

Iron Supplementation from Moringa Leaves in the form of Steeping reduces Iron Deficiency Anemia in Adolescent Girls

Erina Nur Afni^{1*}, Budiyono², Onny Setiani³, Bagoes Widjanarko⁴, Adi Heru Sutomo⁵

Abstract

Introduction: Iron (Fe) supplementation in adolescent girls has not maximally reduced the prevalence of anemia, its implementation is very dependent on the level of individual compliance. This study aims to determine the effect of moringa leaf Fe supplementation in the form of steeping on the decrease in the prevalence of IDA in adolescent girls aged 12-18 years.

Method: The research design was divided into two stages, pre-test, and post-test control group design, with 70 female adolescents as subjects. They are female students at Tarbiatul Mubtadin Islamic Boarding School, Tangerang Regency, Indonesia, aged 12-18 years. Determination of Iron Deficiency Anemia (ADB) is based on indicators of hemoglobin (Hb) <12 mg/dl, and serum ferritin (Sf) <22 g/L. Fe supplementation in the form of steeping with the composition of dry Moringa leaves (2.5 g), dried lemon peel (0.5 g), and 1.5 tablespoons of sugar. Subjects consumed three times per day, two hours after breakfast, lunch, and dinner for 52 days. Data analysis used the Mann-Whitney and Chi-square statistical tests, respectively, to determine the difference in the mean Hb and Sf levels of the two unrelated groups, and to determine the difference in the proportion of IDA decline between the intervention group and the control group.

Results: The mean Hb level in the intervention group increased significantly from 10.72 ± 1.14 g/dl to 11.00 ± 1.34 g/dl, the control group decreased from 9.99 ± 1.89 g/dl to 9.86 ± 1.92 g/dl, and the change in mean Hb levels between groups were significantly different (p = 0.005). Sf levels in both the intervention and control groups increased, respectively from 6.57 ± 5.91 g/l to 11.98 g/l, and from 6.01 ± 5.76 g/l to 11.51 ± 9.19 g/l. The change in mean SF levels between groups was not statistically significant (p = 0.477). The prevalence of IDA in the intervention group decreased by 25.70%, for the control group it was 5.70%, and the decrease in the proportion of IDA prevalence showed a significant difference (p = 0.022).

Conclusion: Fe supplementation from Moringa leaves in the form of steeping was able to reduce the 25.70% prevalence of IDA in adolescent girls aged 12-18 years.

Key Words: Iron, Moringa Leaves, IDA, Adolescents, Women.	
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Introduction

Anemia, especially Iron Deficiency Anemia (IDA) is still a major public health problem in the world.¹ Similarly, in Indonesia, adolescent girls suffering from IDA due to iron deficiency are still high (5.8-15.8%).^{2,3} Efforts to reduce the prevalence of IDA in adolescents by giving iron (Fe) supplementation have not given maximum results⁴, because it has side effects on the digestive tract,⁵ and its implementation is very dependent on compliance in consuming it.⁶ The addition of Multi Micro Nutrient (MMN) fortification into drinks for teenagers is also considered not to cure anemia in adolescents well ⁷.

Corresponding author: Erina Nur Afni

Address: ^{1*,3,4}Public Health Doctoral Program, Faculty of Public Health, Diponegoro University; ²Department of Environmental Health, Faculty of Public Health, Diponegoro University; ⁵Department of Family Medicine, Faculty of Public Health and Nursing, Gadjah Mada University.



Safer ways to deal with ADB include providing natural supplements and food-based interventions.⁸ Moringa (Moringa oleifera) or Moringa plant is a versatile herbal plant that is consumed as human food and an alternative for various medicinal purposes throughout the world,⁵ because Moringa leaves contain a lot of iron (28 mg/100 g dry leaves),⁹ and essential nutrients needed for metabolism.¹⁰

Previous studies revealed that supplementation with Moringa leaf powder significantly increased hemoglobin (Hb) concentration in the intervention group, an average of 8.3 g/dl to 10.9 g/dl (95% CI: 10, 2-11,4) after 6 months of intervention.¹⁰ The diet combines non-heme iron-rich foods and local foods that increase iron absorption (rich in vitamin C), also increase Fe bioavailability,¹¹ and able to increase aroma and taste.¹²

This study analyzed the results of the examination of Hb levels and Sf levels of adolescent girls who experienced IDA before and after being given Fe supplementation from Moringa leaves in the form of steeping, with the aim of knowing the effect of Fe supplementation from dried Moringa leaves on the prevalence of IDA in adolescent girls aged 12-18 years.

