The Efficacy of Mung Bean Drink with Inulin and Iron Tablets in the Erythropoiesis Response of Adolescent Girls with Iron Deficiency Anemia: A Randomized Controlled Trial

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ABSTRACT

Anemia in adolescent girls can have an impact on immunity, reproductive health, and cognitive function. Long-term treatment of anemia with iron tablets can cause gastrointestinal side effects resulting in decreased adherence and increased enteropathogenic bacteria. This study aims to determine the effectiveness of mung bean drinks with inulin and iron tablets in the erythropoiesis response of adolescent girls with iron deficiency anemia. This is a non-blinding randomized controlled trial study, by recruiting 61 female students from junior and high schools in Sleman Regency, Yogyakarta, Indonesia. Randomly assigned into two groups, the treatment group (n=30) received mung bean drink (40 g/250 ml) with inulin (10 g) and iron tablets (containing 60 mg elemental iron and 400 mcg folic acid), whereas the control group (n=31) received iron tablets and palm sugar drink (18 g/250 ml). Mung bean drink, palm sugar drink, and iron tablets were consumed once a day for 12 weeks. Data collection included monitoring of adherence to drinking and blood tests. The results showed that there was erythropoiesis response characterized by a significant increase in hemoglobin levels and change in reticulocytes counts. Hemoglobin levels increased by 1.08 g/dL (p<0.05) after 2 weeks, 1.82 g/dL (p<0.05) after 4 weeks, and 3.28 g/dL (p<0.05) after 12 weeks. Meanwhile, reticulocytes count increased by 0.59% (p<0.05) after 2 weeks, decreased by 0.14% (p<0.05) after 4 weeks, and decreased by 0.1% (p<0.05) after 12 weeks. The change in reticulocytes counts was consistent with the increase in hemoglobin levels. This proves that mung bean drink with inulin enhances the effect of iron tablets and is effective in erythropoiesis response, thus it can be used for the prevention and treatment of iron deficiency anemia in adolescent girls.

Keywords: Mung bean, prebiotic inulin, erythropoiesis response, iron deficiency anemia.

INTRODUCTION

The incidence of anemia in adolescent girls tends to increase because they are in a period of rapid growth that requires more iron, while their food intake contains less iron, and they suffer iron loss through monthly menstruation (WHO, 2011). This can impact on immunity, reproductive health, and cognitive function, which can affect academic achievement (Georgieff, 2017). When anemia occurs, erythropoiesis in the bone marrow increases which is reflected in an increased reticulocytes count in the peripheral blood circulation (Piva, 2015). Therefore, reticulocytes count can be used to describe the productivity...
and activity of erythropoiesis in the bone marrow, diagnosis of anemia, and evaluation of iron therapy in iron deficiency anemia (Suega, 2015a). Anemia is typically treated by supplementing with iron tablets for a certain period of time to increase hemoglobin levels and the body's iron stores (WHO, 2011). However, long-term supplementation with iron tablets can cause gastrointestinal side effects, which may decrease adolescent girls’ adherence to taking the tablets (Urrechaga et al., 2013) and increase the growth of enteropathogenic bacteria (Zimmermann et al., 2010). The Indonesian government has implemented an iron tablet supplementation program since the 1990s, but the prevalence of anemia in women aged 15-24 years remains at 32% (Indonesian Ministry of Health, 2019).

Mung beans are a functional food (Suter, 2013) commonly found in Pakistan, China, South Asia, and Indonesia (Ahmad & Tahir, 2017). They are affordable, making them popular as food and drink ingredients, including in mung bean drinks. The Indonesian government has frequently used mung beans as supplementary feeding in integrated healthcare centers (Posyandu). Mung beans contain a variety of macro and micronutrients that can increase hemoglobin levels (Dahiya et al., 2014; Hou et al., 2019). Inulin is a fructan-type prebiotic (Wilson & Whelan, 2017), which cannot be digested in the stomach and small intestine but can be selectively fermented by probiotic bacteria (Bifidobacteria and Lactobacillus) in the large intestine, thereby enhancing mineral absorption including iron, and inhibiting the growth of enteropathogenic bacteria in the large intestine (Carlson et al., 2017; Slavin, 2013). Inulin is the best natural prebiotic, is more stable, has a sweet taste, and dissolves easily compared to other prebiotics, making it a popular choice for use in mixed drinks (Al-Sheraji et al., 2013). Most of the research in Indonesia has examined the effectiveness of mung bean drinks, with or without iron tablet supplementation, on hemoglobin levels in pregnant women. Various studies have examined adolescent girls (Carolin et al., 2021; Maulina et al., 2022; Olii et al., 2022); however, these gave mung bean drinks only without added prebiotics (Inulin) and the duration of the research was less than 12 weeks. Researchers who have studied reticulocyte count in adolescent girls include Ivana et al. (2019), but their intervention was by giving iron tablets only.

