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The Effect of Giving Edible Amaranth Extract *(Amaranthus tricolor)* and Moringa Leaves *(Moringa oleifera)* Extract on Experimental Pregnant Mice towards Hemoglobin Level

Ayu Jani Puspitasari[1], Efrida, SpPK (K), M. Kes[2], Gusti Revila, M. Kes[3]

[1]Postgraduate Student, Midwifery Science Department, Medical Faculty, Andalas University, Padang, Indonesia
[2]Lecturer, Medical Faculty, Andalas University, Padang, Indonesia
[3]Lecturer, Medical Faculty, Andalas University, Padang, Indonesia

Corresponding Author: Ayu Jani Puspitasari

ABSTRACT

Pregnant woman needs adequate nutrition to maintain maternal health, fetal development, and preparation for lactation. Inadequate nutrition is one of the causes of anemia in pregnant woman. Anemia in pregnant women in Indonesia is mainly caused by iron deficiency. Pregnant woman with anemia can be at risk of giving birth to low birth weight (LBW). One of the forms of fortification for iron needs can be done by consuming green vegetable including edible amaranth (Amaranthus tricolor) and moringa leaves (Moringa Oleifera). This study aimed to determine the effect of giving edible amaranth and moringa leaves extract in experimental pregnant mice on hemoglobin level and birth weight.

This was an experimental research with posttest only control group design on 15 pregnant mice divided into 3 groups (group 1: control, group 2: treatment with edible amaranth extract, group 3: treatment with moringa leaves extract at a dose of 396 mg/200 g of body weight for 17 days. The research was carried out in the Natural Material Chemistry Laboratory of the Faculty of Pharmaceutical, the animal house of the Biomedical Laboratory and the Biochemistry Laboratory of the Faculty of Medicine, University of Andalas. Statistical test with one way ANOVA, the significance is determined if $p<0.05$.

The results showed that the mean of hemoglobin level (g/dL) in the control group: 8,98±0,41, treatment 1: 11,57±0,42, and treatment 2: 12,14±0,43. The mean of birth weight (g) in the control group: 2,99±0,04, treatment 1: 3,09±0,32, and treatment 2: 2,91±0,65. There was an effect of giving edible amaranth and moringa leaves extract in experimental pregnant mice on hemoglobin level with a value of $p=0,00$ ($p<0,05$) and there was no effect on birth weight ($p=0,794$).

The conclusion of this study is that there is an effect of giving edible amaranth and moringa leaves extract on hemoglobin level and there is no effect of edible amaranth extract and moringa leaves on birth weight.

Keywords: Anemia, Edible Amaranth Extract, Moringa Leaves Extract, Pregnant Mice

INTRODUCTION

Pregnant women need adequate nutrition to maintain maternal health, fetal development, and preparation for lactation. Pregnant women are encouraged to eat food that come from vegetables, fruit, and seeds that contain sources of vitamins and minerals. Inadequate nutrition is one of the causes of anemia in pregnant women (Magbool et al., 2019).

Anemia during pregnancy is most commonly caused by iron deficiency (Sabrina et al., 2017). The main cause of iron deficiency during pregnancy is low iron intake along with the increased need for iron as a result of rapid growth, infection, and impaired absorption of iron (Abbaspour et al., 2014).

The incidence of anemia among pregnant women in Indonesia has increased in the last five years. The number of anemia pregnant women in Indonesia in 2018 was 17,686 mothers (48.9%) and Padang City was 1,410 (7.1%) pregnant women with the largest prevalence in the working area of Pauh Community Health Center of 196 pregnant women (26.87%) (Padang Health Office, 2019).

Iron deficiency anemia has a risk to the fetus and pregnant women. The risks that arise in pregnant women are weight loss, placenta previa, eclampsia, and premature rupture of membranes. Risks to the fetus include stunted fetal growth, congenital defects (atresia ani, cleft lip, spina bifida) and low birth weight (LBW) (Pratama et al., 2018).

