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The Effect Of Moringa Leaf (Moringa Oleifera Lam) And Vitamin C Combination Extract In Improving Anemia Indicators Deficiency Of White Rice Iron Male Diet Low Rice Iron

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ABSTRACT

Iron deficiency anemia is still a major health problem in developing countries. Therapy with iron preparations often causes undesirable side effects. Moringa leaf extract which is rich in iron can be used as an alternative therapy in iron deficiency, and combination with vitamin C can increase the effectiveness of iron absorption in the gastrointestinal tract. determine the effect of the combination of Moringa oleifera lam. Extract and vitamin C on the indicator of iron deficiency anemia in white rats (Rattus Novergicus Strain Wistar) who were given a diet low in iron aking rice. True experimental research design using post test only control group design. The object of this study was male white rats (Rattus novergicus Strain wistar) induced by a low-iron rice diet. The study was divided into negative control group, positive control group, the treatment group with Moringa leaf extract dose of 400 mg / kg body weight, 800 mg / kg body weight, 1,600 mg / kg body weight. The treatment group was given vitamin C in a dose of 40 mg. Results and Discussion One wayAnova test, hemoglobin sig. 0,000 ($p < 0.05$), MCV sig 0.27 ($p > 0.05$), MCH sig 0.16 ($p > 0.05$), serum iron sig. 0,000 ($p < 0.05$), transferrin saturation sig. 0.006 ($p < 0.05$), and TIBC sig 0.68 ($p > 0.05$). Pearson correlation for hemoglobin 0.746 (sig.0,000), and linear regression with R2 0.557 (sig.0,000); for serum iron 0.742 (sig.0,000) with R2 0.551 (sig.0,000). There was an increase in hemoglobin and serum iron levels in the treatment group.

Keywords : iron deficiency anemia, moringa leaf extract, vitamin C, hemoglobin, serum iron.

INTRODUCTION

Iron deficiency can cause haematological disorders anemia. Iron deficiency anemia occurs due to low iron levels in the body due to the loss of iron in the body such as in the chronic bleeding process, or on inadequate dietary intake due to increased needs. (Adamson, 2015; Calistania et al., 2014). In iron deficiency anemia, there is a decrease in hemoglobin levels accompanied by a decrease in serum iron (SI) levels and transferrin saturation, while the total iron-binding capacity (TIBC) can be normal or increased (Devkota, 2014; Jimenez, 2015).

Iron deficiency anemia cases often occur in high-risk groups such as toddlers, women with pregnancy and lactation, the low socioeconomic status associated with low dietary intake of iron, patients with infectious diseases and malabsorption disorders.(Miniero R, 2018; Parmar *et al*, 2016).

According to data from the Basic Health Research (Riskesdas) in 2013, Indonesia, as a developing country, had a prevalence of anemia in children aged> 1 year around 27.1%. Based on the age group, the data shows that anemia in toddlers is around 28.1%, and pregnant women are around 37.1% (Riskesdas, 2013). WHO (2015) reports that iron supplementation can eliminate anemia cases in about 42% in children and 50% in women.

Treatment of iron deficiency anemia is carried out by treating the primary disease that caused it, giving an iron-rich diet, and iron preparations. However, administration of oral iron preparations can cause unwanted side effects such as heartburn, nausea, abdominal cramp, constipation, dusky stools, and diarrhoea.(Harper, 2016; Alabsi et al, 2010).

Alternative treatment for deficiency anemia is with natural ingredients which have minimal side effects. The leaves of Moringa (Moringa oleifera lam) are reported to be rich in vitamins A, C, and the mineral iron. (Gophalan, 2010; Kathryn, 2011). Dry Moringa leaves contain high levels of the mineral iron of 32.5 mg per 100 grams (Kathryn, 2011).

