



The effect of red bean powder (*Phaseolus vulgaris* L.) as adjuvant therapy on the blood profile of anemic white rats

Riza Umami^{1*}, Rasipin², Sutopo Patria Jati³

¹ Midwifery Student, Graduate Program, Master Applied of Health, Indonesia

^{2,3} Politeknik Kesehatan Kemenkes Semarang, Semarang, Indonesia

Abstract

Background: The prevalence of anemia in Indonesia is highest in women of childbearing age between the ages of 15-24 years, as many 84.6%. The most common causes of anemia are iron deficiency, resulting from prolonged lack of iron, inadequate intake or absorption of iron, increased need for iron during pregnancy or growth, and increased iron loss as a result of menstruation and intestinal worms. Giving tablets added blood to girls is 76.2%, but those who consume blood-added tablets are only 1.4% due to side effects experienced after consuming these added tablets. Kidney beans are legumes that have complete nutritional content. Flavonoids in kidney beans function for treatment in anemia can work together with iron to ward off free radicals in the form of hydroxy and superoxide so that it protects membrane lipids, prevents cell damage, and protects blood cells.

Objective: To determine the effect of red bean powder on the blood profile of white rats (*Rattus norvegicus*).

Method: This research is *Quasy experiment with pre-posttest design with control group design*. The number of samples in this study were 35 rats divided into 5 groups. The research instrument used a hematology analyzer, preparations, and microscopes.

Results: There was an effect of instant red bean powder on the increase in hemoglobin and reticulocytes with a p value <0.05.

Conclusion: Giving red bean powder is very influential in increasing hemoglobin and reticulocyte levels.

Keywords: kidney beans, blood profile, white rat, anemic

1. Introduction

Anemic is a condition in which the number, size of red blood cells decreases, as well as the concentration of hemoglobin and hematocrit fall below the established normal value, consequently disrupting the blood's capacity to carry oxygen throughout body. Anemia is an indicator of poor health and nutrition [1]. According to WHO the problem of anemia is a public health problem in the world both in low, middle and high income countries [2]. Women of childbearing age and children constitute the group with the highest risk of anemia, the estimated prevalence of anemia in nonpregnant women aged 15-49 years 30.2%, 41.8% pregnant women and in preschool children 47, 5%.³ In 2025 the WHO targets a 50% reduction in anemia in Women of childbearing age [2].

The prevalence of anemia in Indonesia is highest in women of childbearing age between the ages of 15-24, which is 84.6%.⁴ The most common causes of anemia are iron deficiency, which results from prolonged lack of iron, caused by inadequate intake or absorption of iron, increased need for iron during pregnancy or growth, and increased iron loss as a result from menstruation and intestinal worms. Whereas 50% of anemia in women worldwide is caused by iron deficiency [2].

Iron deficiency is the most common nutritional deficiency worldwide, affecting 30 million people in developed and developing countries. In humans and animals, iron is essential for the implementation and maintenance of many vital cellular functions and biosynthetic processes, including oxygen transportation, aerobic cellular activity, intracellular electron transport, and integral enzymatic reactions in body tissues. Iron is not only needed for the formation of

hemoglobin that plays a role in oxygen storage and transport, it is also present in several enzymes that play a role in oxidative metabolism, deoxyribonucleic acid (DNA) synthesis, neurotransmitters and catabolism. Iron deficiency has a lot of impact which is causing disruption of child development, decreasing endurance and decreasing learning concentration [5, 6].

Regarding the importance of iron, intervention should be given to supplement Fe. Iron (Fe) is an important element for cellular homeostasis, oxygen transportation, DNA synthesis, and energy metabolism as well as cofactors for mitochondrial respiratory chain enzymes and nitrogen fixation. In human nutrition, Fe is used mainly in the synthesis of hemoglobin in erythrocytes, myoglobin in muscles, and cytochromes in the liver.

Giving blood added tablets has been going well seen from the data of riskesdas (Basic Health Research) in 2018 giving blood tablets for adolescent girls is quite high at 76.2% compared to those who did not receive it at 23.8%, and young women getting blood added tablets at school by 80.9%, but even though this number is already high, young women who consume ≥ 52 items only 1.4%, while those who consume <52 items are 98.6% [4]. From this data shows that there are still many young women who do not consume blood boosting tablets that have been given at school, causing an increased prevalence of anemia in young women [7].