One of the forms of food fortification to meet iron needs can be done by eating green vegetables, including edible amaranth and moringa leaves. Green edible amaranth is a vegetable that has a higher iron content when compared to other types of vegetables, such as 2.9 mg of mustard greens, 2.7 mg of katuk leaves, 2.5 mg of kale, 2.0 mg of cassava leaves. The iron contained in edible amaranth is useful for the formation of hemoglobin in the blood (Suhada et al., 2019).

The research that has been carried out on 15 mice given edible amaranth leaf extract at a dose of 400 mg/kgbb for 14 days has provided a difference in hemoglobin levels between the control group, namely 14.25 g/dL and the treatment group of 15.95 g/dL ($p < 0.05$) (Aldi et al., 2014).

Moringa leaves have higher nutritional content than edible amaranth leaves. Fresh Moringa leaves contain 7 times more vitamin C than oranges, 4 times more vitamin A than carrots, 4 times more calcium than milk, and twice more protein than yogurt. Moringa leaf powder contains 10 times more vitamin A than carrots, 17 times more calcium than milk, 15 times more potassium than bananas, 25 times more iron than edible amaranth, and 9 times more protein than yogurt (Indriani et al. al., 2019).

The nutritional intake of pregnant women has an important role in the development of pregnancy, the fetus and birth weight. Body weight is the result of an increase/decrease in all tissues in the body including bone, muscle, fat, body fluids and others (Ilza and Siregar, 2015). Proper nutrition during pregnancy should provide the right amount of energy such as protein, fat, carbohydrate, vitamin, and mineral. Micronutrient deficiency in mothers is one of the causes of poor maternal health status, infection, preeclampsia/eclampsia, preterm birth and inhibition of intrauterine development (Maqbool et al., 2019).

Based on this background, the researchers are interested in knowing the effect of giving edible amaranth (Amaranthus tricolor) and Moringa (Moringa oleifera) leaves extracts to experimental pregnant mice on hemoglobin levels.

LITERATURE REVIEW

Nutrition during Pregnancy

Pregnant women experience changes in the mechanisms and functions of organs such as increased physiological, metabolic, and anatomical activity. Changes that occur include hormonal changes, increase in uterine size, increase in placenta, increase in fetal weight and increase in maternal blood volume (Hardinsyah and Suprariasa, 2016).

The various nutrients needed by the fetus depend on the mother's nutrition for growth and development. Maternal nutrition is also the basis of well-being in dealing with childbirth and postnatal recovery (Flynn et al., 2016). Pregnant women are recommended to consume a balanced diet in accordance with the recommended diet where pregnant women must increase their dietary energy intake by no more than 10% in the final period of pregnancy from the recommended energy intake recommended in non-pregnant women (Koletzko et al., 2019).

Hemoglobin

Definition of Hemoglobin

Hemoglobin is a protein molecule that plays a role in transporting red blood cells which functions as oxygen transport (02). Hemoglobin is the main component of red blood cells (erythrocytes), which is a protein that contains lots of iron and plays an important role in transporting oxygen from the lungs to all body tissues (Sutedjo, 2009).

Hemoglobin has functions according to (Ministry of Health of the Republic of Indonesia, 2009) namely: 1) Giving the red color to blood; 2.) Maintaining the shape of red blood cells; 3) Taking oxygen from the lungs then carried throughout the body to be used as fuel; and 4) Carrying carbon dioxide from the tissues of the body as a result of metabolism to the lungs for disposal, to detect whether a person is deficient in blood or not, it can be determined by measuring the hemoglobin level. A decrease in hemoglobin levels from normal is called anemia.

Edible Amaranth

Nutritional Benefits of Edible Amaranth

Edible amaranth has many benefits for the body. Fresh edible amaranth has a nutritional source of water, energy, protein, fat, carbohydrate, fiber, pulp, minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper, manganese, and contains vitamin C, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, folate, vitamin B12, vitamin A, vitamin E (Lalage, 2013).

