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Effect of *Centella asiatica* Nanoparticles as an Adjunct to Iron Supplementation on Hemoglobin Levels among Anemic Adolescent Girls

Jihadiatul Qorimah[1], Runjati Runjati[2], Marsum Marsum[3]
[1]Master of Applied Midwifery, Poltekkes Kemenkes Semarang, Semarang, Indonesia, karimah0299@gmail.com
[2] Midwifery Department, Poltekkes Kemenkes Semarang, Semarang, Indonesia, runjati@poltekkes-smg.ac.id
[3]Radiodiagnostic Engineering and Radiotherapy Department, Poltekkes Kemenkes Semarang, Semarang, Indonesia
mmarsum63@gmail.com

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ABSTRACT

Anemia among adolescent girls remains a major public health problem in Indonesia despite nationwide iron supplementation programs. Poor adherence to iron tablets and limited iron bioavailability reduces treatment effectiveness. *Centella asiatica* nanoparticles may enhance iron absorption and erythropoiesis by improving the bioavailability of bioactive compounds. This study aimed to evaluate whether nanoparticle-based *Centella asiatica* enhances the hematologic response to iron supplementation. A randomized pretest–posttest control group study was conducted from October to November 2025 at the Department of Midwifery, Poltekkes Kemenkes Semarang, Central Java, Indonesia. Forty anemic adolescent girls aged 16–19 years were recruited using purposive sampling and randomly assigned to intervention or control groups. The intervention group received oral *Centella asiatica* nanoparticles (500mg/day; particle size<200 nm) together with iron tablets (60mg/day), while the control group received iron alone for 14 days. Hemoglobin levels were measured before and after the intervention. Statistical analyses included paired and independent t-tests, and effect size was calculated using Cohen's *d*. Ethical approval was obtained from the institutional ethics committee. Hemoglobin levels increased significantly in both groups after the intervention (both $p<0.001$). The intervention group demonstrated a greater increase in hemoglobin concentration (11.19 ± 0.29 to 13.25 ± 0.52 g/dL) than the control group (11.39 ± 0.32 to 13.04 ± 0.46 g/dL), with a large effect size (Cohen's d=1.53). Between-group analysis indicated that the combined intervention produced significantly greater hemoglobin improvement than iron supplementation alone ($p<0.05$). *Centella asiatica* nanoparticles may serve as a promising adjunct to iron supplementation for improving hemoglobin levels in anemic adolescent girls. Larger and longer-term studies are needed to confirm their effectiveness and support integration into adolescent anemia prevention programs.

Corresponding Author:

Jihadiatul Qorimah
Master of Applied MIdwifery, Poltekkes Kemenkes Semarang
Tirto Agung Street, Pedalangan, Banyumanik, Semarang, Central Java, Indonesia. 50268, Phone: (024) 76479189
Email: karimah0299@email.com

INTRODUCTION

Anemia among adolescent girls one of the most prevalent nutritional disorders among adolescent girls worldwide and continues to represent a major public health challenge, particularly in low- and middle-income countries.(1) According to the World Health

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Organization (WHO), anemia in females is defined as a condition characterized by insufficient hemoglobin levels that impair oxygen transport and physiological function, with hemoglobin concentrations below 12.0 g/dL.(8) Globally, nearly one-third of women of reproductive age are affected by anemia, with the greatest burden observed in Africa and Southeast Asia.(1) In Indonesia, national survey data indicate that anemia prevalence among adolescent girls aged 15–24 years has remained at approximately 32%, consistently exceeding that of males.(2) Despite sustained public health efforts, the prevalence of anemia among adolescent girls has shown little decline over recent years and remains persistently high in many low- and middle-income countries.(9) This stagnation underscores the limited impact of current prevention and control strategies. The situation is particularly alarming given that adolescence represents a critical developmental period. In Indonesia, national health reports consistently indicate that approximately 32% of adolescent girls aged 15–24 years are anemic, a prevalence substantially higher than that observed among males.(2) A similar pattern is observed at the regional level, where provincial health records from Central Java report that more than 30% of screened adolescent girls are anemic. Importantly, this proportion has remained relatively stagnant over time, showing little to no meaningful decline, thereby highlighting anemia as a persistent and unresolved public health concern rather than a condition that is gradually improving.(10)