Non adherence of young women in consuming blood-added tablets is caused by many factors, namely the side effects caused after consuming blood-added tablets (Fe) such as nausea, constipation and black stools. Other reasons are bad taste and fishy smell, feeling bored and forgetful, which

causes young women to be lazy to consume Fe tablets [8]. Management of current government programs has not yet reached the maximum results from the lack of non-compliance to take Fe tablets, iron-rich plant material is needed as a companion therapy from blood-added tablets, such as legumes with the highest iron content obtained from whole seeds, in soybean seed research that has been tested and proven to be a good source of iron for animal and human nutrition. In cell culture studies, ferritin is transported from the apical side of polarized intestinal cells through receptor-mediated endocytosis. Iron absorbed by the intestine from intact ferritin is processed intracellularly and is released from the basolateral side and treats iron deficiency in mice. The highest iron levels in rats are found in the liver, kidneys, spleen, and spinal cord [6].

Studies on white rats (*Rattus norvegicus*) have been carried out using extract therapy, which is giving green beans a dose of 18 gr / kgBW / day and a dose of 36 gr / kgBW / day affects the increase in white rat hemoglobin levels carried out by Maulina [9]. Mung beans contain vitamins and minerals. Minerals such as calcium, phosphorus, iron, sodium and potassium, so that green beans can overcome the effects of decreased hemoglobin.

Kidney beans are a legume plant which has long been known to the public as a food ingredient, kidney beans have nutritional content such as carbohydrates, proteins, minerals and vitamin B and in 500 gr/% red beans contain 314 kcal/% of energy, protein 22.1 gr/%, fat 1.1 gr/%, carbohydrates 56, 2 gr/%, calcium 502 mg/%, phosphorus 429 mg /%, iron 10.3 mg/%, vitamin B1 0.4 mg/%, and food fiber 4 gr/% [10].

The application of Fe tablets has a fishy odor and causes nausea need to be supplemented as an antiemetic effect is an antioxidant, antioxidants can be obtained from legumes such as green beans and soybeans which can reduce hyperemesis gravidarum and nausea, vomiting in patients suffering from cancer with chemotherapy cisplatin [11, 12]. Red beans have antioxidant compounds, namely flavonoid compounds for treatment in anemia can work together with iron to counteract free radicals in the form of hydroxy and superoxide so that it protects lipid membranes, prevents cell damage, and protects blood cells. Flavonoids can also form complexes with metal ions, the -OH group and carbonyl can form chelates with iron. If the amount of iron in the plasma decreases, the iron will be released in the form of transferrin and taken to the body parts needed. Transferrin will bind to receptors on the erythroblast cell membrane in the bone marrow and within the mitochondria will be synthesized into heme [13, 14]. Then the administration of red bean powder can be used as an alternative ingredient to reduce the symptoms of nausea and vomiting caused by Fe tablets.

The content of red beans enriched with Fe, is a promising alternative therapy to meet the daily intake of nutrients with essential elements for the body and kidney beans are foods with functional properties that may be effective against anemia. Hemoglobin in pregnant women with a dose of about 800 mg, while the need for 300 mg for the fetus and 500 mg to increase the maternal hemoglobin and from other research studies red beans can increase hemoglobin levels in pregnant women [15, 16].

Kidney beans are a traditional therapy to prevent anemia and the measurement of anemia can be done on the measurement of hemoglobin levels only, so that in this study measurements other than using hemoglobin levels

using erythrocytes, and measurement of reticulocyte counts in the spinal cord, this study utilizes animals try white mice, white mice are widely used because these animals are easily obtained in large quantities, rapid absorption, provide a scientific picture that might occur in humans, and researchers feel interested in examining the administration of instant red bean powder against anemia in white rats.

2. Methods

This type of research uses an experimental research design (Quasy Experiment) with pre-test-post test control group design. Researchers arranged five groups, namely group one, which was given 1.8 ml of red bean powder and 1.08 mg/200 gr BW tablets of rats, two groups were given 3.6 ml of red bean powder and 1.08 mg / 200 gr BW tablets rats, group three were given Fe tablets 1.08 mg / 200 gr BW rats, group four were given Fe tablets 2.16 mg/200 gr BW rats, and group five were given simple feed. The study was conducted for 14 days, where before and after treatment blood samples were taken in all five groups in both the treatment and control groups using a 3 cc syringe and alcohol cotton and stored in an EDTA bottle for examination of hemoglobin levels and reticulocyte count values in the Laboratory.

