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HEMATOPOIETIC EFFECTS OF ETHANOLIC EXTRACT FROM *Alternanthera sissoo* ON ERYTHROCYTE, HEMOGLOBIN, AND HEMATOCRIT LEVELS IN ANEMIA-INDUCED FEMALE WISTAR RATS

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Abstract

Background: Maternal anemia remains a primary global health concern due to its association with adverse pregnancy outcomes and the increased risk of iron deficiency anemia in infants, which may impair neurodevelopment and growth. Although iron supplementation is the standard therapy, its gastrointestinal side effects often limit treatment adherence. Therefore, Brazilian Spinach (*Alternanthera sissoo*), a plant rich in iron, flavonoids, and antioxidants, offers potential as a natural alternative.

Objective: This study aimed to evaluate the hematopoietic effects of the ethanolic extract of *Alternanthera sissoo* on erythrocyte count, hemoglobin concentration, and hematocrit levels in female Wistar rats (*Rattus norvegicus*) induced with anemia.

Methods: A quasi-experimental study using a completely randomized design (CRD) was conducted with three treatment doses (5, 7.5, and 10 mg/kg body weight). Hematological parameters (Erythrocyte count, Hemoglobin (Hb), and Hematocrit (HCT)) were analyzed using a Hematology Analyzer, followed by statistical testing with one-way ANOVA and Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results: The 10 mg/kg BW treatment produced the most significant improvement in erythrocyte count, Hb, and HCT levels compared to the other groups.

Conclusions: Ethanolic extract of *Alternanthera sissoo* effectively enhances hematological indices in iron-deficient rats, showing strong potential as a natural nutraceutical supplement for preventing anemia during pregnancy.

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INTRODUCTION

Anemia is a condition characterized by a reduced number of red blood cells or a decreased oxygen-carrying capacity of these cells, insufficient to meet physiological needs. During pregnancy, anemia is defined as hemoglobin (Hb) levels below 11 g/dL in the first and third trimesters, and below 10.5 g/dL in the second trimester (1). Approximately 40% of maternal deaths in developing countries are associated with anemia during pregnancy, primarily caused by iron deficiency (2). In Bali Province, Indonesia, the prevalence of anemia among pregnant women reached 7.4% (5,305 cases) in 2020 and accounted for 23% of obstetric complications in 2022, making it one of the leading maternal health problems in the region (3,4). Pregnant women are more susceptible to iron deficiency anemia due to the physiological expansion of blood volume, which increases iron demand (5). Inadequate iron storage leads to anemia, which may result in miscarriage, preterm birth, and low birth weight infants (6–8). The diagnostic indicators of iron deficiency anemia include total erythrocyte count, hematocrit (HCT), and hemoglobin (Hb) concentration. Erythrocytes transport hemoglobin that carries oxygen; their normal count in women ranges from 3.9–5.6 million/mm^3, while normal Hb levels are <12 g/dL in women and <11 g/dL in pregnant women. Hematocrit values (37–43%) are also used to determine red cell volume and to identify anemia (9). Anemia management generally involves the consumption of at least 90 iron tablets during pregnancy (10). However, iron supplementation often causes gastrointestinal discomfort, nausea, vomiting, and constipation, which can reduce adherence. Hence, natural-based therapies are increasingly explored as alternative interventions (11).

Brazilian spinach (*Alternanthera sissoo*), used in traditional medicine and as a leafy vegetable. Phytochemical screens of *A. sissoo* leaf extracts report flavonoids, tannins, alkaloids, saponins and tocopherol (vitamin E), and in vitro antioxidant assays (e.g., DPPH/FRAP) have shown notable radical-scavenging activity. Separately, preclinical studies of *Alternanthera* spp. indicate hematinic effects (increased hemoglobin/serum ferritin) in rodent models. These data together motivate evaluation of *A. sissoo* as a candidate hematopoietic/antioxidant intervention in anemia models (12). Flavonoids protect erythrocytes from oxidative damage and can enhance hematological parameters, especially in disease or stress conditions (13). Previous studies reported that flavonoid-rich extracts, such as Beet leaf extract, significantly restored the levels of red blood cells, white blood cells, hemoglobin, and hematocrit in dose- and time-dependent manners (14). Moreover, spinach species are known for their relatively high iron content, comparable to that found in *Syzygium polyanthum* (bay leaf), which also demonstrated hematopoietic effects in similar studies (15). While previous literature has shown that A. sissoo extract contains secondary metabolites and that the extract has antioxidant activity and immunomodulatory effects in animals, no studies have systematically determined the association of A. sissoo extract with anemia recovery through mechanisms of hematopoiesis, iron regulation, or iron absorption. Therefore, this study aims to evaluate the hematopoietic effects of ethanolic extract of Brazilian Spinach (*Alternanthera sissoo*) on erythrocyte count, hemoglobin concentration, and hematocrit levels in female Wistar rats (*Rattus norvegicus*) with iron deficiency anemia induced by aluminum sulfate. The research is expected to provide scientific evidence supporting the potential of Brazilian Spinach extract as a phytopharmaceutical candidate or natural nutraceutical supplement for preventing iron deficiency anemia during pregnancy.