Adolescent girls are particularly vulnerable group for anemia because rapid physical growth, expanding blood volume, and recurrent menstrual blood loss substantially increase physiological iron requirements.(12) These biological demands are frequently compounded by inadequate dietary iron intake, micronutrient deficiencies, parasitic infections, overweight or obesity, and prolonged or excessive menstruation.(13) Chronic anemia during adolescence has been associated with impaired cognitive function, reduced academic performance, decreased physical capacity, and diminished productivity, as well as increased vulnerability to adverse reproductive outcomes later in life. These consequences emphasize the importance of effective preventive and therapeutic strategies targeting this high-risk population.(11)

Iron supplementation remains the cornerstone of adolescent anemia prevention; and has been extensively implemented through national school-based supplementation programs.(14) However, despite the long-standing availability of iron tablets, anemia prevalence has remained relatively unchanged. This apparent paradox suggests that increasing access to iron supplementation alone has not adequately addressed the underlying challenges affecting treatment effectiveness.(9–11) Low adherence to iron tablets, largely attributable to gastrointestinal side effects, forgetfulness, poor acceptability, and limited perceived benefit, substantially reduces the effectiveness of supplementation programs.(15,16) In addition, the physiological response to iron therapy may be limited by suboptimal intestinal absorption and bioavailability, indicating that improving iron utilization may be equally important as increasing iron intake.(3,4)

Recent advances in nanotechnology have attracted considerable interest as an innovative strategy for enhancing the therapeutic efficacy of bioactive compounds. Nanoparticle-based formulations offer a promising solution by improving bioavailability and intestinal uptake of bioactive compounds.(5) Among potential herbal candidates, *Centella asiatica*, a plant rich in flavonoids and vitamin C known to support erythropoiesis, may synergistically enhance hemoglobin synthesis.(6) Therefore, this study hypothesized that supplementation with *Centella asiatica* nanoparticles combined with iron would result in a greater increase in hemoglobin levels compared with iron supplementation alone.(7)

Despite the long-standing implementation of iron supplementation, the problem of adolescent anemia remains unresolved. Although various plant-based interventions have been explored as complementary strategies, their clinical effectiveness has been inconsistent.(6) *Centella asiatica* has been widely recognized for its hematopoietic and

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antioxidant properties, and experimental studies have demonstrated its potential to improve blood parameters and protect red blood cells.(17) However, most existing studies have focused on conventional herbal extracts, which are often limited by poor solubility and low bioavailability, thereby restricting their therapeutic impact.(4) Advances in nanoparticle technology offer a promising approach to overcome these limitations by enhancing absorption, stability, and cellular uptake of bioactive compounds.(5) Nevertheless, a critical research gap remains. To date, no clinical study has evaluated the combined use of *Centella asiatica* nanoparticles and iron supplementation in anemic adolescent girls. Evidence regarding the clinical effectiveness of this approach therefore remains limited. Addressing this gap is important given the persistently high prevalence of anemia and the continuing challenges of adherence to iron supplementation programs.(18)

In this context, combining iron supplementation with a nanoparticle-based herbal intervention represents a rational and strategic approach to overcoming the limitations of existing anemia control efforts. Iron supplementation through national programs has proven efficacy in increasing hemoglobin levels; however, its real-world impact is constrained by poor compliance and suboptimal physiological response when used as a single intervention. Rather than replacing iron therapy, the limited impact of iron supplementation as a standalone intervention, together with the low bioavailability of conventional *Centella asiatica*, makes it clear that neither approach is sufficient on its own; consequently, a nanoparticle-based co-delivery system that enhances iron utilization and optimizes *Centella asiatica* bioactivity emerges as a necessary and logical next step. *Centella asiatica* is a particularly suitable candidate because it contains flavonoids, vitamin C, and essential minerals that promote iron absorption, stimulate red blood cell formation, and reduce oxidative stress on erythrocytes. Nanoparticle technology provides a more rational strategy to enhance the therapeutic potential of *Centella asiatica*, as it improves solubility, stability, and intestinal uptake of bioactive compounds, thereby overcoming the limitations of conventional herbal preparations. Rather than increasing iron dosage which may exacerbate gastrointestinal side effects and reduce adherence this approach aims to enhance iron utilization and therapeutic effectiveness.(5) The integration of *Centella asiatica* nanoparticles with iron supplementation is thus a logical and necessary next step to enhance treatment response and support sustainable anemia management strategies for adolescent girls.

