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The Effectiveness of Combining Iron Supplementation with Sweet Orange in Increasing Hemoglobin Levels

Efektivitas Kombinasi Tablet Tambah Darah dengan Jeruk Manis dalam Meningkatkan Kadar Hemoglobin

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ARTICLE INFO

Received: 31-08-2023 **Accepted:** 05-03-2025 **Published online:** 12-09-2025

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10.20473/amnt.v9i3.2025.412-418

Available online at: https://ejournal.unair.ac.id/AMNT

Keywords:

Anemia, Sweet orange, Adolescents, Iron supplementation, Vitamin C

ABSTRACT

Background: Iron (Fe) deficiency is the leading cause of anemia worldwide due to the impact on hemoglobin (Hb) production. Meanwhile, several nutrients, including protein, iron, copper (Cu), zinc (Zn), vitamin A, and vitamin C, influence Hb levels.

Objective: This study aimed to evaluate the effectiveness of iron supplementation combined with sweet orange in increasing Hb levels.

Methods: A quasi-experimental pretest-posttest design was used with a total of 45 menstruating female students from Grades 8 and 9 as participants. These individuals were divided into two groups, one group received iron supplements (TTD) alone, while the other was given TTD combined with 130 grams of sweet orange, providing 63.7 mg of vitamin C, for 12 weeks. Hb levels before and after the intervention were measured using the Easy Touch GCHb device. An independent t-test was used for statistical analysis.

Results: The results showed that the combination of TTD and vitamin C from sweet orange was significantly more effective in increasing Hb levels in adolescents compared to TTD alone (p-value=0.001).

Conclusions: The combination of TTD and sweet orange was more effective in increasing Hb levels. These results provide valuable insights for government programs aimed at improving adolescent iron supplementation by incorporating vitamin C-rich food sources, such as sweet orange, to enhance iron absorption.

INTRODUCTION

Adolescence is a change from infancy to adulthood indicated by transformations that include different biological, cognitive, and emotional aspects1. These transformations influence both health and nutritional status, as growth requires an adequate intake of essential nutrients. For adolescents, the ideal nutrient intake is used for growth and development². However, an imbalance between needs and sufficiency potentially leads to nutritional disorders such as anemia3. This condition defined as hemoglobin (Hb) levels below 12 g/dL, is caused by a decrease in the formation or the number of erythrocytes and hematocrit, limiting the transport of oxygen to body tissues4. Permaesih & Susilowati (2015) showed that female adolescents suffer from anemia more often than males, with a prevalence of 30% and 20.9%, respectively. This is because female adolescents experience menstruation every month, which is a period of growth that requires more iron (Fe)5.

In general, anemia is caused by the body being unable to produce healthy and ideal red blood cells due to a lack of iron in the food or drink consumed.⁶

Considering the important role in many cellular procedures, iron is essential for life. Approximately 200 billion new red blood cells are produced each day, and iron is an essential part of the production process. The presence of other nutrients, such as proteins and vitamins, affects iron metabolism⁶.

Protein, iron, copper (Cu), zinc (Zn), vitamin C, and vitamin A are nutrients that affect Hb levels. In the long term, iron deficiency anemia in adolescent girls causes stunted growth, decreased school performance, decreased immunity, irregular menstruation, and increased risk of infection. Therefore, adolescent girls who experience iron deficiency anemia must be treated immediately to prepare for the next pregnancy7. Data collected by WHO (World Health Organization) showed that the risk level of anemia in adolescent girls worldwide ranges from 40 to 88%. The number of interventions in developing countries is estimated at 53.7%, increasing from 22.7% in 2013 to 32% in 20188. These results show that anemia is very common in adolescent girls, presumably due to poor diet and lack of exercise. According to an anemia survey conducted in 2005 in four



districts/cities in North Sumatra, 40.5% of adolescent girls experienced iron deficiency anemia8.