MATERIALS AND METHODS

This study was a true experimental design with pre-post test and one factor, namely the administration of *Alternanthera sissoo* extract, consisting of three dosage variations and two control groups. The research was conducted from June to September 2025. Extraction of *A. sissoo* was performed at the UPTD P4TO Bali Province. Animal maintenance and treatment were conducted at the Research Laboratory, Faculty of Medicine and Health Sciences, Warmadewa University. At the same time, hematological examinations were carried out at the Hematology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Denpasar. All procedures involving experimental animals were conducted in accordance with the ethical standards for the care and use of laboratory animals. Ethical approval for this study was granted by the Health Research Ethics Committee of Poltekkes Kemenkes Denpasar, Indonesia, under certificate number DP.04.02/F.XXIV.25/729/2025.

Fresh *A. sissoo* leaves were collected from Gumi Farm Plantation, Baturiti-Bedugul, Bali. The leaves were sorted, washed, and dried in an oven at a temperature below 50°C to produce dried simplicia. The dried sampleswere powdered and macerated in 70% ethanol for seven days. The filtrate was separated and concentrated using a rotary evaporator at 50°C to obtain a thick extract. An organoleptic evaluation (comprising color, odor, and taste) was conducted to ensure the quality of the extract. The sample size used in this study was determined using Federer's formula, which is widely applied in controlled laboratory animal experiments to ensure adequate error degrees of freedom for subsequent statistical analysis. According to this approach, the minimum number of animals required per group is calculated using the expression $(t-1)(n-1) \geq 15$, where t represents the number of treatment groups and n denotes the number of animals per group. With five experimental groups included in this study, the formula yields a minimum requirement of approximately five animals per group to meet the threshold for valid inferential testing. This method provides an appropriate balance between resource efficiency and statistical robustness, and is consistent with established recommendations for animal research design. Therefore, a total of 25 healthy female Wistar rats (*Rattus norvegicus* L.) were allocated across the treatment groups, aged seven weeks and weighing 150–200 g, were used in this study. The animals were selected using purposive sampling and acclimatized before treatment. All rats were induced with aluminum sulfate (Merck®) (67.5 mg/kg body weight, intramuscularly) for seven days to produce iron deficiency anemia. This is a regimen supported by previous toxicology studies demonstrating the hematotoxic effects of aluminum exposure. Although aluminum sulfate is not a universal standard for anemia induction, its use is theoretically justified, as aluminum competitively inhibits iron absorption, interferes with transferrin-mediated iron transport, and suppresses erythropoietic activity through impaired heme synthesis and increased oxidative stress. Previous experimental studies have shown that aluminum salts administered at doses between 60 and 80 mg/kg BW consistently produce microcytic, hypochromic anemia in rodents within one to two weeks, thus providing a reproducible model for evaluating hematological interventions. Therefore, the doses and durations selected in this study are consistent with a mechanistically relevant preclinical model for evaluating the therapeutic effects of A. sissoo extract on iron-related hematological parameters. The animals were then divided into five groups (n=5): K1=Negative control (no treatment); K2=Positive control (iron tablet 5.4 mg); P1=A. sissoo extract 5 mg/kg BW; P2=A. sissoo extract 7.5 mg/kg BW; P3=A. *sissoo* extract 10 mg/kg

BW. The selection of the three extract doses (5, 7.5, and 10 mg/kg BW) was determined based on evidence from previously published studies combined with dose-escalation principles commonly applied in preclinical pharmacological research. Prior investigations on A. sissoo and related phytotherapeutic agents have reported biological activity within a comparable low-to-moderate dose range, indicating that these concentrations are sufficient to elicit hematological and antioxidant responses without inducing toxic manifestations. In addition, the dose interval was structured to follow a graded, proportional increment (approximately 1.5-fold increase per level), which aligns with standard preclinical dose-response design aimed at assessing both efficacy and the potential threshold of pharmacological effect. Where specific dose–response data are limited, such dosing schemes are typically refined through preliminary observations from pilot experiments or derived from analogous plant extracts with similar bioactive profiles. Thus, the three doses employed in this study represent a theoretically justified range intended to capture the lower, intermediate, and upper bounds of anticipated therapeutic activity while maintaining animal safety and experimental feasibility.