Therefore, this study investigated the effect of *Centella asiatica* nanoparticles combined with iron supplementation on hemoglobin levels among anemic adolescent girls. We hypothesized that participants receiving *Centella asiatica* nanoparticles together with iron supplementation would experience a significantly greater increase in hemoglobin concentration than those receiving iron supplementation alone. The findings are expected to provide preliminary clinical evidence supporting nanoparticle-based herbal adjunct therapy as a novel complementary approach for improving adolescent anemia management and informing future public health interventions.

METHOD

This study employed a true experimental study with a randomized pretest–posttest control group approach and was conducted at the Department of Midwifery, Poltekkes Kemenkes Semarang, Central Java, Indonesia, from October 16 to November 6, 2025. From a population of 225 female students, anemia screening was performed once in the morning using a calibrated digital hemoglobinometer (HemoCue® Hb 201+, Sweden) with capillary fingertip blood sampling, following Clinical and Laboratory Standards Institute (CLSI) guidelines; the device was routinely calibrated according to the manufacturer’s instructions and has an acceptable coefficient of variation (<2%). Participants with hemoglobin levels <12 g/dL were classified as anemic.(8) Forty adolescent girls aged 16–19 years met the inclusion criteria (not receiving treatment for chronic or hematological diseases, not using

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other supplements, not menstruating at the time of measurement, and willing to complete the intervention) and were enrolled, while those with a history of hemolytic anemia, pregnancy, acute infection, or gastrointestinal disorders affecting nutrient absorption were excluded. The sample size was determined based on previous experimental studies evaluating hemoglobin response to iron supplementation and herbal adjunct therapy, which reported moderate effect sizes with comparable group sizes, suggesting that approximately 18–20 participants per group would be sufficient to detect clinically meaningful changes in hemoglobin levels. Sample size was estimated assuming an α of 0.05, statistical power of 80%, and an expected moderate effect size based on previous studies. Participants were randomly assigned in a 1:1 ratio using a computer-generated random sequence created with Random Allocation Software by an independent researcher, with group assignments concealed in sealed opaque envelopes, into an intervention group (n=20).

The intervention group received *Centella asiatica* nanoparticles (500 mg/day) in capsule form combined with iron supplementation (60 mg/day). The nanoparticle formulation had a particle size of <200 nm and consisted of a standardized *Centella asiatica* extract rich in flavonoids and vitamin C. The formulation was produced at the Integrated Laboratory, Diponegoro University, Semarang, Indonesia. The control group received iron supplementation alone at a dose of 60 mg/day. Supplements were administered once daily after meals for 14 consecutive days. This duration was selected to allow sufficient time for iron absorption and the early phase of erythropoiesis, as reticulocyte response and initial hemoglobin improvement typically begin within 7–14 days after effective iron therapy. The preparation of the nanoparticle formulation and laboratory analyses were conducted at the Integrated Laboratory of Diponegoro University, while hematological examinations were performed at SIG Laboratory, Semarang, Indonesia. Compliance was monitored through daily consumption logs and supervision by trained enumerators, and participants were observed for potential adverse effects. Hemoglobin levels were measured at baseline and post-intervention at the same time of day using standardized procedures.

Data normality was assessed using the Shapiro–Wilk test, followed by paired and independent t-tests and effect size calculation (Cohen's d) using SPSS version 26.0, with $p < 0.05$ considered statistically significant. Ethical approval was obtained from the Ethics Committee of Poltekkes Kemenkes Semarang (No. 1194/EA/F.XXIII.38/2025). Written informed consent was obtained from participants aged ≥18 years, whereas written parental consent and participant assent were obtained for those younger than 18 years.

RESULTS

A total of 40 adolescent girls met the eligibility criteria, were randomized into the intervention (n = 20) and control (n = 20) groups, and all completed the 14-day intervention. No participants withdrew from the study, and no missing data were recorded. Adherence to supplementation was monitored through daily supervision and participant logs, indicating high compliance throughout the intervention period. No adverse events or clinically significant side effects were reported in either group. The results of the analyses are presented below.