Research that adds prebiotics (inulin) to mung bean drinks and its effect in the erythropoiesis response has never been done. Therefore, this study was conducted to determine the effectiveness of mung bean drink with inulin and iron tablets in the erythropoiesis response, which is characterized by an increase in hemoglobin levels and changes in reticulocyte count and can be used for the prevention and treatment of iron deficiency anemia in adolescent girls.

**MATERIALS AND METHODS**

**Study design**

This is a non-blinding randomized controlled trial study, with pre-test-post-test with control groups. The research was conducted at junior and high schools in Sleman Regency, Yogyakarta, Indonesia. The research period was October 2021 – December 2021.

**Study subject**

The sample size was determined using the formula for the difference in the proportions of the two populations, with a significance degree of 5% and a test power of 80%. The minimum sample size was calculated to be 60 respondents, who suffered from iron deficiency anemia (Hb level <12 g/dL) and met the inclusion criteria of being between 10-18 years old, having normal menstrual periods and menstrual cycles, and being willing to be research respondents by signing informed consent. The exclusion criterion was having diseases that interfered with the process of hematopoiesis. The researchers randomly divided the respondents into two groups using the Random.org application. Thirty respondents in the treatment group were given mung bean drink (40 g/250 ml) with inulin (10 g) and iron tablets (containing 60 mg elemental iron and 400 mcg folic acid), whereas thirty-one respondents in the control group were given iron tablets and palm sugar drink (18 g/250 ml). The mung bean drink and palm sugar drink were taken at a dose of 1 bottle (250 ml) per day for 12 weeks and the iron tablets were taken at a dose of 1 tablet per day for 12 weeks.

**Study approval**

This study has been approved by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Universitas Gadjah Mada, Dr. Sardjito with Protocol Number: KE/FK/0319/EC/2021.
Intervention material and preparation

The mung bean drink was made from the mung bean brand 'Australian Choice Exports' of the Katoomba variety (Australia). In 100 grams of raw mung beans, there is protein (14.6-33 g), iron (5.9 mg), zinc (2.7 mg), vitamin C (3.1 mg), vitamin A (70-100 g RAE), and dietary fiber (16 g) (Dahiya et al., 2014; Hou et al., 2019). Before processing, the mung beans were sorted by selecting bright and compact, then soaked in water for at least four hours, washed until clean, and then drained. To make 1 kg of mung beans, six liters of water were added and boiled until soft. After boiling and softening, the mung beans were blended and strained until mung bean extract was obtained. The mung bean extract was boiled again with the addition of water, granulated sugar (200 g), palm sugar (300 g), and salt as desired, until boiling. After boiling, the mung bean extract was strained again and then cooled until its temperature was below 60°C, measured using a digital food thermometer, and then inulin was added.

Inulin powder from Beneo Orafti® GR was added as much as 10 grams to 250 ml of mung bean drink after the temperature of the mung bean drink was < 60°C. It was then placed in 250 ml packaging bottles and cooled in the refrigerator for four hours. The nutritional content analysis found in the mung bean drink with inulin in this study revealed a total protein content of 1.48%, iron content of 6.3 mg, zinc content of 1.50 ppm, vitamin C content of 31.15 mg, and a total dietary fiber content of 4.68%.

The palm sugar drink was made from a mixture of Javanese sugar and granulated sugar with a ratio of 80:20 (640 grams of palm sugar and 160 grams of granulated sugar). To maintain product standardization, the mung bean drink and palm sugar drink were made by Sari Kedelai Bu Ade with the MD number 232012001125.

Iron tablets containing 60 mg of elemental iron and 400 mcg of folic acid were obtained from the Sleman District Health Office.

Data collection

The data collection included adherence to mung bean drinks and palm sugar drinks and was obtained from the Visual Comstock method form. Data on adherence to taking iron tablets was obtained from record cards for taking iron tablets. Data of hemoglobin levels and reticulocyte counts were measured four times: baseline, two weeks, four weeks, and 12 weeks after intervention. The data were obtained from blood samples from the medial cubital vein as much as 3 ml, which were examined with a hematology analyzer Sysmex XN-1000 with spectrophotometry method for hemoglobin and flow cytometry method for reticulocytes at Dr. Sardjito Hospital Yogyakarta.

Data analysis

Bivariate analysis was carried out with several tests, including the Shapiro-Wilk test to determine the normality of the data distribution; the T-independent test was used to analyze the difference of hemoglobin levels and reticulocyte counts when the data were normally distributed, and the Mann-Whitney test when the data were not normally distributed. Statistical analysis was performed with a significance level of alpha < 0.05.

