Biscuits containing *Moringa oleifera* leaves flour improve conditions of anemia in pregnant women[☆]

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ABSTRACT

Objective: This study analyzes whether anemia in pregnant women improves with indicators of increased hemoglobin, intake of iron and zinc when intervened with biscuits contain *Moringa oleifera* leaf flour.
Method: It was *Quasy experiment* carried out at Antang, Mamajang and Batua Public Health Center in Makassar, South Sulawesi, Indonesia. The subject were the pregnant women of Trimester I and III trimesters which are divided into 2 groups with the intervention Group ($n = 35$) obtained *M. oleifera* leaf flour biscuits with combination iron tablet and the control group ($n = 35$) only accept FE tablets. Intervention group was given biscuits containing 40% moringa leaf flour (2.8 g per biscuits) with a dose of 2 pieces a day containing Moringa leaf flour and a combination of iron tablets with a dose of 2 times 250 mg, while the control group only consumed iron tablets at a dose of 2 times 250 mg. After 60 days, a posttest was carried out to examine hemoglobin, iron and zinc.
Result: The results showed that there was a significance increasing of hemoglobin after intervention (1.04 g/dl, $p = 0.001$), increasing of iron intake significantly (2.51 mg, $p = 0.001$) and zinc intake (0.14 mg, $p = 0.144$) also increas but not statistically significant. The increase in these three indicators was higher than the control group.
Conclusion: Biscuits containing *M. oleifera* leaf flour are able to provide improvement in the condition of pregnant mothers who have anemia, especially in hemoglobin levels, iron and zinc intake.

Introduction

The *World Health Organization (WHO)* reported that iron deficiency anemia is one of the four main nutritional problems in Indonesia the highest persentase cause of maternal mortality is bleeding (28%).[1] Caused by anemia and chronic energy deficiency. Basic Health Research reported cases of anemia in pregnant women in 2018 which is 48.9% higher than the year 2013 which amounted to 37.1% and 2007 amounted 24.5%.[2]

Anemia is a medical condition characterized by red blood cell count or hemoglobin less than normal. Pregnant women can be said to suffer anemia if the level of Hb <11 g/dl in the first and third trimester or <10.5 g/dl in the second trimester.[3] The most common cause of anemia in pregnancy is the low consumption of food containing iron.[4] The impact of anemia in pregnancy is that it can increase the efficacy of mothers and infants, causing premature birth and low birth body.[5]

M. oleifera has the content of macro nutrition and Micro that good for pregnant women. Moringa leaves contain multi elements of micro nutrients that are needed by pregnant mothers such as: beta carotene, thiamin (B1), Riboflavin (B2), niacin (B3), calcium, iron, phosphorus, magnesium, zinc, vitamin C, so it can be an alternative to improving the nutritional status of pregnant women.[6] The results of the study by Dewi (2018) Moringa leaf cookies that can be developed for the prevention of anemia is cookies with substitution 40% moringa flour which is 22.68 ppm iron content.[7] This study analyzes whether anemia in pregnant women improves with indicators of increased hemoglobin, intake of iron and zinc when intervened with biscuits contain moringa oleifira leaf flour.

Materials and methods

Materials

Moringa leaf flour biscuit (cookies) is Consists of moringa leaf flour 100 g, wheat flour 150 g, margarine 175 g, milk powder 30 g, sugar powder 125 g, Salt kitchen 2 g, chicken eggs 1 grain has 22.68 ppm iron content. That dough is processed into 35 chip biscuit with a weight of 17 grams each chip containing 2.8 g of moringa leaf flour each chip. Based on the calculation of food composition nutrient content of each biscuit is 78.3 kcal of energy, Protein of 1.68 g, fat 4.63 g, carbohydrate 8.17 g, iron: 0.99 mg. This formula follows the formula made by previous researchers, namely Dewi (2018).

Design research

The research design uses *quasy experiment* with the design of *nonequaivalent control group Design* where the control and intervention groups are not randomly selected.[8] The study was conducted in March 2020 to June 2020 at the working area of Antang, Mamajang,and Batua Public Health Center Makassar (Makaasar City, South Sulawesi, Indonesia) after receiving ethical approval recommendation from the medical research Ethics Committee of the faculty of medicine of Hasanuddin University Makassar.