Participant Characteristics

Table 1 summarizes the baseline demographic, menstrual, anthropometric, and hematological characteristics of participants in both study groups before the intervention. The table presents the distribution of age, menstrual cycle pattern, menstrual duration, body mass index (BMI), and baseline hemoglobin levels to describe the characteristics of participants prior to the intervention.

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Table 1. Baseline Characteristics of Participants

Variable	Group				Total (f)
	Intervention		Control		
	(f)	(%)	(f)	(%)	
Age (Years)					
16 - 17 Years Old	8	40.0	7	35.0	15
18-19 Years Old	12	60.0	13	65.0	25
Menstrual Cycle	0	0	0	0	
Short Cycle: <21 days	20	100.0	18	90.0	38
Regular: 21-35 days	0	0	2	10.0	2
Long Cycle: >35 days					
Menstrual Duration					
Regular: 3-7 days	14	70.0	14	70.0	28
Irregular: <3 days or >7days	6	30.0	6	30.0	12
BMI					
Underweight: <18,5 kg/m^2	3	15.0	3	15.0	6
Normal: 18,5 – 22,9 kg/m^2	15	75.0	14	70.0	29
Overweight: >23-27,4 kg/m^2	2	10.0	3	15.0	5
Obesity: >27,5 kg/m^2	0	0	0	0	0
Hemoglobin level (g/dL)					
Mild anemia (Hb 11.0–11.9 g/dL)	17	85.0	18	90.0	35
Moderate anemia (Hb 8.0–10.9 g/dL)	3	15.0	2	10.0	5

Notes:
f: frequency
%: percentage

As shown in Table 1, most participants in both groups were aged 18–19 years, accounting for 60.0% of the intervention group and 65.0% of the control group. Regular menstrual cycles (21–35 days) were reported by all participants in the intervention group and by 90.0% of those in the control group, while only two participants in the control group experienced menstrual cycles longer than 35 days. The majority of participants in both groups had a normal menstrual duration (3–7 days), representing 70.0% of each group.

Based on body mass index (BMI), most participants had normal nutritional status (75.0% in the intervention group and 70.0% in the control group), whereas only a small proportion were classified as underweight or overweight. Regarding hematological status, mild anemia (hemoglobin 11.0–11.9 g/dL) predominated in both groups, affecting 85.0% of participants in the intervention group and 90.0% in the control group, while the remaining participants had moderate anemia. No participants presented with severe anemia.

Overall, the demographic, menstrual, anthropometric, and clinical characteristics were generally comparable between groups before the intervention. However, baseline hemoglobin levels were analyzed separately because a statistically significant difference was observed between groups (Table 4).

Baseline Comparability

Prior to hypothesis testing, the normality and homogeneity assumptions were evaluated.

Table 2. Shapiro–Wilk Normality Test for Hemoglobin Levels

Group	Variable	p-value	Interpretation
Intervention	Pretest Hb	0.200	Normal distribution
Intervention	Post-test Hb	0.178	Normal distribution
Control	Pretest Hb	0.244	Normal distribution
Control	Post-test Hb	0.167	Normal distribution

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The Shapiro–Wilk test demonstrated that all hemoglobin measurements in both the intervention and control groups were normally distributed, with all *p*-values exceeding 0.05 (Table 2). Therefore, parametric statistical tests were considered appropriate for subsequent analyses.

Table 3. Levene's Test for Homogeneity of Variance

Variable	p-value	Interpretation
Hemoglobin Levels (Pretest)	0.408*	Homogen

*Levene's test

Levene's test showed homogeneous variance between groups for baseline hemoglobin levels ($p = 0.408$), satisfying the homogeneity assumption required for the independent *t*-test (Table 3).

Effect of the Intervention on Hemoglobin Levels

Table 4 presents the comparison of hemoglobin levels before and after the intervention in both groups.

Table 4. Comparison of Hemoglobin Levels Before and After Intervention Between Groups

Variable		Intervention Group (Mean ± SD)	Control Group (Mean ± SD)	p-value	Effect Size (Cohen's d)
Hemoglobin	Pre	11.19 ± 0.29	11.39 ± 0.32	0.042**	
Levels(g/dL)	Post	13.25 ± 0.52	13.04 ± 0.46	0.049**	1.53***
p-value		0.000*	0.000*		
Δ		2.16 ± 0.36	1.65 ± 0.29	0.000**	

*Pired T-Test
**Independent T-Test
***Cohen"s d Test

At baseline, the mean hemoglobin level was slightly lower in the intervention group (11.19 ± 0.29 g/dL) than in the control group (11.39 ± 0.32 g/dL), and this difference reached statistical significance ($p = 0.042$). Following the 14-day intervention, mean hemoglobin levels increased significantly in both groups according to paired *t*-tests ($p < 0.001$ for each group).