Table 1: Nutritional Composition of Green Edible Amaranth

Nutritional Composition	Total/100g of edible amaranth
Calories	36 cal
Protein	3,5 g
Fat	0,5 g
Carbohydrate	6,5 g
Vitamin A	6,09 mg
Vitamin B1	908 mg
Vitamin C	80 mg
Calcium	267 mg
Phosphor	67 mg
Iron	3,9 mg
Water	86,9 mg

One of the alternatives to meet iron needs can be done by consuming vegetables containing iron in the diet. Iron is found in vegetables, including edible amaranth (Amaranthus sp). Green leafy vegetables like edible amaranth are great sources of nonheme iron. Cooked edible amaranth contains iron as much as 8.3 mg/100 gam (Rohmatika et al., 2016).

Edible amaranth is a vegetable that contains vitamin B6, Vitamin C, riboflavin folate, niacin, fiber and minerals. Edible amaranth is also rich in iron which is useful for preventing several diseases such as osteoporosis and anemia due to iron deficiency. Edible amaranth has benefits as a prevention of indigestion, increase red blood cells, promote growth in children and appetite; healing therapy, and fatigue; as an anticancer agent, and antioxidant (Miano, 2016).

Table 2 Phytochemicals of Green Edible Amaranth Extract and Simplicia

Chemical Substances	Simplicia	Extract
Alkaloid	(-)	(-)
Flavonoid	(+)	(+)
Saponins	(+)	(-)
Quinone	(+)	(+)
Tannins	(-)	(-)
Polyphenolates	(+)	(+)
Steroids and triterpenoids	(+)	(+)

The Relation between Edible Amaranth Consumption and Hemoglobin Levels

Nutritional factors play a role in the pathogenesis of complications in pregnancy such as preterm birth, fetal growth disorders, preeclampsia, anemia, and gestational diabetes mellitus. Maternal malnutrition such as micronutrient deficiency can result in intrauterine inflammation. Several micronutrients such as iron, vitamins A, B6, B12, C, D, E, folic acid, zinc, and docosahexaenoic acid (DHA) affect immune system function and reduce oxidative damage to the placenta. Zinc, vitamins A and D act as regulators of the immune system and have anti-inflammatory effects (Wibowo and Fitriana, 2019).

Iron deficiency has an impact on decreasing hemoglobin levels which causes symptoms of anemia. Iron is a building block for hemoglobin and plays a role in the process of forming red blood cells to

increase endurance and infection (Holdswort et al., 2014). Iron and protein molecules in the body combine to form transferrin. The function of transferrin is to transport iron in the blood, while iron in the intestinal mucosal cells is removed by ferritin. Iron deficiency is associated with increased hemopoiesis and low iron reserves (Almatseir, 2009).

Iron in the body consists of four forms, namely iron in hemoglobin, iron in reserves, especially as ferritin and hemosiderin, iron transported in transferrin, and parenchymal iron or iron in tissues such as myoglobin with several enzymes including cytochromes, catalase, and peroxidase. Sources of non-heme iron derived from plant foods such as green edible amaranth have a ferric bond form (Fe^ (3+)). Iron found in food, initially undergoes a digestive process either in the form of Fe ^ (3+) or (Fe ^ (2+)). Iron in the form of ferric will be reduced by gastric juice (HCl) to form ferrous (Fe ^ (2+)) so that it is more easily absorbed by intestinal mucosal cells (Adyani et al., 2018).

One alternative in meeting iron needs is to eat vegetables that contain iron. One of the vegetables that contains iron is edible amaranth (Amaranthus sp.) which is a source of non-heme iron. Edible amaranth is a vegetable with the highest iron content, namely 3.9 mg/100 gram compared to other types of vegetables, such as 2.9 mg mustard greens, 2.7 mg katuk leaves, 2.5 mg kale, 2.0 mg cassava leaves.

A research that has been carried out on 15 mice given edible amaranth leaf extract at a dose of 400 mg/kgbb for 14 days has provided a difference in hemoglobin levels between the control group, namely 14.25 g/dL and the treatment group of 15.95 g / dL ($p < 0.05$) (Aldi et al., 2014).

Another study at the University of Baghdad, Iraq on 60 pregnant mice given edible amaranth leaf extract at a dose of 100 mg/kgbb given for 20 days showed a difference in mean hemoglobin levels compared to the control group of 11.10 ± 1.58 g/dL and the group treatment of 13.50 ± 1.61 g/dL ($p <0.05$) (Abbas and Hasan, 2019).