RESULTS AND DISCUSSION

Hemoglobin level and reticulocyte count before intervention

The hemoglobin levels in both groups were anemic (< 12 g/dL) before intervention, the level was 9.79 g/dL in the treatment group and 10.78 g/dL in the control group (Figure 1). The reticulocyte count in both groups was normal before the intervention, 0.96% in the treatment group and 1.02% in the control group (Figure 2). Telambenua et al.'s (2014) research on 30 patients with iron deficiency anemia at M. Djamil General Hospital in Padang also found the average of reticulocyte count of the patients before treatment was normal (1.33%). Similarly, the results of Syahendra's (2020) study on 32 patients with iron deficiency anemia at Arifin Ahmad General Hospital in Pekanbaru also found the average of reticulocyte count of the patients before treatment was normal (1.4%). Ivana et al.'s (2019) research on 40 anemic female students in Surakarta also found the reticulocyte count of both groups was normal before intervention, the count was 1.30% in the treatment group and 1.15% in the control group.

The reticulocyte count in both groups was normal because all respondents in this study had iron deficiency anemia. In anemic conditions, the bone marrow will respond by increasing the production of reticulocytes by 15 to 20 times compared to normal conditions due to an increase in the erythropoietin hormone as an effort of the body to maintain homeostasis (Piva et al., 2015). Under these conditions, reticulocytes shorten their maturation time in the bone marrow and prolong their maturation time in the peripheral blood circulation.
The reticulocytes found in people with anemia are also called stress reticulocytes, which refer to an increase in reticulocyte production as an adaptive response of the body to overcome the lack of red blood cells due to iron deficiency (Kiswari, 2014).

The Effect of Intervention in Hemoglobin Levels and Reticulocytes Counts

Two weeks after the intervention, the average hemoglobin level and reticulocyte count increased significantly. The hemoglobin level increased from 9.79 g/dL to 10.87 g/dL (p<0.05) (Figure 2), and the reticulocyte count increased by 0.59% (p<0.05) (Figure 3). In anemic conditions, erythropoiesis activity in the bone marrow increases. The hormone erythropoietin influences hematopoietic stem cells to proliferate and differentiate into mature erythrocytes (Kiswari, 2014). Stem cells need iron (Geisser & Burckhardt, 2011). It is estimated that two weeks after drinking mung bean drink with inulin and iron tablets, the respondents already had iron in their bodies to synthesize hemoglobin in the bone marrow,
resulting in an increase in the average of hemoglobin levels and reticulocyte count for up to 6-10 days or until hemoglobin level was normal (not anemic).

Four weeks after intervention, the average of hemoglobin level continued to increase significantly, from 10.87 g/dL to 11.61 g/dL (p<0.05) (Figure 2), whereas the reticulocyte count decreased by 0.14% from the baseline count (p<0.05), (Figure 3). This was due to the anemia of respondents in the treatment group decreasing from 80% to 60% (Figure 1). Similarly, twelve weeks after the intervention, the average of hemoglobin levels continued to increase significantly, from 11.61 g/dL to 13.07 g/dL (p<0.05) (Figure 2), whereas the reticulocyte count decreased by 0.1% from the baseline count (p<0.05), (Figure 3). This was due to the anemia of respondents in the treatment group decreasing from 60% to 16.7% (Figure 1).

There was a relationship between the percentage of anemia and the reticulocyte count. As the number of respondents who were anemic decreased at four weeks and 12 weeks after intervention, the process of erythropoiesis decreased, leading to a decrease in the reticulocytes count. According to Piva et al. (2015), the reticulocyte count increases in iron deficiency anemia and during treatment with iron and then decreases as the erythropoiesis responses decrease. Therefore, the reticulocyte count can be used to describe erythropoiesis activity and the success of interventions for iron deficiency anemia (Suega, 2015b).

The Effect of Intervention on the Difference in Hemoglobin Levels and The Difference in Reticulocyte Counts after 12 Weeks

After 12 weeks of intervention, the average hemoglobin level of the treatment group (3.28 g/dL) was significantly higher than the control group (2.29 g/dL), (p<0.05) (Table I). Meanwhile, the median reticulocyte counts in the treatment group (0.21%; -1.6-1) was slightly higher than the control group (0.09%; -0.53-0.73), but not significant (p>0.05) (Table I). The reticulocyte count changes in parallel with the decrease in the percentage of anemia, which reflects a decrease in erythropoietic response.