The intervention group showed an increase from 11.19 ± 0.29 g/dL to 13.25 ± 0.52 g/dL, corresponding to a mean change (ΔHb) of 2.16 ± 0.36 g/dL. In comparison, the control group increased from 11.39 ± 0.32 g/dL to 13.04 ± 0.46 g/dL, with a mean change of 1.65 ± 0.29 g/dL.

Between-group analysis using the independent *t*-test demonstrated that the increase in hemoglobin levels was significantly greater in the intervention group than in the control group (ΔHb, $p < 0.001$). Furthermore, the observed effect size was large (Cohen's $d = 1.53$), indicating a substantial clinical effect associated with supplementation using *Centella asiatica* nanoparticles combined with iron.

Although post-intervention hemoglobin levels were significantly higher in the intervention group, these findings should be interpreted with caution because a statistically significant difference in baseline hemoglobin levels was observed between groups.

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Changes in Mean Hemoglobin Levels

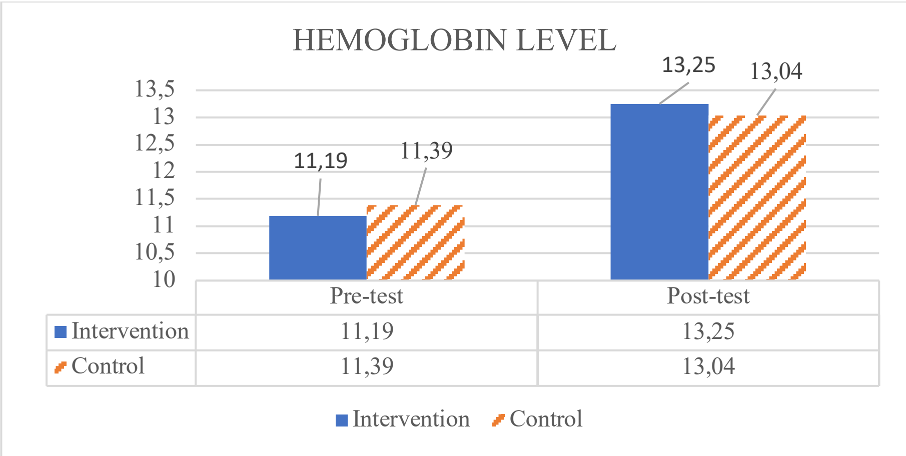

Figure 1. Mean Hemoglobin Levels Before and After Intervention

Figure 1 illustrates the changes in mean hemoglobin levels following the intervention. Both groups experienced increases in hemoglobin concentrations after 14 days of supplementation. However, participants receiving *Centella asiatica* nanoparticles combined with iron supplementation demonstrated a greater improvement than those receiving iron supplementation alone. This pattern is consistent with the statistical findings presented in Table 4, suggesting that adjunctive administration of *Centella asiatica* nanoparticles enhanced the hematological response to iron supplementation.

DISCUSSION

This randomized pretest–posttest study demonstrated that adolescent girls who received *Centella asiatica* nanoparticles combined with oral iron supplementation experienced a significantly greater increase in hemoglobin levels than those who received iron supplementation alone. Although both groups showed significant improvements after the 14-day intervention, the intervention group achieved a larger mean increase in hemoglobin concentration (2.16 ± 0.36 g/dL versus 1.65 ± 0.29 g/dL), accompanied by a large effect size (Cohen's *d* = 1.53). These findings suggest that nanoparticle-formulated *Centella asiatica* may provide a clinically meaningful adjunctive benefit to conventional iron supplementation for adolescent girls with anemia. This study demonstrated that baseline biological characteristics, including age, menstrual cycle pattern, menstrual duration, and body mass index, were well balanced between the intervention and control groups, thereby minimizing potential confounding effects related to demographic and physiological factors. However, a statistically significant difference in baseline hemoglobin levels between groups was observed (p = 0.042), although the absolute mean difference was small and both groups were classified within the range of mild to moderate anemia. This imbalance should be considered when interpreting the intervention effect, as baseline differences in outcome variables may influence post-intervention comparisons. Future studies should apply analytical approaches such as analysis of covariance (ANCOVA) or mixed-effects models to adjust for baseline differences and provide more robust estimates of treatment effects.