The samples in this study were pregnant mothers of trimesters I and III (hemoglobin level <119/DL) that do not consume multivitamins also other minerals than Fe, single fetus and have no genetic disease, inflammatory bowel disease and infectious diseases. There are 10 samples that drop out in each group during the study because it was disobedient in consuming biscuits and was absent when blood taking post intervation. So the total of analyzed samples is 70, 35 samples in Intervention group and 35 samples in control groups. The intervention group is given moringa leaf flour biscuit with dose 2 pieces biscuits in a day whithat have moringa flour content is 2.8 g each chip biscuit and dose of Fe tablets 2 times 250 mg. In the control group only accept tablet Fe dose 2 times 250 mg for 60 days.

Data collection techniques

Data obtained directly from respondents collected through the Baecke Index questionnaire of physical activity and a 24-h food recall. Data of expectant mother anemia then performed measurement of hemoglobin level with blood sampling 3 cc in cubiti vein of both groups by the laboratory officers in Public Health Center then do screening using Hematology Analyzer Sysmex in the Clinical Pathology Laboratory of Education Hospital Hasanuddin University. Iron and zinc intake Data collected using a 24-h food recall questionnaire and analyzed using Nutri survey application.

Data analysis

Data analysis uses the Chi square test to see the homogenization of frequency distribution of the respondents characteristics in both groups. Paired sample *T* test and Wilcoxon test to assess the average difference before and according to the treatment of both groups in pairs. The Independent *T*-test and Mann–Whitney to assess the average difference before and according to the two unpaired groups

Results

Table 1 shows that the majority of respondents in Moringa group is 28 years old amounted to 80% and in the majority control group is 32 years old by 91.43%. Based on the gestational age category, the two both groups of most aged 4–8 weeks (I trimester) are 71.42%. Then in family income variable is known that in both groups have the low-income majority group is under the Recommended Dietary Allowances (RDA) City of Makassar, Both groups of respondents are on average income bitween IDR, 1,000,000.00 – IDR 2,000,000.00 with the average income in the moringa group of IDR. 2,105,714 and the control group amounting to IDR. 2,020,299.

Based on the category of physical activity, in two both groups have a ligh activity habit measured through the score of BAECKE index among 6.25–7.25. Based on the education category, in the moringa and control group majority have last education on Senior high school equal 45.57%. Based on the mothers parity category in both groups equal are multigravida parity. The intervention group amounted to 80% and in the control group of 91.3% (Table 1).

All characteristics of the respondent do not differ significantly in both groups with the value of *p* value >0.05. It means that the characteristic used as sample research is homogeneous (Table 1).

Table 2 shows that the nutritional adequacy rate of protein, calcium, phosphorus, iron, vitamin A, vitamin B1, vitamin C, fiber, vitamin B2, vitamin B6, zinc and folic acid Overall has not achieved minimum needs because the average is still below 80%. It is also known that all of the above nutrient types have *p* value =>0.05, it means before being given the treatment of both groups having comparable or equivalent nutritional intake.

Table 3 shows that the Nutritional substances that fulfill the nutritional adequacy are protein and phosphorus with the average fulfillment above 80%. It is found that some nutrients such as proteins, iron, vitamin B6, zinc and folic acid have a significance of <0.05 which means that the nutrient substance has a significant average difference between the intervention and control group.

Table 4 indicates that the variable Hemoglobin and iron intake increased significantly in the intervention group with *p* value <0.05, while the zink intake did not increase significantly with *p* value >0.05.

Table 5 shows that the hemoglobin level increases significantly in the control group with *p* value of <0.05, while iron and zinc intake does not increase significantly with the *p* value >0.05.

Table 6 depicting from all variables only the hemoglobin which is mean increase differs significantly between the two groups with *p*-value <0.05 which means that giving moringa leaf flour biscuit effectively increases the hemoglobin level.

Discussion

Effect of Moringa leaf flour biscuit on hemoglobin level

The results showed a change in hemoglobin level before and after intervention in both groups. The moringa leaves flour biscuit (cookies) group obtained the mean value before treatment of 10.223 g/dl and increased to 11.227 g/dl, while in the control obtained average before treatment 10.351 g/dl increase to 11.020 mg/dl. Paired sample *t* test on both groups acquired value $p = 0.001 < \alpha = 0.05$.

Although the increase in hemoglobin level in both group is equally significant but the superior one is group given moringa leaf flour biscuit. It can be seen that the average increase is 1.04 while on the control group of 0.66. The Mann Whitney test also showed that the difference of mean before and after administration in both groups was acquired *p* value = 0.001 < 0.05 which means there is statistically difference between the two groups so that it can be inferred in the provision of moringa leaf flour biscuit with a combination of tablets Fe more effectively increase the hemoglobin level in pregnant women anemia compared to the giving iron tablets only.