The Ministry of Health has taken special measures by offering adolescent girls iron supplements (TTD) through the UKS/M at school (junior high school, high school, or equivalent). The supplement is administered simultaneously with one tablet given every week. The purpose of administration is to meet iron needs of girls who will become mothers in the future. Therefore, the possibility of anemia in pregnant women, bleeding during childbirth, malnutrition syndrome (LBW), and short toddlers can be reduced with sufficient iron intake from an early age8. Data from Riskesdas 2018 showed that 76.2% of female adolescents received TTD and 80.9% received at school. The prevalent problem was that TTD consumption was still low, where 98.6% did not consume about 52 tablets. A study conducted at Karanganom State High School found that 65.9% of female adolescents were not compliant with consuming TTD. The occurrence of anemia is also caused by insufficient iron intake from food9.

The availability of vitamin C greatly affects iron absorption, causing an increase in the non-heme form up to 4 times. This is because, in the process of iron absorption, vitamin C can help reduce ferric iron (Fe3+) in the small intestine to produce ferrous (Fe2+). The reduction process increases along with the rise in the acidic stomach pH. Vitamin C can increase iron absorption by up to 30% and adolescent girls need 65 grams per day10. Sweet orange is one of the fruits with a significant amount of vitamin C, amounting to 49 mg per 100 grams of fruit. Orange contains many compounds and helps the absorption of iron which is good for health, and other nutrients such as calcium, magnesium, phosphorus, folic acid, vitamin B6, vitamin C, carbohydrates, flavonoids, and antioxidants. In addition, orange is easy to acquire, cheap, practical, widely consumed, and have no allergies¹¹.

SMP Negeri 1 Atap Tanjung Balai City, North Sumatra, is located in the working area of Kampung Baru Health Center and studies on Hb levels are yet to be conducted at the school. The provision of TTD to female adolescents was carried out for Grade 8 students in January 2022, namely 24 tablets for 6 months. Tanjung Balai City is located in the east coast area and has abundant fish products which are a source of iron. Therefore, this study aimed to examine the differences in Hb levels and iron intake of female adolescents who consume TTD and TTD combined with orange in SMP Negeri 1 Atap Tanjung Balai City.

METHODS

This study was conducted using a Quasi-Experimental design with an unequal control group method at SMP Negeri 1 Atap Tanjung Balai City. This location was selected purposively because students are generally of lower middle economic class, rarely eat fruit, and the school only accepts 1 class per year. A sample of 45 students in Grades 8 and 9 who had started menstruating were divided into two groups based on class. Grade 9, with 22 people, became the control group given government program TTD tablets. Each tablet consisted of 60 mg iron and 0.25 mg folic acid. Meanwhile, 23 students in Grade 8 became the intervention group given TTD combined with vitamin C from sweet orange weighing 130 grams, which contains 63.7 grams of vitamin C. The intervention was given once a week for 12 weeks. In the first week, the samples received TTD and vitamin C directly, similar to the samples given TTD. All of these interventions were consumed directly and supervised. Using Easy Touch GCHb, Hb was measured before and after the intervention, while data were tested using the independent t-test. This study received the KEPK Poltekkes Kemenkes Medan in 2023 with No. 01.1475/KEPK Poltekkes Kemenkes Medan in 2023.

RESULTS AND DISCUSSIONS

The characteristics of the samples collected were age and ethnicity. Characteristic data were distinguished between control and intervention groups. Univariate analysis was used to determine the distribution of data as presented in Table 1. Table 2 shows the results of a bivariate test with a dependent test used to determine the average Hb levels before and after intervention, as well as minimum and maximum values. The number of samples whose Hb increased and decreased were distinguished between controls and interventions.