The difference in hemoglobin levels and the difference in reticulocyte counts after 12 weeks of intervention may be due to the respondents having iron from iron tablets and macro and micronutrients contained in mung bean drinks with inulin to synthesize hemoglobin. In mung bean drinks, some proteins play a role in iron metabolism such as intracellular iron transport, intracellular iron homeostasis, and systemic iron regulation (Suega, 2015). In addition, mung beans contain non-heme iron, which, although its bioavailability is lower than heme iron, is still important as a source of iron for hemoglobin synthesis due to the large amount found in food (Hurrell & Egli, 2010). Vitamin C acts as an enhancer that reduces ferric to ferrous form so that it can enterocytes and be transported by DMT1 and can increase the absorption of non-heme iron four times higher (Suega, 2015). Zinc is a cofactor for the Amino Levulinic Acid (ALA)-dehydratase enzyme that plays a role in heme synthesis when in the cytosol of bone marrow cells (Meagher, 2016). The fiber content in mung beans cannot be hydrolyzed by digestive enzymes, and prolongs transit time in the stomach, thereby increasing enterocyte gene expression for iron absorption (Susanti et al., 2016).

All respondents in this study consumed iron tablets for 12 weeks. Long-term consumption of iron tablets can cause gastrointestinal side effects that can decrease adolescent girls' compliance to consume them (Urrechaga et al., 2013). According to the Indonesian Ministry of Health (2019), many adolescent girls do not consume the iron tablets they receive from school because of feeling they do not need it (26.1%), have unpleasant taste and smell (22.9%), forget to consume it (20%), side effects (8.9%), only take it during menstruation (6.6%), they are not yet finished (3.8%), and other reasons (11.7%). The results of Joshi and Gumasheta's (2013) study in India, Damayanti's (2018) study in Purworejo Regency, and Kantari's (2021) study in Mataram support this finding. Therefore, we need another alternative that can increase iron absorption without causing gastrointestinal side effects.

Inulin can increase iron absorption because its fermentation in the large intestine by Bifidobacteria and Lactobacilli microbiota produces Short Chain Fatty Acids (SCFAs) that lower the luminal pH of fatty acids (SCFAs) that lower the luminal pH of the colon and increase the bioavailability of iron and improve the absorption of iron that is not absorbed in the duodenal enterocytes and proximal jejunum (Carlson et al., 2017; González-Herrera et al., 2015; Parmanand et al., 2019; Wilson & Whelan, 2017).
According to Anderson and Frazer (2017), there is a small amount of iron that can still be absorbed in the distal digestive tract. As a fiber, inulin increases transit time in the stomach, thereby increasing enterocyte gene expression for iron absorption (Freitas et al., 2012; Slavin, 2013).

Long-term supplementation of iron tablets can increase the growth of enteropathogenic bacteria such as Salmonella, Shigella, and Escherichia coli (Zimmermann et al., 2010) because the unabsorbed iron that reaches the large intestine can be utilized by enteropathogenic bacteria for their growth (Parmanand et al., 2019). The fermentation of Bifidobacteria and Lactobacilli in the large intestine produces SCFAs that can inhibit the growth of enteropathogenic bacteria by lowering the colon pH, thereby disrupting the growth of enteropathogenic bacteria that require a neutral or alkaline pH, damaging the cell membrane of enteropathogenic bacteria, and interfering with the replication of enteropathogenic bacteria DNA (Zhang et al., 2020). This is supported by the research of Liutkevičius et al. (2016), which found a decrease in E. coli bacteria after taking SPI (SUPRO XT 219D IP) 20 gr/day and inulin 10 gr/day for 21 days.

There was a significant difference in hemoglobin levels between the treatment and control groups in this study (p<0.05), but there was no significant difference in reticulocytes counts.

Table I. The Effect of Intervention on the Difference in Hemoglobin Levels and the Difference in Reticulocyte Counts After 12 Weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group (n = 30) Mean ± SD (gr/dL)</th>
<th>Control Group (n = 31) Mean ± SD (gr/dL)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin level (K4-K1)</td>
<td>3.28 ± 1.77</td>
<td>2.29 ± 1.23</td>
<td>0.015**</td>
</tr>
<tr>
<td>Reticulocyte counts (K4-K1)</td>
<td>0.21 (-1.6 - 1)</td>
<td>0.09 (-0.53 - 0.73)</td>
<td>0.207b</td>
</tr>
</tbody>
</table>

Intervention: -treatment group: mung bean drink with inulin and iron tablets; control group: iron tablets and palm sugar drink; K1: Baseline; K4: 12 weeks after intervention; *Analysis with T Independent test; b Analysis with Mann Whitney test; Significant (p< 0.05)
between the treatment and control groups (p>0.05) even though both groups experienced changes. This may be due to the small difference in reticulocytes counts between the treatment group (0.21%) and the control group (0.09%), which is only 0.12%, so the data variability is also very small.

CONCLUSION

Based on the results of the study it can be concluded that the intervention of mung bean drink with inulin enhances the effect of iron tablets and is effective in erythropoiesis response, thus it can be used for the prevention and treatment of iron deficiency anemia in adolescent girls without causing side effects on the digestive tract.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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