

The Effect Of Giving Extracted Moringa Oleifera Leaves Plus Royal Jelly Supplement On Erythrocyte Index Of Anemia Pregnant Women In Takalar District

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Abstract: Background: This study aims to determine the effect of giving moringa oleifera leaves extract plus royal jelly supplement on increasing erythrocyte index in anaemia pregnant women.

The method of the research: The research method used is randomized controlled design. The research was conducted in Polongbangkeng Utara Sub district (Puskesmas Polongbangkeng Utara, Ko'mara and Towata) Takalar Regency with a Haematology Analyze examination at the Clinical Pathology Laboratory of the UNHAS Hospital. Subject of the study is 63 anemia pregnant women consisting of 21 subjects of Moringa Leaf Extract plus Royal Jelly (MLERJ) group with the intervention of 1 capsule in the morning with a dose of 500 mg MLE + 10 mg RJ, 21 subjects of Moringa Leaf Extract (MLE) with the intervention 1 capsule in the morning with a dose of 500mg MLE and the Placebo group 21 subjects with the intervention 1 placebo capsule in the morning with a dose of 500mg starch flour, where all of the subjects consumed Fe tablets at night with a dose of 1x60mg. Data were analyzed using the Chi-Square, Mann-Whitney, Wilcoxon, Paied T-Test, Kruskal-Wallis and Post-Hoc Mann Whitney tests.

Results: Based on the characteristics of research subjects based on age, education, occupation, income and parity, the three research groups were not different or evenly distributed. The results showed an increase in the erythrocyte index levels (MCV, MCH and MCHC) of the three study groups. Where in the MLERJ group the levels of MCV, MCH and MCHC experienced a significant increase, in the MLE group who experienced a significant increase in MCV and MCHC levels, while in the Placebo group who experienced a significant increase only in MCV levels.

Conclusion: Supplementation Moringa Leaf Extract Plus Royal Jelly is more effective at increasing the erythrocyte index levels (MCV, MCH and MCHC) in anemic pregnant women so that it can be used as an alternative treatment to treat anemia.

Keywords: Moringa leaves extract, Royal jelly, MCV, MCH, MCHC, Anaemic pregnant women

1. INTRODUCTION

The gestation period is a phase of increasing energy metabolism due to the increase of energy needs and other nutrients. This increase is needed for fetus growth and development,

changes in the composition and metabolic processes of the mother's body. Thus, if there is a lack of nutrients that are needed during pregnancy, it can result in an imperfect fetal growth process^[1]. During pregnancy, there are physiological changes, namely hemodilution, where there is an imbalance of the increase in blood volume with a decrease in blood plasma and an increase in blood cells, causing blood thinning, especially in the third trimester of pregnancy. If these nutritional needs are not met, there will be a decrease in hemoglobin so that adequate nutrition is needed during pregnancy^[2].

Anemia is one of the many global health cases that occur in 56 million women worldwide, where 66-70% of these cases are in the Asian continent. In Indonesia, most anemia occurs due to iron deficiency so that the need for iron (Fe) for the erythropoiesis process is insufficient^[3,4,5]. A person's anemia status can be assessed by examining the levels of hemoglobin (Hb), hematocrit, and red blood cells (erythrocytes). Erythrocyte index or Mean Corpuscular Value is an average value that can provide information about the average erythrocyte and the amount of hemoglobin per erythrocyte^[6,7]. Examination or calculation of the erythrocyte index (MCV, MCH and MCHC) is used to diagnose the type of anemia and can be linked to determine the cause/morphology of anemia. One of the alternatives to prevent anemia is by consuming vegetables, especially Moringa Oleifera leaves. To increase the erythrocyte index level in anemic pregnant women, besides consuming Moringa Oleifera Leaves, we can also consume royal jelly. Royal jelly can increase the number of erythrocytes in human blood and can increase hemoglobin level. Royal jelly acts as an energy restoring tonic, relieves pain and improves appetite^[8,9].