The present study examined the effect of *Centella asiatica* nanoparticles combined with iron supplementation on hemoglobin levels among anemic adolescent girls. Univariate analysis indicated that most participants were late adolescents aged 16–19 years, a developmental stage characterized by rapid physical growth, hormonal changes, and increased nutritional demands. These physiological conditions, combined with menstrual blood loss, increase vulnerability to iron deficiency anemia, as consistently reported in previous studies.(19,20) The homogeneity of menstrual characteristics and nutritional

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status between groups suggests that biological variation beyond hemoglobin levels did not substantially influence differences in hemoglobin outcomes. Previous evidence has shown that irregular menstrual patterns and suboptimal nutritional status are important contributors to anemia in adolescent girls.(21) Therefore, the relatively homogeneous baseline characteristics strengthen the interpretation that the greater improvement in hemoglobin levels was primarily associated with the intervention rather than differences in participant characteristics.

Bivariate analysis revealed significant increases in hemoglobin levels in both groups following the intervention period. Iron supplementation alone significantly improved hemoglobin status, which is consistent with prior clinical studies demonstrating the effectiveness of iron tablets in improving hemoglobin levels among anemic adolescents.(22) The intervention group receiving *Centella asiatica* nanoparticles combined with iron supplementation exhibited a greater increase in hemoglobin levels (2.16 ± 0.36 g/dL) compared with the control group (1.65 ± 0.29 g/dL). Although an independent t-test was used in accordance with the predefined analytical plan, the large observed effect size (Cohen's d = 1.53) suggests a clinically meaningful benefit beyond the modest baseline difference. However, the additional improvement observed in participants receiving *Centella asiatica* nanoparticles suggests that this herbal formulation may enhance the physiological response to iron supplementation rather than replacing conventional therapy. Accordingly, the present findings support the concept of nanoparticle-formulated *Centella asiatica* as an adjunctive intervention designed to optimize the effectiveness of existing iron supplementation programs.

The greater hemoglobin response observed in the intervention group may be partially explained by the bioactive constituents of *Centella asiatica*, including flavonoids, triterpenoids, saponins, tannins, and vitamin C. These coumpounds are known to support erythropoiesis, enhance iron absorption, and reduce oxidative stress.(6,7,17,23) Several experimental studies have demonstrated that *Centella asiatica* possesses antioxidant and anti-inflammatory properties, stabilizes erythrocyte membranes, and protects red blood cells from oxidative damage. In vitro studies have reported membrane-stabilizing effects of *Centella asiatica* extracts on human erythrocytes(, while animal studies have shown increases in hemoglobin and hematocrit levels following administration of *Centella asiatica* extract.(24,25) However, these findings should be interpreted cautiously, as results from in vitro and animal models may not fully translate to clinical outcomes in humans.(26)

In addition to the pharmacological properties of *Centella asiatica*, the nanoparticle formulation itself may have contributed to the observed therapeutic response. Nanotechnology has been shown to improve the bioavailability, stability, and cellular uptake of herbal compounds by reducing particle size and increasing solubility.(27) Studies by Nor Atiqah et al. demonstrated that nano-emulsified *Centella asiatica* significantly increased antioxidant activity and reduced oxidative stress.(28,29) While nano-encapsulation has been reported to enhance the absorption and biological efficacy of *Centella asiatica* extracts.(18) Given that oxidative stress can impair hemoglobin synthesis and increase erythrocyte fragility, improved antioxidant delivery through nanoparticle formulations may contribute to hematologic recovery.(30) Nevertheless, direct clinical evidence linking nanoparticle-based *Centella asiatica* supplementation to hematological outcomes remains limited. Therefore, the mechanisms proposed in this study should be regarded as biologically plausible rather than conclusively established.