Moringa Leaves *(Moringa oleifera)*

Nutritional Composition of Moringa Leaves

Table 3 Nutritional Composition of Moringa Leaves

Nutritional Analysis	Unit	Per 100 grams of ingredients		
		Pod	Fresh Leaf	Leaf Powder
Water content	%	86,9	75	7,5
Calories				
Protein	Cal	26,0	92,0	205,0
Fat	Gram	2,5	6,7	27,1
Carbohydrate	Gram	0,1	1,7	2,3
Fiber	Gram	3,7	13,4	38,2
Mineral	Gram	4,8	0,9	19,2
Calcium	Gram	2,0	2,3	-
Magnesium	Mg	30,0	440,0	2003,0
Phosphor	Mg	24,0	24,0	368,0
Potassium	Mg	110,0	70,0	204,0
Copper	Mg	259,0	259,0	1324,0
Iron	Mg	3,1	1,1	0,6
Oxalic acid	Mg	5,3	7,0	28,2
Sulfur	Mg	10,0	101,0	-
Vitamin A –	Mg	137,0	137,0	870,0
Carotene	Mg	0,10	6,80	16,30
Vitamin B –	Mg			
Choline	Mg	423,00	423,00	-
Vitamin B1 –	Mg	0,05	0,21	2,60
Thiamin				
Vitamin B2 –	Mg	0,07	0,05	20,50
Riboflavin		0,20	0,80	8,20
Vitamin B3 –				
Nicotinic acid				
Vitamin C –	Mg	120,00	220,00	17,30
Ascorbic acid				
Vitamin E –	Mg			
Tocopherols		-	-	113,0
Acetate				

The Relation between Moringa Leaves Consumption and Hemoglobin Leaves

Moringa plants have many benefits ranging from leaves, bark, fruit to seeds. Moringa can be processed as daily necessities such as vegetables, medicinal raw materials and can be traded. The habit of using Moringa plants is also influenced by socio-cultural factors (Bora, 2017).

Moringa oleifera leaves are an alternative to prevent anemia in pregnant women because moringa leaves contain 7 times higher vitamin C content than oranges, 4 times higher vitamin A content than carrots, 4 times higher calcium content than milk, 3 times higher iron content than edible amaranth, and 2 times higher protein content than yogurt (Aisha et al., 2013).

Moringa leaves are rich in nutrients and are a source of beta carotene, vitamin C,

iron and potassium. The Fe content in Moringa leaves from 1 kg of simplicia can produce an iron content of 54.92 mg. Iron (Fe) is a micromineral that is tremendously important for the formation of red blood cells, namely the process of hemoglobin (Hb) synthesis and can also activate several enzymes, one of which is the antibody-forming enzyme. Iron deficiency will result in anemia which is a nutritional problem in Indonesia (Hamzah and Yusuf, 2019).

The research that was conducted on 18 mice given Moringa leaf extract at a dose of 300 mg/Kgbb for 21 days has been effective in increasing hemoglobin levels of female mice significantly ($p < 0.05$) (Ibrahimiyah, 2014). Another study at the University of Indonesia with 15 white mice for 6 days given Moringa leaf extract at a dose of 396 mg/200gbb can significantly increase hemoglobin levels and erythrocyte number ($p < 0.05$) (Mun'im et al., 2016).

MATERIALS & METHODS

This research is experimental study with post-test only control group design. The population in this study were all pregnant mice (Rattus norvegicus strain wistar) that had met predetermined criteria. The placement of mice in each treatment group in this study used a random method (simple random sampling).

The tools used include a place to drink and eat for mice, a mouse cage consisting of a plastic tub covered with wire on the top of as many as 9 cages. Glass tools (Pyrex), mouse observation cages, measuring cups, filter paper, masks, microtubes, capillary tubes, gloves, injection syringes, oral syringes, rotary evaporators (Heidolph type Heizbad WB), analytical scales and material scales.