Treatments were administered orally for 21 consecutive days. The 21-day observation period was selected based on established principles of sub-acute toxicity and pharmacodynamic evaluation in rodent models. According to OECD guidelines and widely accepted laboratory animal protocols, a 21- to 28-day duration is considered sufficient to allow repeated oral administration of a test compound to exert measurable physiological, hematological, and metabolic effects without entering the prolonged adaptive phase typically seen in chronic exposure studies. For hematopoietic endpoints in particular, rats require approximately 10–14 days to complete a full erythropoietic cycle; therefore, a 21-day duration enables the assessment of cumulative treatment effects across at least one complete cycle of red blood cell turnover. This period is also commonly adopted in phytopharmacology studies evaluating plant extracts to ensure adequate bioactive compound accumulation and stabilization of systemic responses, while minimizing confounding deterioration in animal health. Thus, the 21-day treatment period provides a scientifically justified timeframe to capture the sub-acute physiological impact of the interventions and to generate reproducible and biologically relevant hematological outcomes. On day 22, rats were euthanized, and blood samples (5 mL) were collected via the orbital sinus . Sampling was conducted on day 1 (before anemia induction), day 8 (after induction), and day 22 (after treatment), producing a total of 75 samples. Blood samples were analyzed for total erythrocyte count ($\times 10^6/\mu L$), hematocrit (Ht, %), and hemoglobin (Hb, g/dL) using an Auto Hematology Analyzer (Sysmex®). The study employed a Completely Randomized Design (CRD) with five treatment groups and five replications per group. Data were analyzed using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level to determine the most effective extract dosage in improving total erythrocyte count, hematocrit, and hemoglobin levels in anemic Wistar rats.

RESULTS AND DISCUSSION

The results of the simplicia moisture content test on Alternanthera sissoo (Brazilian spinach) leaves showed a value of 4.76%, indicating that the simplicia met the quality standard for dried raw materials, specifically a moisture content of less than 10%, as per BPOM Regulation No. 32 of 2019. Low moisture content is a key indicator of good simplicia

quality, as it minimizes microbial growth, enzymatic degradation, and oxidation that could damage bioactive components (16). The obtained value demonstrates that the drying process was optimal, reducing both free and bound water without damaging thermolabile compounds. A moisture content below 5% also indicates high stability during storage, ensuring the material remains suitable for extraction and pharmaceutical use.

The extract yield obtained using 70% ethanol as solvent was 7.075%, which falls within the ideal range for ethanol extracts of herbal leaves (5–10%). Extraction yield reflects the efficiency of solvent penetration and the solubility of phytochemical compounds in the solvent. Extraction yield is a direct indicator of how successfully bioactive compounds are isolated from plant materials. High yields of flavonoids, alkaloids, saponins, tannins, and polyphenols are consistently reported when extraction parameters (such as solvent type, concentration, temperature, and extraction method) are carefully optimized (17). A yield that is not excessively high also implies that unwanted non-polar substances, such as waxes or resins, were minimally co-extracted, confirming the selectivity of 70% ethanol as a polar solvent.

Table 1. Results of Characteristic Test of Brazilian Spinach Extract

Extract Yield (%)	Taste	Odor	Color
7,075	Bitter	Characteristic aroma	Dark green

The organoleptic evaluation of the thick extract revealed a bitter taste, a characteristic aroma, and a dark green color. The bitter taste corresponds to the presence of flavonoids and alkaloids, while the characteristic aroma indicates that volatile compounds were well preserved during solvent evaporation. The dark green coloration suggests that the successful extraction of chlorophyll and other polar pigments occurred, indicating that the extraction temperature and solvent polarity were appropriate and did not induce pigment degradation (18). Collectively, these characteristics confirm that the extract possesses acceptable physicochemical quality for further biological testing.