To our knowledge, this study is among the first randomized controlled investigations evaluating nanoparticle-formulated *Centella asiatica* as an adjunct to oral iron supplementation for improving hemoglobin concentrations in anemic adolescent girls. While previous clinical trials have reported physiological and metabolic benefits of standardized *Centella asiatica* extract in adults, studies specifically evaluating hematological outcomes, particularly hemoglobin levels in anemic adolescents, remain scarce.(31) This study aimed

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to address an important clinical gap by providing preliminary human evidence on the potential role of *Centella asiatica* nanoparticles as an adjunct to iron supplementation in improving hemoglobin levels. These findings contribute to the emerging evidence supporting the use of nanoparticle-based herbal adjuncts in anemia management.(32) However, confirmation through larger multicenter randomized controlled trials remains necessary before this approach can be recommended for routine clinical practice.

The findings of this study suggest that *Centella asiatica* nanoparticles may serve as a promising adjunct to iron supplementation for improving hemoglobin levels in adolescent girls with anemia. This approach may be particularly relevant for anemia prevention programs where adherence to iron tablets remains a persistent challenge. Integration of this strategy into existing adolescent health initiatives such as school-based health programs, School Health Units (UKS), and adolescent integrated health posts (Posyandu Remaja) could potentially strengthen the effectiveness of current iron supplementation efforts.In primary health care settings, this adjunct approach may offer a complementary strategy to enhance iron utilization without increasing iron dosage, which is often associated with gastrointestinal side effects and reduced adherence. If future studies confirm its efficacy and safety, nanoparticle-based herbal adjuncts could contribute to strengthening public health policies aimed at improving adolescent anemia prevention and control.

Several limitations should be acknowledged when interpreting these findings. First, the 14-day intervention period was sufficient to detect early hematopoietic responses but was unlikely to reflect complete restoration of body iron stores, as iron repletion generally requires several weeks. Second, purposive sampling and recruitment from a single educational institution may limit the generalizability of the findings. Third, the absence of participant and investigator blinding may have introduced performance bias, although objective hemoglobin measurements reduced the likelihood of measurement bias. Fourth, statistical comparisons between groups relied on independent *t*-tests despite a modest baseline difference in hemoglobin concentration. Future studies should therefore consider ANCOVA or mixed repeated-measures analyses to account for baseline variability more appropriately. Finally, hematological assessment was limited to hemoglobin concentration without evaluation of additional biomarkers such as serum ferritin, transferrin saturation, reticulocyte count, or inflammatory markers. Future randomized controlled trials involving larger and more diverse populations, longer intervention periods, comprehensive hematological biomarkers, and economic evaluations will be essential to establish the long-term effectiveness, safety, and feasibility of nanoparticle-formulated *Centella asiatica* for large-scale adolescent anemia prevention programs.

CONCLUSION

This randomized controlled study provides preliminary evidence that *Centella asiatica* nanoparticles (500 mg/day) combined with iron supplementation (60 mg/day) improve hemoglobin levels more effectively than iron supplementation alone in adolescent girls with anemia. Although both groups experienced significant increases in hemoglobin concentrations, the intervention group demonstrated a greater improvement with a large effect size, suggesting that nanoparticle-based *Centella asiatica* may enhance the hematological response to iron therapy. These findings indicate that nanoparticle-based *Centella asiatica* has potential as a promising adjunct to existing adolescent anemia prevention strategies, particularly in settings where adherence to iron supplementation remains suboptimal. However, given the short intervention period and modest sample size, the results should be interpreted as preliminary evidence rather than definitive confirmation of long-term effectiveness. Future multicenter randomized controlled trials with larger samples, longer follow-up periods, and comprehensive hematological biomarkers—including serum ferritin, transferrin saturation, reticulocyte count, and mean corpuscular volume—are warranted to confirm efficacy, evaluate long-term safety, and determine the feasibility of integrating nanoparticle-based herbal adjuncts into adolescent anemia prevention programs.

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AUTHOR CREDIT STATEMENT

All authors contributed substantially to the conception and design of the study. **JQ** was responsible for study conceptualization, methodology development, data collection, data analysis, and drafting the original manuscript. **RR** contributed to data analysis, interpretation of results, and critical revision of the manuscript for important intellectual content. **MM** provided supervision, validated the methodology, and reviewed and edited the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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DECLARATION OF COMPETING INTEREST

There is no conflict of interest.

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