Moringa plants can be found in Indonesia, which has woody, round, branched, black spots and dirty white grey. Compound leaves and green. Leaves 20-60 cm long. Leaflets are ovoid. Flat-leaf edge with a notched tip. Pinnate leaf repetition. Compound interest, shaped panicles. Flowers are located in the axillary of the leaves. Flower length 10-30 cm. Small stamens and pistils. The flower crown is white-cream. Fruit in the form of blackish-brown capsules with a length of 20-45 cm, each fruit contains 15-25 seeds. Seeds are round, three-winged and black. The taproot is dirty white. (Napitupulu, 2008)

In this study, rats were induced by iron deficiency by giving aking rice feed. Moringa leaf extract's dietary treatment in combination with vitamin C. Vitamin C is expected to increase iron absorption from Moringa leaf extract.

MATERIAL AND METHOD

Moringa leaf extract and vitamin C

In this study, Moringa leaf extract was combined with vitamin C. Moringa oleifera lam. The extract was obtained from the Materia Medica Batu Laboratory. In the initial stage, a determination is carried out, after which the extract is made into a liquid using 70% ethanol as a solvent. Meanwhile, vitamin C used is in the form of generic vitamin C tablets.

Study design

This research is a true experimental study using post-test only control group design. This study's object was active male white rats (Rattus novergicus Wistar strain), aged 2-3 months, weighing 150-200 grams. The treatment was given a combination of moringa leaf extract in various doses with vitamin C. In this study, there were five treatment groups: one normal control group that was only given standard feed and drink, positive control with the induction of a low iron diet of aking rice, and three treatment groups. Moringa leaf extract at a dose of 400 mg / 200 g BW, 800 mg / 200 g BW, 1,600 mg / 200 g BW, combined with 40 mg of vitamin C. The treatment was given for 40 days.

Anemia induction

Anemia induction is done by giving low iron feed in the form of aking rice. Aking rice is leftover rice which is then dried in the sun to dry. The rats were given a 35 g/day rice diet.

Parameter measurement

Hemoglobin, MCV, and MCH levels were measured using a Sysmex autoanalyzer. Serum iron, transferrin saturation, and TIBC were measured using the Cobas C 311 apparatus.

RESULT AND DISCUSSION

The results of the study The effect of the combination of Moringa oleifera lam and Vitamin C extract in improving iron deficiency anemia indicators in male white rats on a low iron rice diet showed an increase in hemoglobin levels, serum iron, transferrin saturation in the treatment group compared to the positive control group who did not receive Moringa leaf extract and vitamin C. The average binding capacity of TIBC iron was almost the same; the MCH index appeared to be increasing, while the MCV index was relatively the same between the treatment group and the positive control.

Table 1. Mean hemoglobin levels (g / dl), mean corpuscular volume (fl), mean corpuscular hemoglobin (pg), serum iron (μg / dl), total iron-binding capacity (μg / dl), and transferrin saturation (%)

GROUP	Hb		MCV		MCH		SI		TIBC		ST	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
K-	15,06	1,05	60,00	3,33	18,66	1,07	256,8	54,50	522,00	44,49	54,80	8,25
K+	12,78	0,59	53,04	2,77	16,90	0,71	148,8	18,40	481,00	81,69	37,20	5,11
PI	13,80	0,47	55,50	3,22	17,34	0,58	174,0	23,40	470,80	42,02	49,40	5,50
PII	15,06	0,80	53,26	2,56	18,04	0,96	208,6	21,30	443,60	81,22	52,40	8,84
PIII	15,21	1,22	51,98	2,48	17,76	0,96	214,4	47,00	489,60	20,12	47,00	3,31

Notes :

K- : Negative control group

K+ : Positive control group

PI : Moringa leaf extract group had a dose of 400 mg/kg BW, and vitamin C 40 mg

PII : Moringa leaf extract group had a dose of 800 mg/kg BW, and vitamin C 40 mg

PIII : Moringa leaf extract group had a dose of 1600 mg/kg BW, and vitamin C 40 mg