The population in this study was white rats (*Rattus norvegicus*) female made with anemia obtained from the Biology Laboratory of Semarang State University. Determination of the minimum sample size using a technique probability sampling with a method simple random sampling and is based on the inclusion and exclusion criteria of 5 white rats (*Rattus norvegicus*) divided into five groups with each 1 white rat (*Rattus norvegicus*) in group.

In this study, researchers conducted data collection by observation, identification and filling in the observation sheet. The collected data were analyzed through the IBM SPSS program version 24.0, and continued with a different test, namely the parametric test (paired t test and test One Way Anova). The processed data is used as a basis for discussing statement matters, which are then presented in tabular form so that conclusions can be drawn.

3. Results

Table 3.1: Differences in hemoglobin levels before and after treatment in groups I, II, III, IV and control

Group	Hemoglobin Levels		P value
	Before treatment	After treatment	
	Mean±SD	Mean±SD	
Group I	8.786±0.698	14.385±1.411	0.001
Group II	8.814±0.552	17.450±1.310	0.001
Group III	8.557±0.642	0.573±10.790	0.001
Group IV	9.057±0.687	11.940± 0.784	0.001
Control group	9.414 ± 0.595	9.450 ± 0.559	0.518

*Paired t test

Description

Group I: 1.08 ml Fe tablet and 1.8 ml red bean instant powder

Group II: 1.08ml Fe tablet and 3.6 ml red bean instant powder

Group III: 1.08 ml Fe tablet

Group IV: 2.16ml Fe tablet

Group Control: Only simple feeds were given.

Based on the table above, the results showed that the amount of hemoglobin before and after treatment in groups I, II, III and IV had a significant difference with a p value of 0.001 (<0.05). Where as the control group showed a p value of 0.518 (> 0.05) meaning that there was no significant difference.

Table 3.2: Differences in reticulocyte levels before and after treatment in groups I, II, III, IV and control

Groups	Reticulocyte Levels		P value
	Before treatment	After treatment	
	Mean±SD	Mean±SD	
Group I	0.459±0.166	0.961±0.044	0.001
Group II	0.622±0.227	1.715±0.343	0.001
Group III	0.765±0.139	0.868±0.166	0.001
Group IV	0.535±0.255	0.871±0.136	0.001
Control group	0.220±0.553	0.565±0.268	0.782

*Paired t test

Description

Group I: 1.08 ml Fe tablet and 1.8 ml red bean instant powder

Group II: 1.08ml Fe tablet and 3.6 ml red bean instant powder

Group III: 1.08 ml Fe tablet

Group IV: 2.16ml Fe tablet

Group Control : Only simple feeds were given.

Based on the table above, the results showed that the number of reticulocyte levels before and after treatment in groups I and II were significant differences with p values of 0.000 (<0.05), but in groups III and IV also occurred a significant difference with the p value of 0.001 (<0.05). Where as the control group showed a p value of 0.782 (> 0.05) meaning that there was no significant difference.

Table 3.3: Analysis of differences in hemoglobin and reticulocyte levels between groups I, II, III, IV and control group in anemic white rats.

Variable	Group					P value
	Control	Group I	Group II	Group III	Group IV	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Hemoglobin						
Pre	9.414±0.595	8.557±0.642	9.055±0.687	8.786±0.698	8.814±0.552	0.150
Post	9.450±0.559	10.79±0.573	11.942±0.784	14.385±1.411	17.45±1.310	0.001
Δmean	0.035±0.137	5.600±0.877	2.241±0.412	8.635±0.840	2.885±0.620	0.000
Reticulocyte						
Pre	0.553±0.220	0.765±0.139	0.535±0.255	0.459±0.166	0.622±0.227	0.094
Post	4.177±0.465	5.055±0.549	6.544±0.741	0.961±0.044	1.715±0.343	0.001
Δmean	0.035±0.137	0.502±0.191	1.092±0.376	0.102±0.073	0.335±0.143	0.000

*One way anova test

Description:

Group I: 1.08 ml Fe tablet and 1.8 ml red bean instant powder

Group II: 1.08ml Fe tablet and 3.6 ml red bean instant powder

Group III: 1.08 ml Fe tablet

Group IV: 2.16ml Fe tablet

Group Control: Only simple feeds were given.

Based on the table above, the analysis showed differences in hemoglobin and reticulocyte levels in groups I, II, III, IV

and in the control group there were significant differences after treatment with a p value of 0.001 (<0.05).

Table 3.4: Analysis of differences in hemoglobin and reticulocyte levels between the treatment and control groups in anemic white rats.