Table 2. Results of Total Erythrocyte Levels, Hematocrit Levels, and Hemoglobin Tests

Hematology Summary by Treatment Group

Values are Mean ± SD (n = 5 per group)

	Day 1 (Post-acclimatization)			Day 8 (Pre-treatment)			Day 22 (Post-treatment)		
Group	RBC ($\times 10^6$/µL)	Hb (g/dL)	HCT (%)	RBC ($\times 10^6$/µL)	Hb (g/dL)	HCT (%)	RBC ($\times 10^6$/µL)	Hb (g/dL)	HCT (%)
KN	6.15 ± 0.35	12.16 ± 0.88	36.20 ± 2.29	5.35 ± 0.19	10.84 ± 0.68	32.00 ± 0.93	5.85 ± 0.16	11.12 ± 0.71	33.52 ± 0.84
KP	6.74 ± 0.71	12.74 ± 0.57	38.20 ± 2.21	5.75 ± 0.30	10.44 ± 0.65	31.00 ± 1.09	7.37 ± 0.46	12.38 ± 0.47	34.80 ± 0.75
P1	6.69 ± 0.49	13.02 ± 0.99	38.00 ± 2.67	5.66 ± 0.38	10.70 ± 0.46	31.80 ± 1.83	6.77 ± 0.38	11.30 ± 0.45	34.90 ± 1.84
P2	6.65 ± 0.32	12.78 ± 0.48	38.28 ± 1.81	5.42 ± 0.17	10.72 ± 0.43	32.56 ± 0.67	7.11 ± 0.12	12.52 ± 0.39	37.36 ± 0.67
P3	6.44 ± 0.21	12.32 ± 0.73	36.24 ± 1.33	5.72 ± 0.20	10.90 ± 0.44	31.22 ± 1.19	8.02 ± 0.23	13.52 ± 0.38	37.92 ± 0.99

Abbreviations: RBC = red blood cell count; Hb = hemoglobin; HCT = hematocrit; SD = standard deviation.
Groups: KN = negative control; KP = positive control; P1–P3 = treatment groups.

Observation of hematological parameters was conducted at three time points: post-acclimatization (day 1), pre-treatment (day 8), and post-treatment (day 22). At baseline, all test animals exhibited normal erythrocyte counts (6–9 × 10^6/µL), hemoglobin (12–16 g/dL), and hematocrit (36–48%), indicating a healthy physiological status before induction (19). After induction using aluminum sulfate ($Al_2(SO_4)_3$), all treatment groups experienced a marked decline in erythrocyte, Hb, and HCT levels, consistent with the mechanism of aluminum toxicity that disrupts iron metabolism and erythropoiesis (20).