The fresh leaves of Moringa contain vitamin C seven times more than oranges, Vitamin A four times more than carrots, calcium four times more than milk, potassium three times more than bananas and proteins twice as much than yogurt. Traditionally used for anemia.[9] The results of this study in line with research conducted by Nadimin (2015) also showed that moringa leaf extracts significantly increased hemoglobin levels in pregnant women anemia. Vitamin C content in the leaves of moringa extract accelerates the process of absorption of iron.[10] Iskandar (2015) found that the giving *Moringa oleifera* extract capsules in pregnant women can increase hemoglobin levels and prevent the decline of serum ferritin up to 50%.[11]

The difference in the significant hemoglobin concentration between the two groups is also due to the adequacy of different nutritional intake levels, we can see in Tables 3 and 4. It is known

Table 1
Frequency distribution of respondents characteristick.

Characteristics of respondents	Moringa biscuit			Control			p-Value
	n = 35	%	Mean ± SD	n = 35	%	Mean ± SD	
Age (year)							
<20	4	11.42		3	8.57		
20–35	28	80	28.29 ± 5.89	32	91.43	29.71 ± 5.22	0.270[b]
>35	3	8.57		0	0		
Gestation age (week)							
4–8	25	71.42		25	71.42		
9–12	5	14.28	9.83 ± 7.83	5	14.28	9.80 ± 7.83	0.878[b]
28–32	5	14.28		5	14.28		
Family income (IDR)							
1,000,000.00–2,000,000.00	27	77.14		21	60		
2,100,000.00–3,000,000.00	5	14.28	2,105,714.29 ± 805,474.96	8	22.85	2020299.43 ± 855825.68	0.208[b]
3,100,000.00–4,000,000.00	1	5.71		4	11.42		
4,100,000.00–5,000,000.00	2	0.86		2	5.71		
Physical activity							
6.5–7.25	27	77.14	6.79 ± 0.41	27	77.14	6.79 ± 0.41	0.090[b]
7.5–8.0	8	22.86		8	22.86		
Education							
Elementary school	6	17.14		2	5.71		
Junior high school	5	14.28		10	28.57		
Senior high school	17	48.57	–	17	48.57	–	0.232[a]
Diploma 3	2	5.71		2	5.71		
Bachelor	5	14.28		4	11.42		
Parity							
Primigravida	7	20	–	3	8.7	–	0.172[a]
Multigravida	28	80		32	91.3		

[a] Chi square test.
[b] Mann–Whitney, *Source*: Primary Data, 2020.

Table 2
Distribution of nutritional adequacy of macronutrient and micronutrient in moringa biscuit group and control before treatment.

Nutritional substances	RDA	Mean ± SD						
		Moringa	% RDA	Control	% RDA	Mean difference	p-Value
Protein	61	31.39 ± 13.64	51.45	30.63 ± 12.59	50.21	0.76	0.746[a]
Calcium (mg)	1200	486.37 ± 297.16	40.53	532.25 ± 409.40	44.35	−45.92	0.902[b]
Phosphorus (mg)	700	473.45 ± 299.09	67.63	508.52 ± 310.82	72.64	35.07	0.698[b]
Iron (mg)	18	5.36 ± 3.18	29.78	5.27 ± 3.29	29.28	0.09	0.643[b]
Vitamin A (mg)	900	443.18 ± 401.74	49.24	435.75 ± 285.93	48.42	7.43	0.507[b]
Vitamin B1 (mg)	1.6	0.42 ± 0.33	26.25	0.31 ± 0.16	19.37	0.11	0.076[b]
VitaminC (mg)	85	28.86 ± 18.36	33.95	28.33 ± 17.17	33.32	0.53	0.792[b]
Fiber (mg)	34	4.68 ± 2.13	13.76	3.82 ± 2.13	11.23	0.86	0.746[b]
Vitamin B2	1.4	0.57 ± 0.39	40.71	0.54 ± 0.44	38.57	0.03	0.538[b]
Vitamin B6	1.9	0.87 ± 0.94	45.79	0,70 ± 0.25	36.84	0.17	0.463[b]
Zinc	10	6.11 ± 2.76	61	4.86 ± 1.75	48.6	0.03	0.060[b]
Folic acid	600	91.15 ± 33.25	15.19	88.27 ± 56.28	14.71	2.88	0.134[b]

[a] *t* independent test.
[b] Mann–Whitney test, *Source*: Primary Data, 2020.