Various studies regarding the effect of Moringa Oleifera Leaves on erythrocyte levels, which in a study conducted by Suzana et.al (2017) on the effectiveness of Moringa Leaf Extract on the hematological and biochemical values of blood in women suffering from anemia it obtained differences in hemoglobin, erythrocyte, hematocrit, MCV levels (Mean Corpuscular Volume), MCH and RDW in anemic women taking Fe only^[10]. Giving Moringa Leaf Extract as much as 600 mg/day for 30 days can increase erythrocyte levels by about 30%^[11]. A research conducted by Tinna by giving Moringa capsules combined with Fe tablets can increase erythrocyte levels in anemic pregnant women when compared to pregnant women who only consume Fe tablets^[12]. In contrast to the research conducted by Muliani (2019), the results showed that the supplementation of Moringa flour and bee bread in the intervention group I and supplementation of Moringa flour in the intervention group II had less effect on increasing the erythrocyte index (MCV, MCH and MCHC) in adolescent girls who have anemia^[13]. The research conducted by Hadju, et. al (2015) administration of moringa oleifera can increase erythrocyte levels, but it is not statistically significant. Another study regarding the effects of royal jelly conducted by Saritaş et. al (2017) in athletes who were given a mixture of royal jelly (RJ) + honey (H) with a dose of 5g RJ + 45g H mixed into a dosage (50g/day) given for 8 weeks, it was found that there was a significant increase in MCHC levels, and there was an increase in MCV and MCH levels although not statistically significant^[14]. In contrast to research conducted by Nazmi et al., (2014) giving royal jelly to swimming athletes can increase levels of MCV, MCH, MCHC, erythrocytes (RBC), leukocytes (WBC) and platelets (PLT), but it can reduce HCT levels, CHCM, CH and HDW^[15].

The provision of capsule supplementation that combines Moringa Oleifera Leaves extract and royal jelly has never been done before, so it is necessary to do a research on "The Effect of giving extracted Moringa Oleifera Leaves capsule supplement Plus Royal Jelly on Erythrocyte Index in Anemia Pregnant Women" as one alternative to treat anemia.

2. MATERIALS AND METHODS

Location and research design

The research lasted for 60 days and was carried out in Polongbangkeng Utara Subdistrict (North Polongbangkeng Puskesmas, Ko'mara Puskesmas and Towata Puskesmas) Takalar District, and blood subject examinations were carried out at the Clinical Pathology Laboratory at UNHAS Hospital. This study used the True Experiment type, with a pre-post test design with a control group. The research group was divided into 3 with the Randomized Controlled Double Blind Design, which means that the parties involved in the study, such as researchers, recipients and enumerators, did not know the difference in the supplements given.

Population and Subject

The population of this study were all anemia pregnant women with gestational age of 20-32 weMLEs who visited Antenatal Care (ANC) or who performed antenatal care at Puskesmas in Polongbangkeng Utara District (Puskesmas Polut, Puskesmas Ko'mara and Puskesmas Towata). The sampling technique used in this study was the simple random sampling technique which met the inclusion criteria of pregnant women with gestational age 20-32 weeks, Hb < 11 gr/dl, single fetus and willing to become respondents by signing an informed consent sheet. Subjects in this study was 63 obtained using the Sopiudin Dahlan formula^[16]. This study has received a recommendation of approval from the Ethical Commission for Health Research of medical Faculty, Hasanuddin University Makassar with Number 439/UN4.6.4.5.31/PP36/2020 and Protocol Number UH20070314.

Method and data analysis

Data collection used a questionnaire. data for age, parity, gestational age, parity, education, occupation and income were measured by interview using a questionnaire. The data were processed using SPSS for windows version 25. Then, the data were analyzed using the Chi-Square, Mann-Whitney, Wilcoxon, Kruskal-Wallis and Post-Hoc Mann Whitney tests.