The ingredients used were pregnant white mice (Rattus novergicus strain wistar) weighing 150 - 180 grams, green edible amaranth (Amaranthus tricolor), Moringa oleifera leaves, ethanol, distilled water, ether, pellet standard feed or daily feed, green edible amaranth extract, and moringa leaf extract.

The research data were collected in the form of an observation sheet which would function as technical guidance for the implementation of the intervention in the form of sample codes, edible amaranth leaf extract, hemoglobin levels and birth weight. Data collection was carried out by researchers with assistance by laboratory instructors related to the guidance and direction of laboratory assistants.

The data that have been collected is continued with data processing and analysis (Notoatmodjo, 2010) include editing, coding, entry, and cleaning. In editing, the researchers rechecked the completeness of the observation sheet so that it could be processed further. The things that must be considered include: completeness of content, writing, suitability of answers, and uniformity of units of measure. In coding, the research data will be classified according to uniformity in the form of codes to make it more concise. In entry process, the data were processed into coded form with coding techniques and then the data were entered into statistics. Last, in cleaning, the research data that have been entered were re-checked to ensure that there were no incorrect or missing data. Checking can be done by making the frequency distribution and cross tabulation manually and checking the consistency between variables.

Statistical Analysis

After checking the hemoglobin level, the data normality test was carried out with Shapiro Wilk, then the homogeneity of variance was carried out. If $p > 0.05$, the data is normal and the variation of each sample is homogeneous. Analysis of the effect of extracts on hemoglobin levels used the one-way ANOVA (Analysis of Variance) test with a 95% degree of confidence with a value of $p \leq 0.05$ (significant). The significant results were followed by a multiple statistical test (post hoc test) for the Tukey HSD and Gomes-Howell types. The data analysis process used computer software.

RESULT

The Effect of Giving Green Edible Amaranth (Amaranthus tricolor) and Moringa (Moringa Oleifera) Leaves Extracts on Hemoglobin Levels in Pregnant Mice (Rattus norvegicus)

This study conducted a normality test of hemoglobin levels using the Shapiro Wilk test with normally distributed data results (p> 0.05).

Table 4 Hemoglobin Levels of Pregnant Mice (Rattus novergicus) Control and Treatment Group

Group	n	Hemoglobin Levels Mean±SD	p-value
Control	5	8,98±0,41	0,000
Group 1	5	11,57±0,42	
Group 2	5	12,14±0,43	

One way ANOVA test

Remarks:

Control: without treatment

Group 1: given green edible amaranth extract (396mg/200gbb)

Group 2: given Moringa leaf extract (396mg/200gbb)

Table 4 above shows that the mean hemoglobin level (g/dL) in the control group was lower than the treatment groups 1 and 2. The mean hemoglobin level of the treatment group 2 was higher than the control group and the treatment group 1. The difference in hemoglobin levels between the control group and the treatment group 1 and 2 was statistically significant (p = 0.000). The difference between each group was carried out by a Multiple Comparison test (post hoc test) for the Tukey HSD type as shown in table 5.

Table 5 One way ANOVA Test Results of Hemoglobin Levels in Pregnant Mice (Rattus novergicus) in Control and Treatment Groups

Group	Significance Level of Hemoglobin Levels		
	Control	Treatment 1	Treatment 2
Control	-	0,000*	0,000*
Treatment 1	0,000*	-	0,127
Treatment 2	0,000*	0,127	-

One way ANOVA test

Based on Table 5, it can be seen from the test results that there is a significant difference between the control group with treatment 1 (p = 0.000) and the control group with treatment 2 (p = 0.000).

DISCUSSION

The Effect of Giving Green Edible Amaranth (Amaranthus tricolor) and Moringa (Moringa oleifera) Leaves Extracts on Hemoglobin Levels of Pregnant Mice (Rattus novergicus)

After analyzing one way ANOVA test, it was found that there was a significant effect of giving green edible amaranth extract (Amaranthus tricolor) and Moringa (Moringa Oleifera) leaf extract on hemoglobin levels in pregnant mice (Rattus novergicus). The results of the Multiple Comparison test (post hoc test) showed a significant difference between the control group and treatment groups 1 and 2, and there was no significant difference between treatment 1 and treatment 2.