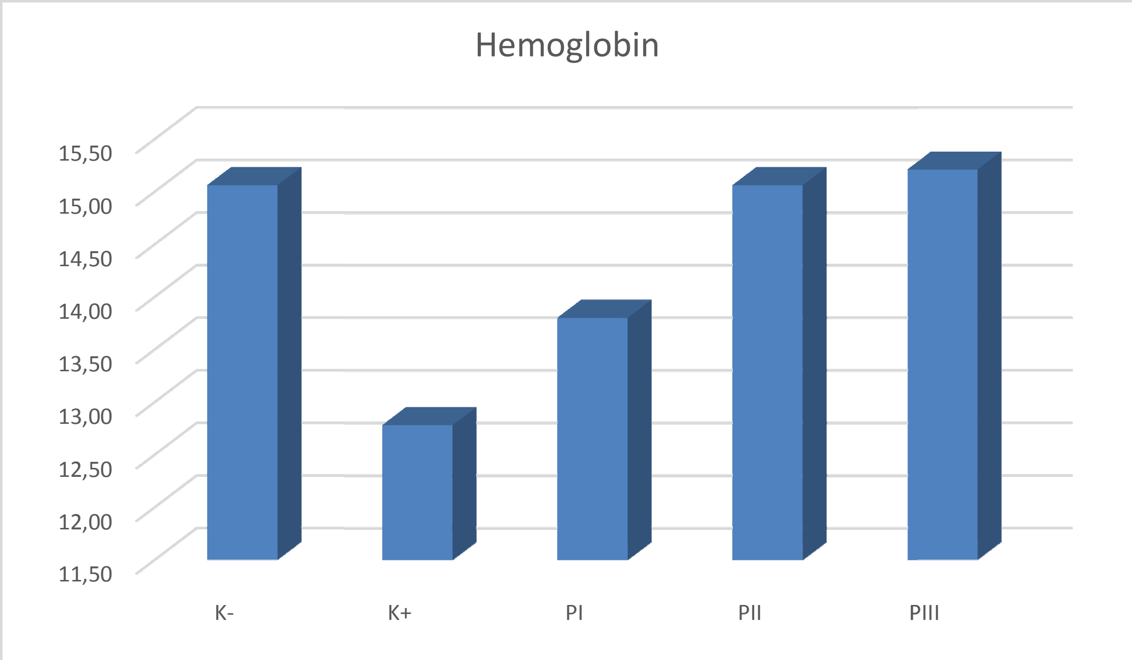

Figure 1. Column graph of hemoglobin levels (g/dl)

