate and vitamin A promote erythroid cell differentiation (Silvestri & Nai, 2021). The combined effect of these nutrients increased Hb by 27.32% and Hct by 22.40% after

two weeks of treatment (P2), with the RBC count reaching 7.07 × $10^6$/µL in the third week.

Vitamin C in *V. unguiculata* functions as an enhancer of non-heme iron absorption by reducing $Fe^{3+}$ to $Fe^{2+}$ and forming soluble complexes under the duodenal alkaline pH, thereby improving bioavailability in a dose-dependent manner. Folate and β-carotene contribute to DNA synthesis and erythroid proliferation, while anthocyanins and flavonoids serve as potent antioxidants protecting erythrocyte membranes from lipid peroxidation and cell deformation (An et al., 2016; Bonarska-Kujawa et al., 2012). This micronutrient synergy exerts stronger anti-anemic effects than individual nutrients alone (Imran Hussain et al., 2024; Imungi & Potter, 1983; Sodedji et al., 2022).

The differences in hematological recovery patterns among groups indicate distinct phases of erythropoiesis dynamics (Table 2). The P1 group (1 week) showed a 14.53% increase in Hb (from 9.98 to 11.43 g/dL) and a 15.41% rise in Hct (from 31.8% to 36.7%), with an RBC count of 5.705 × $10^6$/µL. This reflects early bone marrow activation within 36–48 hours, marked by increased reticulocyte counts, although one week was insufficient for full iron store restoration (Chiabrando et al., 2014; Hematology, 2016). The P2 group (2 weeks) exhibited the greatest improvement—Hb increased by 27.32% (from 9.92 to 12.63 g/dL) and Hct by 22.40% (from 31.7% to 38.8%)—indicating an optimal balance between active erythropoiesis and iron accumulation (Halterman & Segel, 2023; Jimenez et al., 2015). The P3 group (3 weeks) showed a 23.29% Hb increase (from 9.92 to 12.23 g/dL) and a 19.87% Hct rise (from 31.7% to 38.0%), with the highest RBC count of 7.075 × $10^6$/µL. The slight decrease in Hb and Hct increments compared to P2 suggests a plateau phase of erythropoiesis, during which hemoglobin synthesis slows as Hb levels approach normal, while absorbed iron begins to be stored as ferritin and hemosiderin (Kiss et al., 2015; Cogan et al., 2024; Correnti et al., 2022).

The positive control (Fe) group showed Hb and Hct increases comparable to those of the treatment groups, but at a slower rate in the second week, indicating that the natural iron bioavailability of Nagara Bean can rival that of inorganic Fe supplements. Moreover, its protein and antioxidant components may minimize gastrointestinal side effects and maintain erythrocyte stability (Ansari et al., 2015). In contrast, the negative control (K−) group, which received no treatment, showed a 36.64% increase in Hb by week three, likely due to spontaneous physiological recovery after cessation of $NaNO_2$ exposure (Gluhcheva et al., 2012). However, this recovery was partial and less stable than in the treatment groups.

Regression analysis yielded $R^2$ values of 0.141 for Hb and 0.129 for Hct, indicating that only part of the hematological variation was explained by treatment type and duration. These values remain meaningful in biomedical contexts, given the complex nature of physiological systems (Chicco et al., 2021). This finding aligns with Gupta et al. (2024), who reported that most medical studies exhibit $R^2$ values below 25%, including those on anemia, stroke, and sepsis. Thus, $R^2$ values in the 10–20% range are considered clinically valid, as statistical significance is more influenced by p-values and effect sizes than by the absolute $R^2$ magnitude. ANOVA results ($p < 0.05$) confirmed that the combination of treatment type and duration significantly affected Hb and Hct levels. Treatment duration had a significant effect on Hb ($p = 0.004$) and Hct ($p = 0.007$), showing a time-dependent relationship, whereas treatment type did not differ significantly ($p > 0.05$), likely due to biological variability and limited sample size (Anand et al., 2005; Andrade, 2019; Jachno et al., 2022).

Overall, these findings are consistent with previous *in vivo* studies demonstrating the anti-anemic potential of the genus *Vigna*. Supplementation with *V. unguiculata* flour increased Hb and Hct levels toward normal in diabetic Wistar rats (Njapndounke et al., 2021) and improved erythrocyte counts and hemoglobin levels in anemic rats (Daniel Ikechukwu et al., 2024). Furthermore, cowpea-based snack formulations increased Hb by 3.35 g/dL and significantly elevated ferritin concentrations, confirming their ability to stimulate erythropoiesis (Dewi & Sajiman, 2023). Collectively, these results indicate that *Vigna unguiculata* subsp*. cylindrica* is a promising source of bioavailable plant-based iron and natural antioxidants, effectively accelerating anemia recovery within 2–3 weeks, with outcomes comparable to or more stable than Fe supplementation.

This study was limited by a small sample size and a short intervention period. Further research should evaluate iron storage biomarkers (ferritin, transferrin receptor, and transferrin saturation), assess the bioavailability of iron from Nagara Bean compared with standard iron supplements using a crossover design, and analyze the bioactive compound profile (anthocyanins, flavonoids, vitamin C, and folate) in relation to hematological responses.

## CONCLUSION

Nagara Bean (*Vigna unguiculata* subsp. *cylindrica*) supplementation improved hemoglobin, hematocrit, and red blood cell counts in sodium nitrite-induced anemic mice. The optimal improvement was observed after two weeks of intervention, indicating a time-dependent hematological response. Based on these findings, Nagara Bean can be recommended as a potential plant-based source of bioavailable iron and an alternative to synthetic iron supplements. Further research is needed to evaluate its effectiveness in human subjects, identify the bioactive compounds responsible for its activity, and explore its incorporation into functional food formulations for community-based anemia prevention programs.

## ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Poltekkes Kemenkes Banjarmasin and the Faculty of Mathematics and Natural Sciences (FMIPA) Laboratory at Lambung Mangkurat University, Banjarmasin, for providing facilities, technical assistance, and institutional support that made this research possible.

## FUNDING

This study was financially supported by Poltekkes Kemenkes Banjarmasin, Indonesia.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the conduct of this research or the publication of its findings.

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