Procedure Of Haematocrit Level Examnitaion

Study group were divided into 3 groups, 1 group got Moringa leaves extract plus royal jelly (MLERJ) capsule, 1 group got Moringa leaves extract (MLE) capsule and 1 group got placebo. The blood samples of the subjects in intervention and control group had been taken in vena mediana cubiti as many as 3 cc before consuming the supplementation, the respondents then got explanation to consume 1 capsule a day with MLERJ group got 1x500 mg MLE + 10 mg RJ, MLE group got 1x500mg MLE and the Placebo group got 1x500mg starch flour doses for 60 days. After 60 days the blood samples were taken as post-test as many as 3 cc, and the blood samples were put into microcentrifugation tube contains EDTA as anticoagulant, then the blood samples were saved in in -700°C refrigerator before sent to laboratory to get haematocrit examination. Measurement of the erythrocyte index (MCV, MCH and MCHC) was measured using the Sysmex Xs-800i Hematology Analyzer.

3. RESULTS

Respondent Characteristics

Table 1 Frequency Distribution of Respondent Characteristics

Respondent Characteristics	Moringa Oleifera Leaves Extract plus Royal Jelly	Moringa Oleifera Leaves Extract	Placebo (control)	Total	P-Value

	n	%	n	%	n	%	N	%	
Age									
Low risk	16	33.3	16	33.3	16	33.3	48	100	1.000 ^a
High risk	5	33.3	5	33.3	5	33.3	15	100	
Education									
Low	6	28.6	7	33.3	8	38.1	21	100	0.807 ^a
High	15	35.7	14	33.3	13	31	42	100	
Occupation									
Unemployment	15	29.4	18	35.5	18	35.3	51	100	0.265 ^b
Employment	6	50	3	25	3	25	12	100	
Income									
Low < UMK	17	30.4	19	33.9	20	35.7	56	100	0.158 ^b
High ≥ UMK	4	57.1	2	28.6	1	14.3	7	100	
Parity									
Primigravida	11	33.3	10	30.3	12	36.4	33	100	0.826 ^a
Multigravida	10	33.3	11	36.7	9	30	30	100	

^aChi-Square; ^bMann-Whitney

Table 1 shows that most of the respondents in both the intervention and control groups are 20-35 years old or in the low-risk age group. In terms of education, most mothers have higher education. Most of the respondents do not work and have a low family income or below the UMK < Rp. 3.100.000. Meanwhile, on parity characteristics, most of the respondents are primigravida mothers.

Erythrocyte Index Analysis

Table 2 Analysis of Differences in Erythrocyte Index (MCV, MCH and MCHC) Levels Before and After Intervention

Erythrocyte Index	n	Mean ± SD		P-Value	Difference in mean ± SD	95% CI	P-Value
		Pre (fl)	Post (fl)				
MCV							
Moringa Oleifera Leaves Extract plus Royal Jelly	21	77.06 ± 1.85	83.38 ± 2.22	0.000^a	6.31±2.60	5.12-7.50	0.004^b
Moringa Oleifera Leaves Extract	21	76.80 ± 2.11	80.81 ± 1.99	0.000^a	4.01±2.65	2.80-5.22	
Placebo	21	76.47 ± 3.30	79.89 ± 3.44	0.001^a	3.41±3.22	1.94-4.88	
MCH							
Moringa Oleifera Leaves Extract plus Royal Jelly	21	29.20 ± 1.89	30.08 ± 1.75	0.001^c	0.87±0.96	0.43-1.31	0.014^b
Moringa Oleifera Leaves Extract	21	29.03 ± 2.75	29.48 ± 2.54	0.088 ^c	0.44±1.14	-0.07-0.96	
Placebo	21	28.74 ± 2.42	28.83 ± 2.68	0.609 ^c	0.09±0.79	-0.27-0.45	