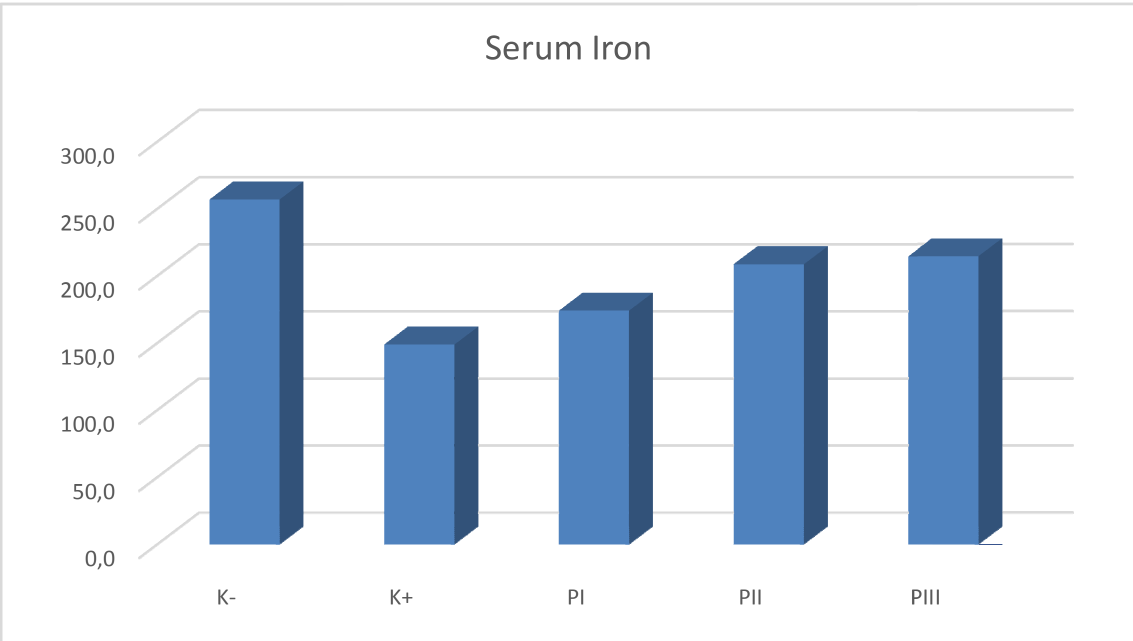

Figure 2. Column graph of erum iron level (μg/dl)

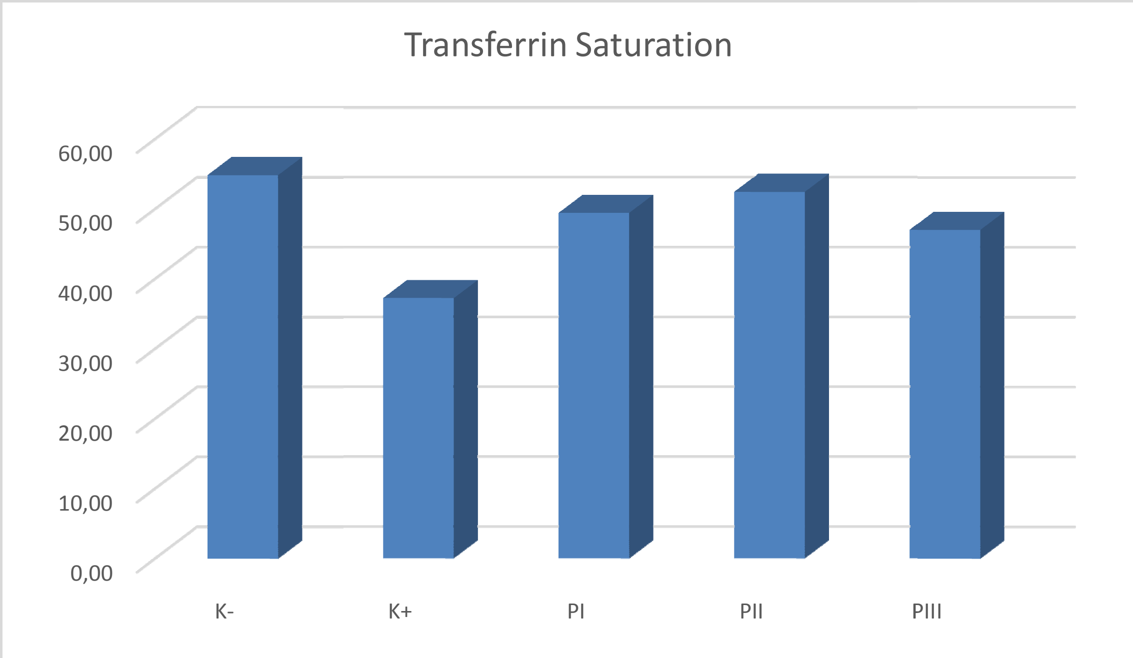

Figure 3. Column graph of transferrin saturation levels (%)