Method

Using an experimental design with two stages, namely Pre and Post-Test. The sampling technique is purposive because it aims to collect subjects with symptoms of IDA determined based on the indicators of Hb < 12 g/dl, and Sf < 22 g/L.

The first step was to determine the research subjects, namely 329 female students who met the inclusion criteria at Tarbiatul Mubtadin Islamic Boarding School, Tangerang Regency, Indonesia. The inclusion criteria were that the subject had no problem drinking tea, did not suffer from or had ever suffered from thalassemia, was not allergic to Moringa leaves, was willing to participate in the study to completion, and had a Hb level <12 g/dl.

Of all that, 138 subjects were obtained who met the criteria, then to ensure that the low Hb level was not caused by infection or impaired liver function, further examination was carried out on the concentration of C-reactive protein (CRP) <10 mg/L, and serum concentration Glutamic Pyruvic Transaminase (SGPT) = 0-35 g/L). The results of

the examination, 4 subjects tested positive for CRP, 5 subjects had SGPT levels >35 g/L, so 9 subjects were excluded and 129 subjects were confirmed.

Of the 129 subjects who met the inclusion criteria for the second stage, Sf levels were then examined by the Prodia Clinical Laboratory, Tangerang Regency, and obtained 77 subjects with Sf levels <22 g/L. So, there were 77 subjects who met the ADB indicators (Hb < 12 g/dl and Sf < 22 g/L), with a distribution of 38 subjects living in the lower dormitory, and 39 in the upper dormitory.

Determine the group that gets the intervention and the control group by a simple random method based on the place of residence (lower and upper dormitories). The selected and determined students who live in the lower dormitory are the intervention group, while those who live in the upper dormitory are the control group.

The intervention group was given Fe supplementation from Moringa leaves in the form of steeping. The composition of the intervention material in each tea bag contains dried Moringa leaves (size 0.5-1.0 mm) weighing 2.5 g, plus dried lemon peel weighing 0.5 g, thus the intervention material resembles a tea bag that is usually consumed by teenage girls in the cottage or at home. How to serve each bag of intervention ingredients brewed with 200 ml of boiling water with added 1.5 tablespoons of sugar. The infusion of Moringa leaves is consumed 3 times per day, ie every two hours after breakfast, lunch, and evening (breaks in the morning, afternoon, and evening). The control group was given 2.5 g of Indonesian brand jasmine tea (Sariwangi, Indonesia), also added with 0.5 g of dried lemon peel, and 1.5 tablespoons of granulated sugar brewed in 200 ml of boiling water. The intervention was given for 52 days, from March 2 to April 22, 2021.

The intervention was carried out after the research protocol was evaluated and approved by the Ethics Committee of Diponegoro University Semarang (No. 09/AE/KEPK-FKM/2020), and all subjects had given written consent to participate voluntarily in the study. However, during the implementation of the intervention 7 (seven) subjects were excluded due to health reasons that made them unable to continue as research subjects.

The research implementation process can be seen in the following flow chart (figure 1).





Figure 1. Flowchart of research implementation

Statistical Analysis

To achieve the objectives of this study, several stages were carried out, namely, examination of Hb and Sf levels at the Prodia Clinical Laboratory, data management and analysis using the Statistical Package for the Social Sciences (SPSS 16) software, descriptive analysis was carried out with absolute (n) and relative (%) frequencies), Mann-Whitney test to determine the difference in the mean levels of Hb and Sf from the two groups, as well as the

Chi-Square test, to see whether or not there are differences in changes in the proportion of IDA.