Table 1. Sample characteristics based on age and ethnicity

	Group					
Variables	Control (n=23)	%	Intervention (n=22)	%		
Age						
13	0	0	11	50.0		
14	13	56.5	9	40.9		
15	10	43.5	2	9.1		
Ethnic group						
Java	8	34.8	5	22.7		
Batak	8	34.8	11	50.0		
Malay	4	17.4	3	13.6		
Minang	3	13.0	3	13.6		

e-ISSN: 2580-1163 (Online)

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Table 2. Mean Hb levels before and after intervention, increase in Hb levels, minimum and maximum values, number of samples with increase and decrease in Hb between controls and interventions

	G			
Variability	Control (n=23)	Interventions (n=22)	p-value	
Hemoglobin				
Before	12.70 gr/dL	12.69 gr/dL	<0.001*	
After	13.48 gr/dL	14.63/dL		
Hemoglobin After Treatment				
Minimum value	10 gr/dL	12.3 gr/dL		
Maximum value	16.3 gr/dL	17.3 gr/dL		
Number of Samples				
Hemoglobin increases	22 people	22 people		
Hemoglobin down	1 person	0		

^{*}Dependent t-test, p-value < 0.05

Table 1 shows that the highest ages in the control and intervention groups were 13 and 11 years, respectively. The highest ethnicity was Batak followed by the Javanese and the lowest was Minang. These results show that the sample originated from various ethnicities. Table 2 shows that the average Hb levels before the intervention were almost the same between the intervention group and the control group. After treatment, Hb levels of the intervention group increased,

an average of 0.78 g/dL in the control group and an increase of 1.94 g/dL in the intervention group, with a minimum increase of 0.8 g/dL and a maximum increase of 3.9 g/dL. The minimum and maximum values in the control group were 10 gr/dL and 16.3 gr/dL, lower than the intervention group, with 12.3 gr/dL and 17.3 gr/dL. Therefore, there was a significant difference in Hb levels before and after the intervention in both groups.

Table 3. Mean increase in Hb, minimum, maximum values, and standard deviation between control and intervention groups

Variables	Group	Mean	Minimum	Maximum	SD	p-value
Hemoglobin Level	Control (n=23) Interventions (n=22)	0.78 1.94	-0.8 0.8	1.9 3.9	0.52 0.84	<0.001*

^{*}Independent t-test, p-value < 0.05

Table 3 shows that the intervention group had a high increase in Hb levels on average compared to the control group, with a difference of 1.16 g/dL. In the control group, the increase obtained was a minimum and maximum value of -0.8 mg/dL and 0.8 mg/dL, lower than the intervention group whose minimum and maximum values were 1.9 gr/dL and 3.9 gr/dL. The statistical test results showed that there was a difference in the average increase in Hb levels between the control and the intervention group (p-value=0.05).

Each TTD contains 60 mg of iron, while the requirement for adolescents is approximately 15 mg per day. The observed increase in Hb levels of students at SMP Negeri 1 Atap underscores the importance of iron supplementation. Adolescent girls can lose iron due to bleeding during menstruation; hence, supplementation of TTD is very helpful. To maintain balance, women need an additional TDD of 1 mg per day because during menstruation an average of 60 ml of blood is released each month, which is equivalent to 30 mg of iron¹². For patients with iron deficiency anemia, supplementation using TTD is a cheap and effective method to restore iron balance.

The results showed that during 12 weeks of using TTD supplementation, the average blood Hb level increased by 0.78 mg/dL before and after the use of TTD (p-value<0.05). A study by Tonasih et al. (2019) reported a significant increase in Hb levels both before and after iron supplements. Additionally, Suparningsih (2018) found that iron supplementation effectively raised Hb

levels in both pregnant and non-pregnant women¹³. A study conducted by Kusdalinah et al. (2023) showed that administering vitamin C and TTD increased Hb levels of pregnant women at Wundulako Health Center, Kolaka Regency, by 2.37 times¹⁴. Agusmayanti et al. (2020) found that pregnant women with anemia who were given 50 mg vitamin C tablets combined with TTD every day for 14 days had increased Hb levels after lunch¹⁵.