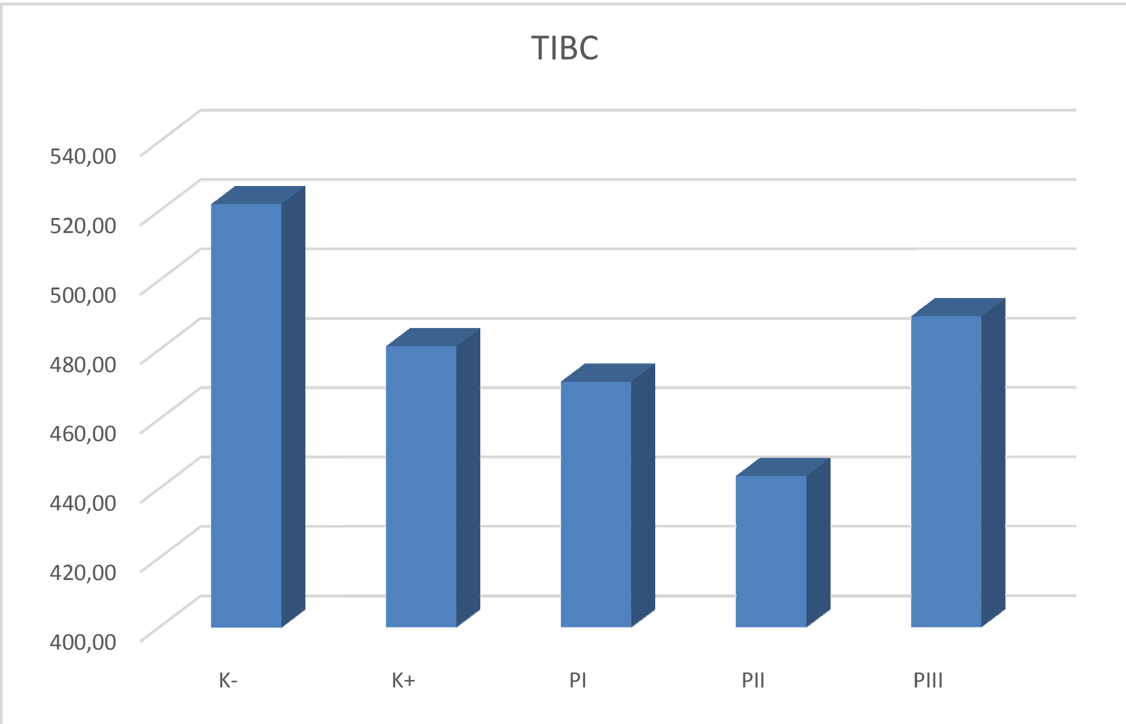

Figure 4. Total iron binding capacity column graph(μg/dl)