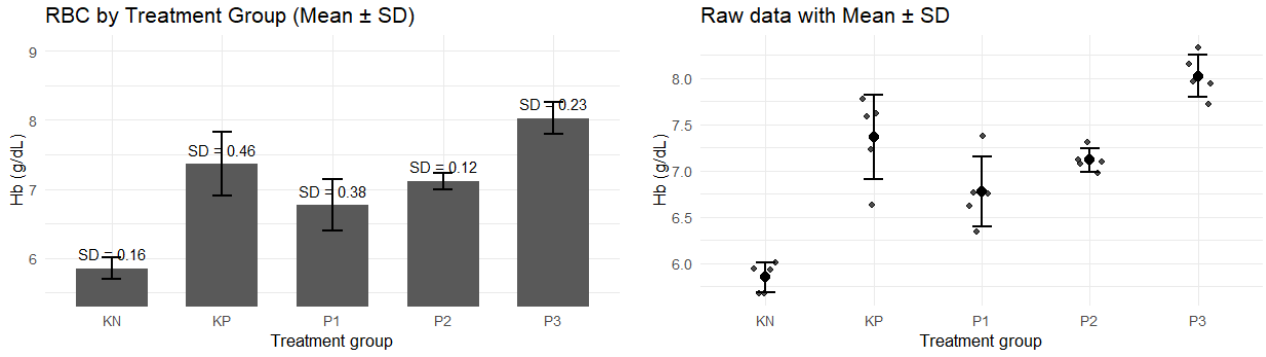

Figure 1. Post-Treatment Total Erythrocyte Levels

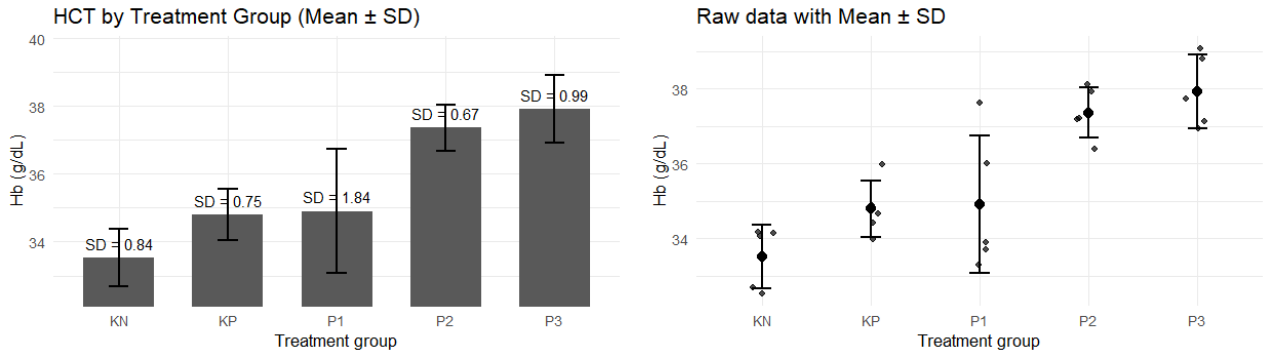

Figure 2. Post-Treatment Hematocrit Levels

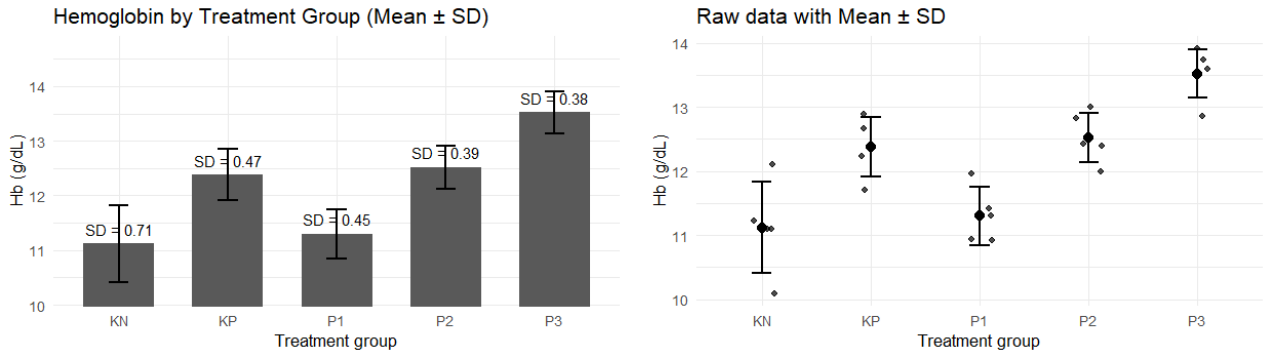

Figure 3. Post-Treatment Hemoglobin Levels

Following administration of *Alternanthera sissoo* ethanol extract, there was a dose-dependent increase in erythrocyte, HCT, and Hb levels, with the most pronounced effect observed in group P3 (10 mg/kg BW). This increase reflects the hematopoietic potential of the extract, likely due to its rich composition of flavonoids, alkaloids, saponins, and vitamin E. These compounds are known to stimulate erythropoiesis, enhance iron absorption, and protect red blood cells from oxidative damage (21,22)

The Kolmogorov–Smirnov normality test yielded *p-values greater than 0.05 for all groups, confirming that the* data were normally distributed. Therefore, analysis proceeded using One-Way ANOVA, which revealed significant differences among treatment groups (p = 0.000 < 0.05) in all hematological parameters (erythrocyte count, Hb, and HCT). Subsequent Duncan's Multiple Range Test (DMRT) identified that group P3 (10 mg/kg BW) exhibited the highest mean erythrocyte count (8.022 × 10^6/µL), hemoglobin concentration (13.52 g/dL), and hematocrit level (37.92%). These values formed separate subsets in the post-hoc analysis, indicating statistically significant differences compared to other treatments. In contrast, P1 (the lowest dose) consistently showed the lowest hematological parameters. At the same time, the P2 and control groups shared similar subset groupings,

indicating a moderate improvement, although not statistically significant, compared to the control.