MCHC Moringa Oleifera Leaves Extract plus Royal Jelly	21	33.48 ± 0.91	33.92 ± 0.92	0.001^a	0.43±0.51	0.20-0.67	0.008 ^b
Moringa Oleifera Leaves Extract	21	33.42 ± 1.41	33.78 ± 1.22	0.004^a	0.35±0.63	0.06-0.64	
Placebo	21	33.86 ± 1.04	33.96 ± 0.94	0.662 ^a	0.10±0.48	-0.12-0.32	

^aUji Wilcoxon; ^bKruskall-Wallis; Paired T-Test

Based on the data in table 2 on MCV levels after the Wilcoxon test, there is an increase in the mean MCV before and after treatment with MLERJ, MLE and Placebo intervention groups with a value of $p < 0.05$, which means that there is a difference in MCV values before and after treatment, where the highest mean increase in MLERJ intervention group with a mean difference of 6.31. Then performed the Kruskal-Wallis test statistically the p value of the three groups, namely 0.004, where the p value < 0.05 , which means that there is a significant difference between the three groups and based on the value of the confidence interval the value does not exceed zero, which means that clinically there is a difference in MCV in the three groups.

In the MCH after the Paired T-Test was carried out in the MLERJ intervention group, the mean MCH before and after treatment had an increase in the mean of MCH before and after treatment with a value of $p = 0.001 < 0.05$, which means that there was a significant difference before and after treatment, while the MLE and Placebo intervention groups also experienced an increase in the mean MCH before and after the intervention with a p value > 0.05 , which means there is no significant difference before and after treatment. Then performed the Kruskal-Wallis test statistically the p value of the three groups, namely 0.014, where the p value < 0.05 , which means that there is a significant difference between the three groups, but the statistical and clinical significance of increasing MCH is in the MLERJ intervention group.

At the MCHC level after the Wilcoxon test, there was an increase in the mean MCHC before and after good treatment in the MLERJ, MLE and Placebo intervention groups with an increase in the mean MCHC in the MLERJ intervention group with a mean value of 0.43. However, there was a significant difference in MCHC before and after treatment in the MLERJ intervention group with $p = 0.001 < 0.05$ and the MLE intervention group with a value of $p = 0.004 < 0.05$. Then performed the Kruskal-Wallis test statistically the p value of the three groups, namely 0.008, where the p value < 0.05 , which means that there is a significant difference between the three study groups, but the statistical and clinical significance of increasing MCHC is in the MLERJ and MLE intervention groups.

Table 3 Post-Hoc Analysis of Differences in Erythrocyte Index (MCV, MCH and MCHC) Levels Between Groups

Groups	Difference in Mean ± SD	Median (Min-Max)	95% CI	P-Value
MCV Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Moringa Oleifera Leaves Capsule	2.30 ± 3.95	3.70 (-6.00-7.70)	0.50-4.09	0.005*

Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Placebo	2.89±4.48	3.90 (-5.50-11.10)	0.85-4.93	0.004*
Extracted Moringa Oleifera Leaves Capsule vs Placebo	0.59±4.08	-0.10 (-6.50-10.80)	-1.26-2.45	0.860*
MCH Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Moringa Oleifera Leaves Capsule	0.42±1.25	0.20 (-1.30-2.80)	-0.14-0.99	0.312*
Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Placebo	0.78±1.50	0.40 (-0.06-6.30)	0.10-1.47	0.004*
Extracted Moringa Oleifera Leaves Capsule vs Placebo	0.35±1.52	0.20 (-2.50-5.00)	-0.33-1.05	0.063*
MCHC Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Moringa Oleifera Leaves Capsule	0.08±0.84	0.10 (-1.60-2.00)	-0.30-0.46	0.849*
Extracted Moringa Oleifera Leaves Capsule plus Royal Jelly vs Placebo	0.33±0.73	0.50 (-1.40-1.90)	0.00-0.06	0.007*
Extracted Moringa Oleifera Leaves Capsule vs Placebo	0.25±0.76	0.50 (-1.80-1.80)	-0.09-0.60	0.009*

*Post-Hoc-Mann Whitney

In table 3 we can see based on follow-up tests using the Post-Hoc Mann Whitney test to see which intervention group showed significant clinical differences. In the table above, MLERJ vs Placebo and MLERJ vs MLE groups were statistically and clinically significant. So it can be concluded that giving MLERJ vs Placebo has an effect on increasing the erythrocyte index (MCV, MCH and MCHC) while giving MLERJ vs MLE only has an effect on increasing MCV.