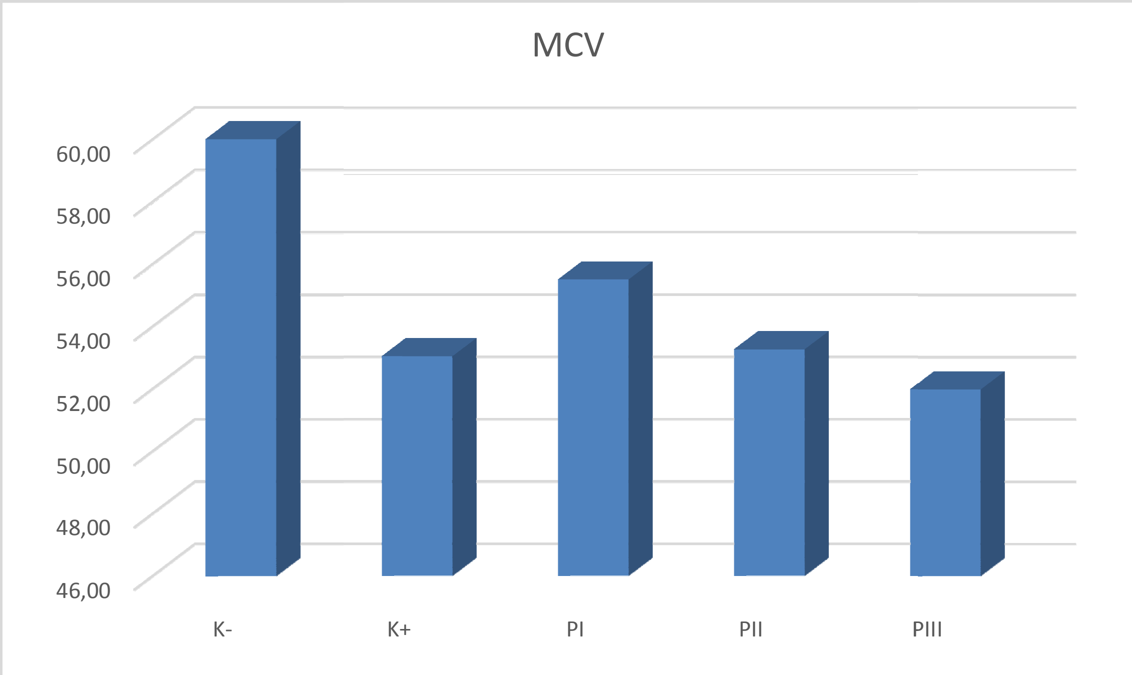

Figure 5. Column graph of Mean corpuscular volume(fl)

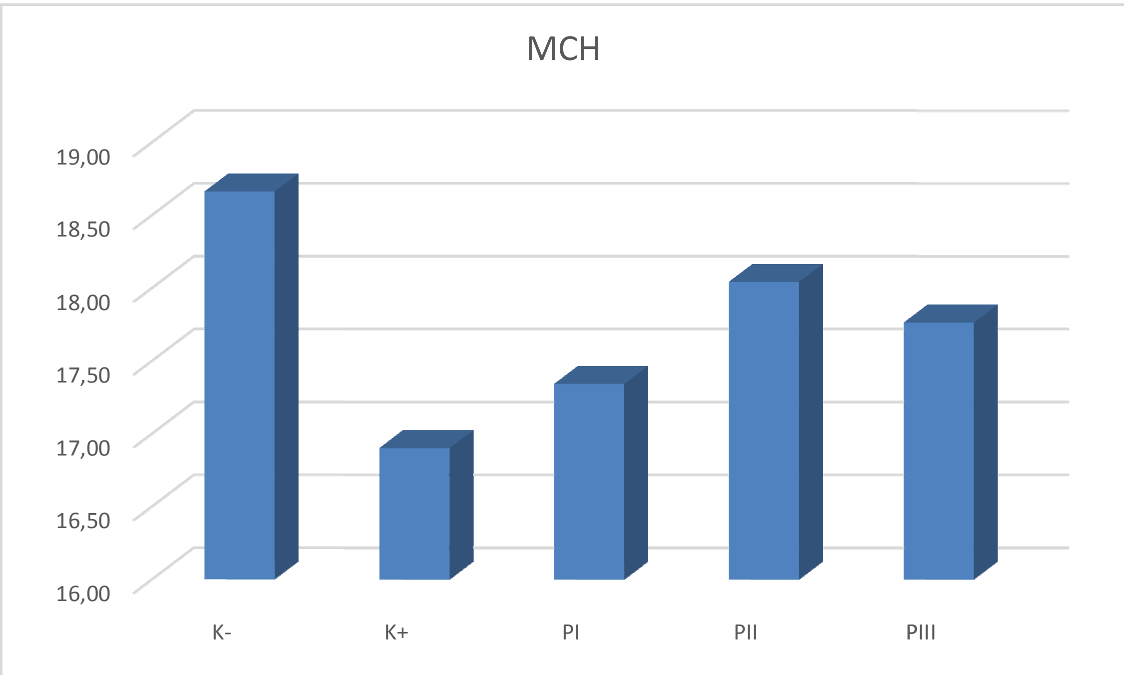

Figure 6. Column graph of mean corpuscular hemoglobin(pg)

The data are normal and homogeneous, then with One wayAnova analysis the following results are obtained: hemoglobin sig. 0.000 ($p < 0.05$), MCV sig 0.27 ($p > 0.05$), MCH sig 0.16 ($p > 0.05$), serum iron sig. 0.000 ($p < 0.05$), transferrin saturation sig. 0.006 ($p < 0.05$), and TIBC sig 0.68 ($p > 0.05$). Based on the one way ANOVA analysis above, there are significant mean differences between groups on the variables of hemoglobin, serum iron, transferrin saturation, but not different in the TIBC, MCV, and MCH variables.