These results demonstrate a dose-dependent effect, where the 10 mg/kg BW dose achieved optimal hematopoietic stimulation. The presence of bioactive compounds, particularly flavonoids and vitamin E, appears to enhance erythropoiesis by protecting erythrocyte membranes from oxidative stress and improving iron utilization in hemoglobin synthesis (23). Alkaloids and triterpenoids may further contribute through modulation of erythropoietin activity in bone marrow, supporting increased red blood cell production (24). The significant hematological improvements observed in the P3 group confirm the hematopoietic and antioxidant effects of *Alternanthera sissoo* ethanol extract. The synergistic action of flavonoids, saponins, and vitamin E likely contributes to restoring erythrocyte numbers and hemoglobin levels by mitigating oxidative damage caused by aluminum-induced anemia. Flavonoids function as free-radical scavengers, preventing erythrocyte membrane lysis and promoting iron absorption, while vitamin E acts as a lipophilic antioxidant stabilizing cell membranes. Saponins and tannins provide anti-inflammatory support, maintaining normal erythropoietic microenvironments, and alkaloids enhance hepatic iron storage through ferritin metabolism. Collectively, these effects contribute to the normalization of hematological profiles, as shown by significantly higher values in erythrocyte, Hb, and HCT parameters post-treatment. The findings are consistent with prior studies reporting the hematopoietic effects of *Alternanthera* species, where ethanolic extracts stimulated erythrocyte regeneration and increased hemoglobin concentration in anemia-induced models(19,22). Thus, *Alternanthera sissoo* extract demonstrates strong potential as a natural hematopoietic agent in correcting anemia through both antioxidant protection and erythropoietin stimulation mechanisms.

CLINICAL IMPLICATION

Iron deficiency anemia (IDA) remains a major maternal health issue, contributing to adverse pregnancy outcomes such as preterm birth, low birth weight, and increased maternal morbidity. The findings of this study demonstrate that the ethanolic extract of *Alternanthera sissoo* (Brazilian Spinach) significantly improves erythrocyte count, hemoglobin, and hematocrit levels in Wistar rats with iron deficiency anemia. These results suggest that *A. sissoo* possesses hematopoietic and antioxidant properties that may enhance erythropoiesis and iron bioavailability. From a clinical perspective, this plant extract holds promise as a natural nutraceutical or complementary therapy for preventing and managing iron deficiency anemia in pregnant women, especially in populations with poor tolerance to conventional iron supplementation due to gastrointestinal side effects. Further preclinical and clinical studies are needed to establish its safety profile, optimal dosage, and efficacy in human subjects before it can be integrated into prenatal nutritional interventions.

LIMITATIONS

This study has several limitations that should be acknowledged. First, the research was conducted using an *in vivo* model on Wistar rats, which may not fully represent the physiological responses of humans, particularly pregnant women. Second, the study focused solely on hematological parameters (erythrocyte count, hematocrit, and hemoglobin), without assessing biochemical indicators of iron metabolism such as serum ferritin, transferrin saturation, or total iron-binding capacity. Third, the relatively short

treatment duration (21 days) may not capture the long-term hematopoietic effects of *Alternanthera sissoo* extract. Moreover, histopathological examinations of the liver, spleen, and bone marrow were not included, limiting insight into the mechanism of erythropoiesis.

CONCLUSIONS

The results of this study demonstrated that the ethanolic extract of *Alternanthera sissoo* exhibits significant hematopoietic activity in anemia-induced female Wistar rats. Administration of the extract effectively increased erythrocyte count, hemoglobin concentration, and hematocrit levels in a dose-dependent manner. The highest dose (10 mg/kg BW) produced the most optimal improvement, restoring hematological parameters close to normal physiological values.

These findings suggest that *Alternanthera sissoo* contains bioactive compounds—such as flavonoids, alkaloids, saponins, and vitamin E—that act synergistically to stimulate erythropoiesis, enhance iron utilization, and protect erythrocytes from oxidative stress. Therefore, the ethanolic extract of *Alternanthera sissoo* has strong potential to be developed as a natural nutraceutical supplement for preventing anemia during pregnancy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper. All authors have contributed equally to the conception, design, data collection, analysis, and manuscript preparation, and have no financial, personal, or professional relationships that could influence the work reported in this study.

AUTHOR CONTRIBUTIONS

GAMR conceived and designed the study, supervised the research process, and prepared the manuscript. NH performed the extraction procedure and conducted the laboratory analyses. DGDA handled the experimental animals, administered treatments, and collected the data. AASAA conducted data processing, statistical analysis, and interpretation of the results. PAS contributed to the literature review, manuscript drafting, and organization of references.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

No artificial intelligence (AI) tools were used in the preparation of this manuscript.

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