4. DISCUSSION

In this study, respondents in the intervention group I were given 1 supplement of Moringa Leaf Extract plus royal jelly at a dose (500mg of MLE + 10mg RJ)/day in the morning and combined with Fe tablet at a dose of 1x60mg at night. The intervention group II was given 1 moringa oleifera leaves extract supplement with a dose of 500 mg of Moringa Leaf Extract/day in the morning and combined with Fe tablets at a dose of 1x60 mg at night, while the control group was given 1 placebo capsule with a dose of 500 mg of starch/day in the morning and administration of Fe tablet with a dose of 1x60mg at night. From the results of the research that has been done, it was found that in the moringa oleifera leaves plus royal jelly supplement group, it could increase the MCV, MCH and MCH erythrocyte index levels and statistically significant effect on increasing erythrocyte index levels in Anemia pregnant women with $p = 0.000 < 0.05$. From table 2, we can see that the highest increase in erythrocyte index levels is in the intervention group Moringa Leaf Extract supplement plus royal jelly.

In line with Adegbite et. al where there was a significant increase in the value of MCV levels after administration of Moringa leaf flour^[17]. A research conducted by Suzana et.al on the administration of Moringa Leaf Extract capsules at a dose of 700 mg/capsule shows that it can affect MCV levels^[10]. The increase in erythrocyte index levels occurred because Moringa Oleifera contains alkaloids, phytosterols, saponins and flavonoids which are known to have hemotopoietic properties. In addition, the content of vitamins A, B, C and minerals such as iron can contribute to the formation of erythrocytes, especially the formation of hemoglobin in the blood. A research related to the erythrocyte index conducted by Sri Muliani (2019) in Anemia teenage girls shows that moringa leaf flour and bee bread supplementation can increase the erythrocyte index (MCV, MCH and MCHC)^[13]. Other research conducted by Hadju, et. al (2015) administration of moringa oleifera can increase erythrocyte levels, but it is not statistically significant^[18].

In contrast to a research conducted by Bhalchandra W., et. al (2018), giving a combination of honey and royal jelly to male albino wistar rats with a dose of honey (500 mg/kg/day) and royal jelly (100 mg/kg/day) given orally every day for 15 days showed a significant increase in hematological parameters, hematocrit, MCV and MCHC^[19]. A research conducted by Kausar & More (2019), giving royal jelly to male albino swiss mice could increase levels of MCV, MCH and MCHC^[20].

Another study conducted by Saritaş et. al (2017) in athletes who were given a mixture of royal jelly (RJ) + honey (H) with a dose of 5 g of royal jelly + 45 g of honey mixed into a dosage (50 g/day) given for 8 weeks, there was a significant increase in MCHC levels and there was an increase in MCV and MCH levels although not statistically significant^[14]. In contrast to studies conducted by Nazmi et al., (2014) giving royal jelly to swimming athletes could reduce levels of HCT, CHCM, CH and HDW and increase levels of MCV, MCH, MCHC, erythrocytes (RBC), leukocytes (WBC) and platelets. (PLT)^[15]. A research conducted by Morita et. al (2012) to 31 people in the intervention group and 30 people in the control group, where the intervention group was given 100 ml of liquid mixed with 3000 mg of royal jelly every day for 6 months and the control group was given the same fluid 100 ml/day for 6 months without royal mixture jelly obtained results that consuming RJ for six months in humans could

improve erythropoiesis (the process of forming erythrocytes/red blood cells), glucose tolerance and mental health^[21].