In the intervention group, administration of TTD combined with vitamin C increased the absorption of TTD. Several nutrients increase the bioavailability of iron, including vitamin C, organic acids, fish and meat proteins, as well as peptides from partially digested muscle tissue. Vitamin C is the most effective enhancer of iron absorption, increasing the absorption of ferric (Fe3+) and ferric ions (Fe2+). The reducing properties of vitamin C allow iron to dissolve in a wide range of pH and be absorbed through the iron transporter, namely the divalent metal transporter 1 (DMT1) in the small intestine¹⁶.

Vitamin C belongs to a group of nutrients useful for increasing or enhancing iron absorption. It is recommended that adolescent girls consume 65 grams daily¹⁵, while an intake of 100 mg can be absorbed at an efficiency of 80-100%. In pregnant women, iron absorption may be increased by 37.5-46% through the administration of vitamin C in tablets. Vitamin C plays an important role in the absorption of iron, specifically nonheme iron found in plant foods¹⁷.

Daily intake of vitamin C is highly recommended, specifically when consuming iron-rich foods as it facilitates the formation of ascobate iron clusters that dissolve in the high pH of the duodenum [19]. This mechanism enables the reduction of non-heme iron to ferric ions (Fe3+ and Fe2+). Apoferritin binds ferritin (Fe3+) after being absorbed by mucosal cells, and the bond will be released in the serum. Furthermore, iron is transported through transferrin, which is an iron chain with 3-4 mg of protein. This bond is then stored in the spleen, bone marrow, and liver. The body utilizes 20-25 mg of iron daily for Hb synthesis and the replacement of damaged Hb, with these two functions accounting for 60-70% of total Hb production¹⁷. Vitamin C is often used to increase iron absorption and metabolic mechanisms. However, when vitamin C consumption is insufficient or iron intake is unbalanced, the function of vitamin C in the body will be disrupted, resulting in decreased Hb levels. Agusmayanti and Anggraini (2020) stated that adolescents who do not consume enough vitamin C experience anemia¹⁵.

Nutrient interaction influences iron absorption, for example, consuming foods that contain various nutrients allows for a synergistic effect that enhances bioavailability. Orange provides not only iron but vitamin C, which forms soluble iron ascorbate complexes, readily absorbed. Additionally, orange is rich in folate, which helps the body make new red blood cells. Vitamin C also increases the absorption of iron in the body and contributes to the delivery into the blood, specifically the mobilization of hemosiderin iron stores in the spleen¹³.

The results showed that the treatment group given TTD with orange weekly for 12 weeks experienced an average increase in Hb from 12.69 grams/dl to 14.63 grams/dl or an increase of 1.94 grams/dL. Meanwhile, the control group who only consumed iron supplementation experienced a lower increase in Hb, namely from 12.70 grams/dl to 13.48 grams/dl, or an increase of 0.78 mg/dL. The independent t-test analysis with a 95% confidence level showed that there was a significant difference in Hb levels between the two treatment groups (pvalue=0.001). TTD together with various micronutrients (micronutrients) were more effective in increasing Hb levels than only iron supplements alone. Therefore, iron supplementation should be accompanied micronutrients such as vitamins A, C, and D, or calcium to improve iron absorption by the body. Similar results were reported by Wahyuni (2021) who stated that administering TTD and vitamin C at a dose of 50 mg daily increased Hb levels for 14 days with p-value=0.00018. Another study by Kusdalinah et al. (2023) found that the supplementation of TTD, vitamin C, and protein to 14year-old female students increased Hb levels¹⁹.

Several factors can inhibit iron absorption, including tannin compounds found in tea. These compounds have the ability to bind various metals, such as aluminum, calcium, and iron, creating complex chemical bonds. The presence of iron and calcium compounds in foods makes iron absorption more difficult, causing a reduction in iron²⁰. Iron status is influenced by the absorption of heme iron, which is generally more efficient than non-heme. Therefore, ferritin is used as a measure of iron status¹⁸.