Results

Characteristics

The characteristics of the research subjects were determined based on the age of the subjects, Body Mass Index (BMI), daily intake of Fe and vitamin C, and the average length of menstruation (table 1).



1857

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Variable	intervention group			trol group	moon difference (IK 0E%)	n
Variable	n	Mean±SD	n	Mean±SD	mean unierence (ik 95%)	p
Age (th)	35	15,43 ± 1,52	35	14,86 ± 1,42	0,57 (-0,13 – 1,27)	0,108
BMI (Kg/m ²)	35	23,55 ± 3,82	35	22,09 ± 2,68	1,46 (-0,12 – 3,04)	0,070
Fe intake (mg)	35	8,06 ± 4,05	35	8,46 ± 4,47	-0,39 (-2,43 – 1,64)	0,518
Intake of Vit-C (mg)	35	6,08 ± 11,76	35	7,59 ± 9,68	-1,51 (-6,65 – 3,63)	0,145
Menstruation (hr)	35	6,91 ± 0,45	35	6,94 ± 0,68	0,14 (-0,30 – 0,25)	0,836
			-			

Table 1. Characteristics of research subjects

Description : BMI = Body Mass Index SD = Standard Deviation.

Based on the characteristics of the variables of age, BMI, Fe intake, vitamin C intake, and duration of menstruation, statistically, there was no significant difference between the subjects of the intervention group and the control group, with the overall pvalue > 0.05.

Levels of Hb and Sf Levels

Hb and Sf levels between the intervention group and the control group before and after the intervention were analyzed using the Mann-Whitney test (Table 2).

Table 2. Hb and Sf levels during pre-test and post-test

Indicators that shocked	n	Mean (± SD)					
indicators that thetked	11	Pre-test	Post-test	Change	Score-p		
Hb level (g/dl)							
Intervention group	35	10,72 (± 1,14)	11,00 ± 1,34	$\textbf{0,}280 \pm \textbf{0,}853$	0,045°		
control group	35	9,99 (± 1,89)	9,86 ± 1,92	$\textbf{-0,}134\pm0,\!750$	0,362°		
Score-p	:	0,210 ^a	0,005 ^b	0,033 ^a			
Sf Level (μg/l)							
Intervention group	35	5,42 ± 4,07	$11,\!98\pm7,\!91$	6,57 ± 5,91	0,001c		
control group	35	5,72 ± 5,54	$11,51 \pm 9,19$	6,01 ± 5,76	0,001c		
Score-p	:	0,599ª	0,477 ^a	0.769 ^a			

Description: a= Mann Whitney test b. Independent t test c. Wilcoxon test

The average assessment of Hb levels in the group after the intervention in the intervention group experienced a mean increase (0.280 ± 0.853) to 11.00 ± 1.34 g/dl, statistically significantly different (p = 0.045), while the control group decreased (p = 0.045). -0.134 \pm 0.750), resulting in 9.86 \pm 1.92 g/dl, although statistically not significantly different from the mean at screening (p = 0.362). The mean Hb level between groups at the end of the intervention, in the intervention group was higher (11.00 ± 1.34 g/dl), than in the control group (9.86 ± 1.92 g/dl), and the difference was statistically significant (p = 0.005).

SF levels after the intervention for two months, in the intervention group, increased $(6.57 \pm 5.91 \text{ g/l})$ to 11.98 g/l, statistically, the increase was statistically significant (p = 0.001), as well as in the control increased (6.01 ± 5.76 g/l) to 11.51 ± 9.19 g/l, statistically, the increase was significant (p = 0.001). There was no statistically significant difference between the groups of SF levels at the end of the intervention (p = 0.477).

Changes in Hb and Sf Levels

After two months of intervention, the proportion of Hb levels increased in the intervention group (77.14 %), and in the control group (34.29 %). For Sf levels in the intervention group, the proportion increased by 97.14%, and in the control group, it was 91.43% (table 3).