This study is in accordance with the research that was carried out on 15 mice given edible amaranth leaf extract at a dose of 400 mg/kgbb for 14 days, which gave a difference in hemoglobin levels between the control group, namely 14.25 g/dL and the treatment group of 15.95 g/dL (p <0.05) (Aldi et al., 2014).

Another study conducted at the University of Baghdad, Iraq on 60 pregnant mice given edible amaranth leaf extract at a dose of 100 mg/kgbb given for 20 days showed a difference in hemoglobin levels compared to the control group of 11.10 ± 1.58 g/dL and the treatment group of 13.50 ± 1.61 g/dL (p <0.05) (Abbas and Hasan, 2019).

Moringa leaves have more nutrition than green edible amaranth. Nutrition that can increase hemoglobin levels include protein, iron, and vitamin C. The protein content in green edible amaranth is 3.5 g/100 grams of edible amaranth; iron by 3.9 mg/100 grams of edible amaranth and vitamin C by 80 mg/100 grams of edible amaranth. Green edible amaranth contains bioactive substances such as flavonoids, tannins, quinones, polyphenolates, steroids and triterpenoids (Mauliandani et al., 2017).

Moringa leaves have protein nutrients of 27.1 g/100 grams, 28.2 mg of iron/100 grams, however, vitamin C is less

than green edible amaranth, namely 17.30 mg/100 grams (Ministry of Health of the Republic of Indonesia, 2015). Moringa leaves contain active ingredients such as flavonoids, tannins, phytates, and polyphenols (Sally et al., 2014).

The results of this study are in line with research conducted on 18 white mice given Moringa leaf extract at a dose of 300 mg/Kgbb for 21 days, which significantly increased hemoglobin levels in female mice ($p < 0.05$) (Ibrahimiyah, 2014). Another study at the University of Indonesia with 15 white mice for 6 days given Moringa leaf extract at a dose of 396 mg/200gbb can significantly increase hemoglobin levels and erythrocyte ($p < 0.05$) (Mun'im et al., 2016).

The absence of a significant difference in hemoglobin levels between treatment 1 and treatment 2 could be due to low non-heme iron derived from vegetables and very dependent on other types of food or food variations. Iron from animal food such as meat can absorb iron as much as 20-30%, while iron from plant food is only about 5% (Wirakusumah, 2010).

Iron absorption is influenced by enhancer and inhibitor. The enhancers include protein and vitamin C. The inhibitors include tannins in tea and coffee, in foods that contain large amounts of calcium, phosphate, tannins and phytates which will interfere with the absorption of iron (Widari and Pratiwi, 2018).

The protein nutrition in Moringa leaves is greater, namely 17.1 g/100 grams and green edible amaranth of 3.5 g/100 grams. Protein plays a role in the formation of essential bonds, including hemoglobin. Protein in red blood cells as a means of transporting iron because iron is not freely available in the body. Iron and protein combine to form transferrin. Transferrin will carry iron to the bone marrow and combine to form hemoglobin (Musyabiq et al., 2019).

CONCLUSION

Based on the research results on the effect of giving green edible amaranth (Amaranthus tricolor) and Moringa (Moringa oleifera) leaves extracts on hemoglobin levels in pregnant mice (Rattus novergicus), it can be concluded that 1) the mean hemoglobin level of experimental pregnant mice in the group given Moringa oleifera leaf extract was higher than the group given green edible amaranth extract (Amaranthus tricolor); 2) the mean birth weight of mice in the group given green edible amaranth extract (Amaranthus tricolor) was higher than the group given Moringa (Moringa oleifera) leaf extract; and 3) there is an effect of giving green edible amaranth (Amaranthus tricolor) and Moringa (Moringa oleifera) leaf extracts to experimental pregnant mice on hemoglobin levels.

Research that has been conducted on 51 pregnant women in trimesters II and III in the Purwanegara Community Health Center working area by checking protein and hemoglobin levels, it was found that there was a relation between protein adequacy levels and hemoglobin levels ($p = 0.005$) with a Spearman correlation value of 0.388 which means the higher protein adequacy, the better the hemoglobin level (Yuliati et al., 2017).