Moringa Oleifera Leaves are one of the local foods that are rich in micronutrients and widely available, however, they have not been fully utilized. Moringa leaves contain multi-micronutrient/micronutrient elements that are needed by pregnant women, including iron, phosphorus, magnesium, zinc, vitamin C, beta carotene, thiamin (B1), riboflavin (B2), niacin (B3) and calcium^[22]. MCV (Mean Corpuscular Volume) or VER (Mean Erythrocyte Volume) is the mean volume of an erythrocyte expressed in units of femtoliters (fl)^[23]. The MCV is an index for determining the size of red blood cells. MCV indicates the size of a single red cell as either Normocytic (normal size), Microcytic (small size < 80 fl), or Macrocytic (small size > 100 fl)^[7]. Where the MCV value is the parameter that most influences changes in erythrocyte levels when compared to MCH and MCHC levels to determine the possibility of anemia due to iron deficiency^[10]. Pregnant women may experience changes in the erythrocyte index based on MCV levels which can increase up to 4 fl. However, if the MCV level and MCH level has decreased, it is a sign of iron deficiency, and when it is followed by a decrease in MCHC levels, it will worsen anemia.

Hematological changes in pregnancy cause the need for red blood cells to increase due to the increased physiological requirements for fetus growth and development. The mechanism of red blood cell production is very complex and involves hormonal mediators such as erythropoetin, human placenta lactogen, estrogen and progesterone. Although red blood cells increase during pregnancy, their increase is in contrast to the increase in blood plasma volume resulting in physiological anemia in pregnancy^[24]. The increase in erythrocyte index levels occurs because Moringa Oleifera contains alkaloids, phytosterols, saponins and flavonoids which are known to have hematopoietic properties. In addition, the content of vitamins A, B, C and minerals such as iron can contribute to the formation of erythrocytes, especially the formation of hemoglobin in the blood.

Dried Moringa leaves have high levels of protein, iron, vitamin A and vitamin C so they are very effective in treating iron deficiency anemia. In addition, these leaves do not contain harmful substances so they have no side effects. So far, there has never been a case of poisoning due to consuming Moringa leaves. Fe content in honey and royal jelly can increase the number of erythrocytes in human blood and can increase hemoglobin levels^[8]. Besides that, the nutritional content of honey is almost the same as royal jelly which contains iron (Fe), which is a very important micro-mineral in the body because it can function as a builder of red blood cells. Iron content can synthesize the formation of heme which can spur hemoglobin levels.

In addition to low iron intake, anemia is caused due to low intake of nutrients that act as absorbers (enhancers). Vitamin C is an important enhancer compound to increase iron absorption. The presence of vitamin C in food consumed by pregnant women will increase the iron absorption process^[25]. The content of vitamin C in both Moringa Leaf Extract and royal jelly plays an important role in iron absorption. If moringa leaf flour is combined with royal jelly, the iron absorption process will be faster which will affect the increase in hemoglobin levels and erythrocyte index levels.

Besides increasing levels of the MCV erythrocyte index, when consuming Moringa Leaf Extract supplements plus royal jelly, respondents felt better sleep and increased appetite. This is the effect of royal jelly contained in supplements, where royal jelly has a tonic effect to restore energy, relieve pain and increase appetite and royal jelly can relieve various problems including fatigue/lack of energy, insomnia, anxiety, mild depression. and stamina problems^[9,26].

5. CONCLUSIONS

There is an increase in MCV, MCH and MCHC levels before and after treatment at the groups supplement Moringa Oleifera Leaves Extract plus Royal Jelly, Moringa Oleifera Leaves Extract and Placebo. However, the highest average increase in erythrocyte index (MCV, MCH and MCHC) is in the Moringa Leaf Extract plus royal jelly capsule group. Thus, the supplementation of Moringa Leaf Extract plus royal jelly can be used as a non pharmacological alternative treatment to treat anemia and increases erythrocyte index.

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