On the hemoglobin variable, with the One wayAnova test, the sig value was 0.000 ($p < 0.05$), indicating a significant difference in the mean hemoglobin levels between groups. In the Post Hoc Bonferroni test, it appears that there is a significant difference between the negative control group and the positive control (sig. 0.03, $p < 0.05$), but there is no difference between the treatment groups ($p > 0.05$). The positive control group appeared significantly different from the negative control group, the PII and PIII treatment groups; but not different from the PI group. The three treatment groups appeared to show no difference. In the Pearson correlation test, the correlation value was 0.746 with sig. 0.000 ($p < 0.05$), indicating a significant relationship between moringa leaf extract dosage and hemoglobin levels. The strength of giving Moringa leaf extract on the increase in hemoglobin levels was measured by regression to get R2 = 0.557. This equation is in accordance with the Anova Regression, where the Sig ANOVA regression value = 0.000 ($p < 0.05$), which means that the regression model fits significantly. This is also supported by the t-table coefficient test which shows that the value of Sig (constant) = 0.000 and the value of Sig (dose) = 0.000 which means it is smaller than the p-value (0.05). The R2 value formed in this equation is 0.557, meaning that the effect of moringa leaf extract dosage on the increase in hemoglobin levels is 55.7%. While the remaining 44.3% is influenced by other factors outside of Moringa leaf extract.

In the serum iron variable, the One way ANOVA test found sig. 0,000 ($p < 0.05$), indicating a significant difference in the mean serum iron levels between groups. In the post Hoc Benferoni test, there appeared a significant difference between the negative control group and the positive control group (sig. 0.000, $p < 0.005$) and the PI treatment group (sig 0.04, $p < 0.05$), but it was not different from the PII group. And PIII. The positive control group was significantly different from the negative control group and PIII, but not different from the PI and PII groups. The three treatment groups PI, PII, and PIII, showed no significant differences. In the Pearson correlation test, the correlation value was 0.742 with sig. 0.000 ($p < 0.05$), indicating a significant relationship between the dose of Moringa leaf extract and serum iron levels. The strength of giving Moringa leaf extract on the increase in serum iron levels was measured by regression to get R2 = 0.551. This equation is in accordance with the Anova Regression, where the Sig ANOVA regression value = 0.000 ($p < 0.05$), which means that the regression model fits significantly. This is also supported by the t-table coefficient test which shows that the value of Sig (constant) = 0.000 and the value of Sig (dose) = 0.000 which means it is smaller than the p-value (0.05). The R2 value formed in this equation is 0.551, meaning that the effect of moringa leaf extract dosage on the increase in hemoglobin levels is 55.1%. While the remaining 44.9% is influenced by other factors outside of Moringa leaf extract.

The variable transferrin saturation with the One-way ANOVA test was obtained sig.0.006 ($p < 0.05$), indicating a significant difference in the mean transferrin saturation levels. In the post-Hoc Benferoni test for transferrin saturation, there was a significant difference between the negative control group and the positive control group (sig. 0.004, $p < 0.05$), but there was no difference between all treatment groups. The positive control group looked different from the negative control and the PII group, but not different from the other groups. The three treatment groups PI, PII, and PIII, showed no significant differences. In the Pearson correlation test, the correlation value obtained was 0.360 with sig. 0.12 ($p > .05$), indicating that Moringa leaf extract was less related to transferrin saturation.

In the post-Hoc Benferoni test for mean corpuscular volume (MCV), there were significant differences between the negative control group and the positive control group, the PII and PIII treatment groups ($p < 0.05$), but there was no difference with the PI group. The positive control group differed only from the positive control but did not differ from the three treatment groups. The three treatment groups PI, PII, and PIII, showed no significant difference.