Vitamin C in Moringa leaves is higher at 220 mg/100 g compared to green edible amaranth, which is 80 mg/100 g. Vitamin C is a water-soluble vitamin. Iron absorption occurs mostly when it is in the jejunum and only 5-10% experience homeostasis from the entire intake that enters the body. Iron absorption can increase three to five times when the body is deficient in iron (Krisnanda, 2020).

A research that has been conducted on 90 trimester III pregnant women divided into group 1 with supplementation of Fe, vitamin C and accompanied by mentoring and counselling, group 2 with supplementation of Fe, vitamin C and mentoring, and group 3 with supplementation of Fe tablets accompanied by assistance and counselling for 30 days showed that there was a significant difference in hemoglobin levels of pregnant women in the group that was given iron

supplementation, vitamin C supplemented with assistance and counselling with the group that was given supplementation with Fe tablet, vitamin C accompanied by mentoring and the group that was given supplement of Fe tablet accompanied by assistance and counselling (p = 0.004) (Hadi et al., 2017).

The calcium in Moringa leaves is higher than green edible amaranth, which is 440 mg/100 g and 80 mg/100 g. Calcium and iron are divalent ions. Absorption of calcium and iron in the intestine using Divalent Metal Transforter 1 (DMT 1). The similarity of transporters between iron and calcium results in the absorption of iron and calcium influencing each other (competitive barriers). Consumption of calcium> 2500mg/day will interfere with the absorption of iron and other ions that have a positive 2 charge (Almatsier, 2009).

In a study with 30 anemic white female mice given iron and calcium tablets for 7 days, it was found that 50 mg/day of calcium administration began to inhibit iron absorption and 75 mg/day of calcium had a very significant impact on iron absorption (Hermawan, 2015).

Green edible amaranth and moringa leaves contain tannins. Tannins are known as anti-nutritional compounds because of their ability to form complex bonds with proteins. The oxidized tannins turn into tannic acid. Substances containing tannic acid have a negative effect on the gastric mucosa, namely the mucous membrane that lines the stomach, causing problems in the stomach (Hidjrawan, 2018).

Research that has been conducted on 244 trimester II pregnant women in Muara Enim Regency, South Sumatra, namely assessing the intake of iron and its inhibitors with hemoglobin levels, the results obtained from tannin intake above 10.5 grams/day have a risk of anemia 2.21 times than pregnant women who have an intake tannins less than 10.5 grams/day (Riswanda, 2017).

The bioactive content in green edible amaranth and moringa leaves can affect hemoglobin levels. Green edible amaranth and moringa leaves contain bioactives, namely flavonoids. Flavonoids are secondary metabolite compounds that are found in plants and have bioactive effects such as anti-virus, anti-inflammatory, cardioprotective, anti-diabetes, anti-cancer, anti-aging and antioxidant properties (Arifin and Ibrahim, 2018).

Flavonoids are compounds found in vascular plants as glycosides and plavonoid aglycones which are one type of antioxidant. Antioxidants are molecules that are able to slow down or prevent the oxidation of other molecules (Hamid et al., 2010). The types of flavonoids in edible amaranth and moringa leaves are lutein and quercetin, which are powerful antioxidants that can trap superoxide free radicals and inhibit the oxidation of LDL cholesterol (Latifah and Susilawati, 2019).

Flavonoids form iron compounds Fe^{3+} (ferric) will decrease to iron Fe^{2+} (ferro) in plasma then converted into transferrin form and brought to the body where it is needed. Transferrin will join and bind to receptors in the spinal cord, namely the erythoblast cell membrane and will be synthesized into heme in mitochondria so that it can protect red blood cells from lysis and increase the amount of hemoglobin (Wirawan et al., 2015).

People who only eat vegetables are not always detrimental to the body if nutritional needs are fulfilled. The prevention of iron deficiency can be optimized by consuming vitamin C and other factors that can facilitate absorption of nonheme iron. Eating wheat, nuts, seeds, dry fruits, cereals and green leafy vegetables can provide adequate iron intake (Musyabiq et al., 2019).

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