Table 3. Distribution of Status Changes in Hb and Sf Levels from pretest to post-test

Indicator chacked	n	Go	on	Not Up		
multator thetkeu	11	f	%	f	%	
Hb level (g/dl)						
Intervention group	35	27	77,14	8	22,86	
control group	35	12	34,29	23	65,71	
Sf Level (µg/l)						
Intervention group	35	34	97,14	1	2,86	
control group	35	32	91,43	3	8,57	

Figure 2, describes the average change in Hb levels after a two-month intervention. In the intervention group, the pattern increased (from 10.72 g/dl to 11



g/dl), while in the control group, the pattern decreased (9.99 g/dl to 9.86 g/dl). Meanwhile, SF levels in the intervention group increased from

 5.42 ± 4.07 g/l to 11.98 ± 7.91 g/l, and in the control group also increased from 5.72 ± 5.54 g/l) to 11.51 ± 9.19 g/l.



Figure 2. Average changes in Hb (g/dl) and Sf (μ g/l) levels from pre-test to post-test

ADB Prevalence Change

In this study, the determination of ADB status was based on indicators of Hb < 12 g/dl, and Sf < 22 g/L. Before the intervention 70 (100%) research subjects were adolescent girls with ADB. After two months of intervention, from 35 subjects in the intervention group, 9 (25.70%) became no IDA,

while in the control group only 2 (5.70%) changed to no IDA. Statistical test results obtained a p value = 0.022, meaning that at = 5% it can be concluded that there is a significant difference in the decrease in the proportion of IDA prevalence between the intervention group and the control group (see table 4).

1859

Table 4. ADB	Prevalence	Distribution
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	St	atus					
Group	No ADB		ADB		Total	Score p	OR (95% CI)
	n	%	n	%			
Intervention	9	25,70	26	74,30	35	0 0 2 2 3	5,71 (1,14-28,75)
Control	2	5,70	33	94,30	35	0,022ª	1

Description: a = *Chi Square*

Discussion

In this study, Fe supplementation from Moringa leaves was given in the form of steeping (tea), with added slices of lemon peel. It is given in the form of steeping so that it is identical to drinking tea because tea is a simple and common drink for consumption¹³ including in Indonesia¹⁴ which is ranked as the 22nd largest tea consumer, Therefore, it is expected that the subject adheres to drinking regularly. The addition of orange peel slices in addition to containing vitamin-c, with the aroma of oranges can function to prevent nausea, and the potential for functional food can be optimized to promote local wisdom, culture, and Indonesian cuisine¹⁶.

Fe Content Analysis

Two grams of dried Moringa leaves at the Bogor Agricultural Center (BBIA) contain 11.6 mg Fe per 100 g¹⁷. Fe content is slightly smaller than Moringa leaf extract which contains 14.67 mg per 100 g Fe¹⁸, while the content of vitamin C in every 100 g of dried moringa is 773 mg¹⁹.

The intervention materials for this study were dried Moringa leaves and lemon peel, which were



then placed in a container (teabag). Each bag contains 2.5 g of moringa and 0.5 g of dried lemon zest. Based on the literature review on Fe and Vitamin C content, it can be estimated that each Moringa tea bag contains 0.29 mg of the mineral Fe and 3.87 mg of vitamin C.

Hemoglobin Levels

The average Hb levels of adolescent girls between groups before the intervention did not show a statistically significant difference. The end of the intervention (Post-test), showed that the intervention group had an increase of 0.28 g/dl precisely on the 52nd day of the intervention period, the Hb level of the intervention group increased significantly (mean Hb levels became 11.00 1.34 g/dl), while the Hb levels in the control group were still lower (mean Hb levels were 9.86 \pm 1.92 g/dl).

These results are relevant to the results of the study in Tanzania, the average Hb level of the intervention group increased from 8.3 g/dl during the pre-test, to 10.9 g/dl (95% CI: 10.2-11.4), and was statistically significantly higher than the control group, which was 9.4 g/dl (95% CI: 7.8-10.1). As for the difference, a study in Tanzania used the intervention of Moringa leaf powder as a dietary supplement, with the research subjects being children under two years, with an intervention period of six months.