This study is in line with research by Anisa N et al. (2019), where there was a significant increase in haemoglobin levels in anemic young women who were given a combination of Moringa leaves 250 mg x 2 per day and vitamin C 50 mg x 2 per day for three months. Research by Dossou I et al. (2011) reported a significant increase in hemoglobin levels in anemic women who were breastfeeding after being given dry powder of Moringa leaves for three months. Still, it was unable to replenish their iron reserves. Another study by Suzana D et al. (2017) reported a significant increase in hemoglobin levels in anemic patients by administering ferrous sulfas preparations 200 mg/day plus moringa leaf extract 1400 mg/day. Yulianti (2016) also reported a significant increase in adolescent girls' haemoglobin levels after being given Moringa leaf extract. Meanwhile, Mun'im (2016) reported that giving 792 mg / 200 g BW / day of Moringa leaf extract can increase hemoglobin levels and significantly improve the morphology of aniline-induced rat erythrocytes. Osman HM (2012) reported a significant increase in albino rats' haemoglobin levels induced with aluminium ($AlCl_3$) and given 300 mg/kg moringa leaf extract for 21 days.

Moringa leaf extract significantly increased serum iron levels and increased transferrin saturation. Serum iron is iron in circulating blood bound by transferrin. A decrease in serum iron parameters and transferrin saturation is a sign of iron deficiency. (Devkota, 2014). The increase in serum iron and transferrin saturation in this study was due to Moringa leaf extract administration, and vitamin C. Moringa leaf extract is rich in iron. Still, the iron in plants is in non-heme iron, where its absorption is less good compared to heme iron from animal protein foods. Only 10% of non-heme iron can be absorbed. Iron absorption itself is a complex process that occurs in the proximal duodenum and jejunum. In the brush border of enterocytes, there are several iron transport proteins and specific absorption pathways for the two ionic forms (Fe 2+ and Fe3 +, both of which come from non-heme iron molecules), and for the heme molecule (heme iron). Non-heme iron is associated with various protein reserves including ferritin, while heme iron is in hemoproteins such as Mb AND Hb. At acidic pH in the stomach, heme breaks down from hemoprotein, while non-heme iron is stable in its reduced form (Fe2 +). Non-heme iron is captured, but some complexes can affect its absorption such as plant-derived phytates or tannins. Ascorbic acid or vitamin C and other acidic components derived from the diet can increase iron absorption. (Waldvogel, 2014). Ascorbic acids or vitamin C have been reported to increase absorption and bioavailability of ferrous sulfas preparations in rats (Vicent, et al., 2008). Vitamin C works to reduce and catalyze iron absorption inhibitors, thus increasing non-heme iron absorption (Teucher B, 2004). Foods that contain low to moderate levels of inhibitors require additional vitamin C with a molar ratio of 2: 1 (example: 20 mg vitamin C: 3 mg iron). Diets with high inhibitor levels require vitamin C with a molar ratio of more than 4: 1, which may not be practised. (Teucher, 2004). Research by Gallaher (2017) on the bioavailability of moringa leaf extract in foods commonly consumed by Ugandans suspects that giving Moringa leaves does not improve the iron status due to high levels of phytic acid which can bind iron so that it cannot be absorbed.

In this study, Moringa leaf extract and vitamin C did not appear to affect the total iron-binding capacity (TIBC), MCV and MCH. There has not been any improvement in the parameters of TIBC and MCH, probably because iron reserves are not filled. Iron therapy generally takes three months to improve hemoglobin levels and replenish iron stores (Harper, 2016; Goddard, 2011).

CONCLUSION

The combination of Moringa leaf extract and vitamin C affected the increase in hemoglobin and serum iron levels in white male Wistar rats on a low iron diet of rice aking. Vitamin C increases the bioavailability of intestinal iron absorption.

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