The loss of iron between 12.5-15 mg per month or 0.5 mgFe/ml is associated with the monthly blood volume caused by menstruation in adolescent girls, which ranges between 30 and 50cc²¹. The body stores are reduced when iron loss exceeds intake. This occurs because the iron stored in the form of ferritin and hemosiderin is depleted, reducing availability for standard needs. It also interferes with the supply of iron to the apotransferrin transport protein. Therefore, transferrin saturation decreases, and receptors in the circulation increase. Iron deficiency causes decreased production of red blood cells and Hb. Deficiencies in nutrients important for erythropoiesis, including folic acid and vitamin B12, can also cause anemia²² but vitamin B12 and folic acid intake were not assessed in this study.

The administration of TTD with vitamin C led to increased Hb levels among adolescents. Adolescents require approximately 65 mg of vitamin C daily, and consuming vitamin C-rich fruits, such as sweet orange (130 grams), which provide 63.7 mg, significantly enhances iron absorption. Susilo Wirawan et al. (2015) showed that iron supplements alone were less effective in increasing Hb levels compared to treatment using TTD together with various micronutrients²³. Therefore, iron supplementation must be combined with micronutrients such as vitamins A and C to increase iron absorption. Rista Andaruni, NQ & Nurbaety (2018) found that the administration of TTD and vitamin C increased Hb levels by 2–20% in adolescent girls, with a significantly different result compared to those given TTD alone¹⁰. In general, iron absorption increased by about 10% with the administration of tablet (60 mg) and vitamin C (100 mg). Iron and the water-soluble and easily digested ascorbate complex are formed by vitamin C. Foods such as tea, coffee, and chocolate are not to be consumed together due to the ability to bind iron. Polyphenols including tannic acid and tannin also bind minerals such as iron when oxidized, inhibiting iron absorption²⁴.

A study by Li et al. (2020) performed an intervention with 100 mg oral TTD combined with 200 mg vitamin C (vitamin C, 100 mg/tablet) every 8 hours daily, while the control group received 100 mg TTD (ferrous succinate, 100 mg/tablet) every 8 hours daily25. The results showed that oral iron supplementation alone increased Hb and absorption of iron stores in patients with deficiency anemia. Patients with iron deficiency anemia can use oral iron, a cheap and effective method to restore iron balance. The only food ingredient other than animal tissue that has been shown to increase nonheme iron absorption in humans is vitamin C. Some clinicians recommend taking vitamin C supplements in addition to oral TTD to accelerate anemia treatment. However, the absolute dose of vitamin C does not appear to have a directly proportional effect on iron absorption²⁶. The addition of vitamin C to iron supplementation in the management of nutritional deficiency anemia showed statistically significant but clinically insignificant results with an increase in serum Hb of 0.14 g/dL. There was also a statistically significant increase in serum ferritin of 3.23 μg/L, but the clinical relevance remains uncertain. The strength of this analysis is the number of randomized controlled trials (RCTs) included. The conclusions are limited by the heterogeneity of interventions such as the

use of different formulations/doses across studies. The results do not provide strong evidence to support the addition of vitamin C to iron supplementation²⁷.

CONCLUSIONS

The provision of TTD intervention combined with vitamin C showed better results for increasing adolescent Hb compared to intervention using TTD alone. To enhance the efficacy of iron supplementation in schools, it is necessary to provide vitamin C-rich food alongside tablets to improve absorption. Furthermore, the success of TTD provision program requires direct supervision from the school and the Health Center to achieve even distribution and proper consumption.

ACKNOWLEDGEMENT

The authors are grateful to the Principal of SMP Negeri 1 Atap Tanjung Balai City and the staff who have helped in implementing this study. The authors are also grateful to the respondents who cooperated towards the completion of this study.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest in this article. This study is self-funded by the authors.

AUTHOR CONTRIBUTIONS

The authors contributed to the writing of this article. US as the primary author contributed to: conceptualization, investigation, methodology, supervision, writing review and editing; HN: methodology, writing-original draft and formal analysis; NM: formal analysis, resources, and editing.

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e-ISSN: 2580-1163 (Online) p-ISSN: 2580-9776 (Print)

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