Absorption of iron (Fe) varies, from 1% to 40%, and adequate intake of Fe in the diet can be increased by modifying consumption patterns to optimize the addition of Fe absorption and reduce Fe absorption inhibitors.²⁰ Previous studies have confirmed that iron absorption inhibitors, such as tannins, should be eliminated or minimized during a diet to increase Hb.²¹ A healthy diet that combines non-heme iron sources with local foods can increase Fe absorption and increase Fe bioavailability,^{11,22} Increasing the absorption of Fe, such as vitamin C can increase the absorption of iron by reducing the possibility of the return of Fe Ferry to Ferro.²³

Citrus fruit peels contain abundant phenolic compounds, flavonoids, and vitamin C. The pulp and seeds of the lemon also contain 49.8 mg of total phenol and 110.4 mg of vitamin C per 100 g.²⁴ The effect consuming Moringa leaves and lemon peel as tea supplements has been shown to increase Hb levels.

Sustained interventions can correct micronutrient deficiencies if the supplements are natural and

food-based.²⁵ This study recommends Moringa leaves as a potential solution to increase Hb and improve ADB conditions, especially for adolescent girls. Consuming moringa plants not only benefits an abundant plant-based diet but is also more affordable and considered more sustainable.²⁶

Moringa leaves are rich in iron,⁸ Dried moringa contains 28.2 mg/g of iron. In this study, the Fe compound in Moringa was 11.6 mg/100 g,¹⁷ and the content of vitamin C in every 100 g of dried moringa is 773 mg.²⁷ This study confirmed that three times a day intake of 0.29 Fe from dried Moringa leaves plus 3.87 mg of vitamin C from dried lemon peel, added 1.5 g of sugar (as a tea drink) was able to increase Hb levels for 52 days of consumption in adolescents woman.

Based on Table 1, the characteristics of the subjects (age, BMI, Fe intake, Vitamin C intake, and duration of menstruation) between the intervention and control groups were not statistically significant, therefore the increase in Hb levels in the intervention group averaged 0.28 g/dl. allegedly due to the influence of Moringa leaf supplementation in the form of steeping.

Each tea bag containing 2.5 grams of dry Moringa leaves contains 0.29 mg of Fe. This content is relatively low when compared to Fe content in the Fe supplementation program for adolescent girls, but with lower doses, it has been able to reduce the prevalence of IDA, this is presumably because Fe supplementation from Moringa leaves in the form of steeping can optimize fractional Fe absorption.,²⁸ thereby reducing gastrointestinal exposure to unabsorbed Fe and reducing supplemental side effects.²⁸ Consuming drinks containing Vitamin C can increase iron absorption,²⁹ the addition of lemon peel is estimated to contain vitamin C of 3.87 mg/bag, this additional ingredient helps increase iron absorption optimally.³⁰

The control group in this study also consumed lemon peel but was not given Moringa tea. They only consume regular Indonesian brand tea (Sariwangi, Indonesia). However, the mean value of Hb levels in the control group did not show any improvement. Lemon peel can help the absorption of Fe, but without Fe supplementation, Hb levels in the control group remained lower than in the intervention group.²⁹

Iron deficiency nutritional supplementation must be provided strategically and sustainably because nutritional interventions in adolescence directly affect adulthood.^{31,32} These results provide new information for those who suffer from IDA,



especially girls, that getting used to drinking dried Moringa leaves added with lemon peel slices can increase Hb and Sf levels which in the long run will prevent iron deficiency or as an alternative natural therapy against ADB incident. However, these results still require further research on its efficacy and effectiveness on a larger scale, not only in adolescent girls but example in the general public.

Conclusion

Moringa leaf iron supplementation in the form of steeping for 52 days increased Hb and SF levels and was able to reduce 25.70% IDA in adolescent girls aged 12-18 years.

Suggestion

The efficacy of Fe supplementation from Moringa leaves in the form of steeping in this study is the result of an ideal condition test, where all factors are controlled, therefore, it is necessary to do further research to test the effectiveness of these findings by adding Moringa leaves and dried lemon peel on research subjects with a different scale bigger.

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Erina Nur Afni et al / Iron Supplementation from Moringa Leaves in the form of Steeping reduces Iron Deficiency Anemia in Adolescent Girls

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