Variable	Group I & Control	Group II & Control	Group III & Control	Group IV & control	Group I & II	Group I & III	Group I & IV	Group I & III	Group II & IV	Group III & IV
	P value	P value	P value	P value	P value	P value	P value	P value	P value	P value
Hemoglobin										
Pre	0.751	0.886	0.175	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Post	0.000	0.000	0.006	0.001	0.001	0.000	0.001	0.000	0.000	0.339
Difference	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.695
Reticulocyte										
Pre	1.000	1.000	0.642	1.000	1.000	0.094	1.000	1.000	1.000	0.457
posts	0.003	0.000	0.232	0.220	0.000	0.001	0.001	0.001	0.001	1.000
Difference	0.001	0.000	1.000	0.067	0.000	0.012	0.001	0.001	0.001	0.448

*Post hoc test

Description:

Group I: 1.08 ml Fe tablet and 1.8 ml red bean instant powder

Group II: 1.08ml Fe tablet and 3.6 ml red bean instant powder

Group III: 1.08 ml Fe tablet

Group IV: 2.16ml Fe tablet

Group Control: Only simple feeds were given.

Based on the table above, the statistical test results showed a significant hemoglobin level in group 1 and control by administering a dose of 1.8 ml of instant red bean powder and judging from the statistical test data obtained on the count of reticulocytes at dose 3, 6 ml is more significant, the effect of a dose of 3.6 ml to increase the regeneration of red blood cells and stimulate bone marrow with high doses.

4. Discussion

Results of statistical analysis together between groups after the intervention was given there were significant differences in hemoglobin, erythrocyte and reticulocyte levels.

Hemoglobin is a dye in the blood that functions to transport oxygen and carbon dioxide in the body. Hemoglobin is a protein substance found in erythrocytes and functions as a carrier of oxygen in the body ^[1]. The mechanism of hemoglobin formation starts from succinyl-CoA which is formed in the Krebs cycle, will bind with glycine to form a pyrrole molecule, then there are four pyrrole will join and form protoporphyrin IX, then join with iron to form heme molecules. In the end the heme molecule, which is already together with a long polypeptide chain, globin, which is synthesized by the ribosome, forms a hemoglobin subunit called the hemoglobin chain ^[1].

Hemoglobin functions as a binding of oxygen in the blood. Hemoglobin plays an important role in the body to carry oxygen in the lungs to the peripheral tissues and to carry carbon dioxide peripheral tissues into the lungs and then excreted out. The more oxygen circulated through hemoglobin, the complete supply of oxygen will be circulated throughout the body ^[1].

The results showed that group one, namely the group treated with Fe tablets of 1.08 mg/200 gr BW rats was more effective than the other groups. This is in accordance with research conducted by Maydianasari which stated that mice given Fe for 7 days showed an average increase in Hb from 9.41 gr/dl to 13.09 gr/dl. Iron has an essential function in the body as a means of transporting electrons in cells and as an enzyme reaction in body tissues. Other studies have shown that ferrous sulfate influences hemoglobin levels in adolescent girls ^[2, 3].

Erythrocytes contain important proteins that function as globulins that are conjugated with hem pigments to form hemoglobin in oxygen binding. Erythrocytes circulate in peripheral blood for 120 days. Every 1 mm³ of blood contains about 5 million erythrocytes. Erythrocyte index is an average value that can give information about the average erythrocytes and about the amount of hemoglobin per erythrocyte ^[4]. According to the theory put forward by Sherwood as age increases, the number of erythrocytes decreases due to lower spinal cord productivity. The number of erythrocytes decreases due to free radicals that attack cells so that the number is not balanced between the number of cells found in the blood circulation with the number of cells synthesized. There are several factors that can affect the number of erythrocytes, namely age, species, food consumption and availability of erythrocyte production material. Raw materials that can be processed by hematopoiesis (iron, vitamin B12 and folic acid) obtained from food.

Research says that plant content in the form of flavonoids for treatment in anemia can work together with iron to counteract free radicals in the form of hydroxy and peroxide to protect membrane lipids, prevent cell damage, and protect

blood cells. Flavonoids can also form complexes with metal ions, the -OH group and carbonyl can form chelates with iron. If the amount of iron in the plasma decreases, the iron will be released in the form of transferrin and taken to the body parts needed ^[5, 6].

The results of this study are in line with research conducted by Yousif which states that flavonoids are antioxidants which have the ability to search for free radicals, anti-inflammatory, and anti-carcinogenic. Flavonoids are able to provide a protective effect against oxidative stress in biological systems, especially in erythrocytes. Erythrocytes are susceptible to oxidative damage as a result of high levels of polyunsaturated fatty acids from the membrane, high oxygen concentrations, and cellular hemoglobin. In healthy erythrocytes, significant oxidative damage can be prevented by an antioxidant system consisting of a number of antioxidant compounds and enzymes ^[7].

Reticulocytes are immature erythrocyte cells and have a level of 1% in the human body. Reticulocytes develop and mature in the spinal cord and are circulated in blood vessels before they become erythrocytes. The number of reticulocytes is calculated on a light microscope with a magnification of 100x10, with a minimum count of 1000 erythrocytes in the field of view of more than 10. The normal value of erythrocytes is 0.5-1.5% ^[8].

Reticulocyte count values are indicators of bone marrow activity and are used to diagnose anemia. The number of reticulocytes in peripheral blood reflects erythropoiesis which is close to accurate numbers. An increase in the number of reticulocytes in peripheral blood illustrates the acceleration of erythrocyte production in the bone marrow ^[8]. Reticulocyte counts describe bone marrow activity. Increased bone marrow activity is characterized by an increase in reticulocytes, whereas a decrease or absence of reticulocytes indicates a failure of bone marrow function. Reticulocyte counts describe the production of erythrocytes in the bone marrow which is used to diagnose anemia ^[9].

Examination of erythrocytes is a series of anemia blood tests together with examination of leukocytes, platelets, and blood sedimentation rates. An increase in reticulocyte count in a normal hemoglobin level indicates erythrocytes are damaged or missing, but the bone marrow has increased erythrocyte production to replace. In the process of forming hemoglobin, after the amount of iron in the plasma falls, the iron will be released in the form of transferrin and carried to the body parts needed. Transferrin will bind to receptors on the erythroblast cell membrane in the bone marrow and within the mitochondria will be synthesized into heme ^[5, 6]. Low hemoglobin levels and an erythrocyte count of 0.5-2.5% indicate an inadequate anemia response. Erythrocyte examination can be done manually by wet or dry preparations ^[8].

According to research from Nijveldt explains that the amount of erythrocytes can also be influenced by active compounds such as saponins, tannins in food or drinks consumed in large amounts. This is in line with the theory expressed by Cheeke explaining that tannin has the ability to bind to proteins. The presence of tannins that can bind to proteins and coat the walls of the small intestine will inhibit protein absorption. This can cause inhibition of making the hormone erythropoetin and reduce the formation of erythrocytes.

Another study put forward by Nadjeeb explains that saponins destroy erythrocytes through hemolysis reactions.

While different things are explained by Francis *et al*, explaining that the ability of saponins to increase membrane permeability will facilitate large molecules such as proteins to be absorbed in the body so that absorption of nutrients increases.

This research is supported by research conducted by Susilo which states that flavonoids in soursop leaves are effective in increasing the amount of hemoglobin and reticulocytes because flavonoids act as antioxidants in blood cells and act as reservoirs of hydroxy and superoxide radicals, thus protecting membrane lipids and preventing damage to blood cells. Flavonoids form complexes with metal ions, -OH and carbonyl groups, so that if the amount of iron (Fe^{2+}) in plasma falls, iron (Fe^{3+}) will be released in the form of transferrin and taken to the parts of the body that need it. Transferrin binds to receptors on the erythoblast cell membrane in the bone marrow and in the mitochondria will be synthesized into heme ^[6].

5. Conclusion

Based on the results of research and discussion that have been described related to the effect of giving red bean powder (*phaseolus vulgaris l*) as an adjuvant therapy to the blood profile of anemic white rats, several conclusions can be formulated, namely:

- 5.1 There are differences in the blood rat hemoglobin levels (*Rattus norvegicus*) before and after red bean powder in each group with a p-value of 0.001
- 5.2 There was a difference in the reticulocyte count of white rats (*Rattus norvegicus*) before and after the administration of red bean powder in each group with a p-value of 0.001.
- 5.3 There were differences in hemoglobin levels in white rats (*Rattus norvegicus*) between the study groups with a p-value of 0.001.
- 5.4 There were differences in reticulocyte levels in the white rat (*Rattus norvegicus*) between the study groups with a p-value of 0.001.
- 5.5 Based on the statistical treatment test the most recommended is in group 1 and control by administering a dose of 1.8 ml of instant red bean powder to increase hemoglobin levels and a more significant 3.6 ml dose, the effect of a dose of 3.6 ml to increase the regeneration of red blood cells and stimulate bone marrow with high doses.

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