Table 3
Distribution of nutritional adequacy of macronutrients and micronutrients in moringa and control group after treatment.

Nutritional substances	RDA	Mean ± SD						
		Moringa	% RDA	Control	% RDA	Mean difference	p-Value
Protein	61	49.59 ± 14.65	81.29	38.63 ± 14.75	63.33	10.96	0.002[a]
Calcium (mg)	1200	491.04 ± 330.81	40.92	545.01 ± 335.50	45.08	−53.97	0.707[b]
Phosphorus (mg)	700	693.78 ± 374.04	99.1	578.65 ± 283.37	82.66	115.13	0.151[b]
Iron (mg)	18	7.87 ± 3.53	43.72	6.33 ± 3.60	35.16	1.54	0.041[b]
Vitamin A (mg)	900	566.599 ± 417.33	62.95	522.82 ± 264.18	61.5	58.09	0.902[b]
Vitamin B1 (mg)	1.6	0.454 ± 0.144	28.12	0.466 ± 0.472	28.75	0.01	0.098[b]
Vitamin C (mg)	85	35.80 ± 43.84	42.11	32.31 ± 24.55	38.01	3.49	0.690[b]
Fiber (mg)	34	6.45 ± 2.45	18.97	5.40 ± 1.74	15.8	2.87	0.062[b]
Vitamin B2	1.4	0.83 ± 0.55	59.28	0.61 ± 0.32	43.57	0.22	0.066[b]
Vitamin B6	1.9	0.98 ± 0.31	51.57	0.79 ± 0.30	41.57	0.19	0.006[b]
Zinc	10	6.25 ± 2.94	48.07	4.88 ± 2.19	37.54	1.37	0.031[b]
Folic acid	600	130.77 ± 57.5	21.79	100.14 ± 69.5	16.69	30.63	0.002[b]

[a] *t* independent test.
[b] Mann–Whitney test, Source: Primary Data, 2020.

Table 4
Mean difference parameters (before and after treatment) in the moringa biscuit (cookies) group.

Parameters	Moringa biscuit (cookies) group			
	Baselin	Post intervation	Mean difference	Sig (2-tailed)
Hemoglobin	10.223 ± 0.28	11.277 ± 0.26	1.04	.001[a]
Iron intake	5.36 ± 3.18	7.87 ± 3.53	2.51	.001[b]
Zinc intake	6.11 ± 2.76	6.25 ± 2.91	0.14	.144[b]

[a] Paired sample *t* test.
[b] Wilcoxon.

Table 5
Mean difference parameters (before and after treatment) in the control group.

Parameters	Control group			
	baseline	Post intervation	Mean difference	Sig (2-tailed)
Hemoglobin	10.351 ± 0.26	11.020 ± 0.39	0.66	0.001[a]
Iron intake	5.27 ± 3.29	6.32 ± 3.61	1.05	0.152[b]
Zinc intake	4.86 ± 1.75	4.89 ± 2.19	0.03	0.277[a]

[a] Paired sample *t* test.
[b] Wilcoxon.

Table 6
Comparison of the Effectiveness of Moringa biscuit compared to the control.

Parameters	Mean difference ± standard deviation		Sig (2-tailed)
	Moringa group	Control group	
Hemoglobin	1.04 ± 0.24	0.66 ± 0.33	0.001[b]
Iron intake	2.51 ± 2.83	1.05 ± 4.64	0.116[a]
Zinc intake	0.14 ± 0.83	0.03 ± 1.33	0.878[b]

[a] Independent *t* test.
[b] Mann–Whitney, source: Primary Data, 2020.

that some nutrients such as protein, iron, vitamin B6, zinc and folic acid have a significance *p* value <0.05 meaning that the nutrients have a significant average difference between the intervention and control group, where the average in the intervention group is bigger compared to the control group even though-average rate of Sufficiency is still under of Standard of nutritional adequacy.

Nutrient intake during pregnancy affects the health of baby as well as the health of pregnant women themselves so that the need for nutrition during pregnancy will increase according to the introduction. Protein is needed for biochemical processes in the formation of HB, vitamin C effectively increases the absorption of iron in food can also play a major role on iron metabolism, vitamin B6 for the formation of red blood cells, fibers facilitate the process of bowel movements and overcoming constipation, folic acid can prevent the power of the onset of pregnancy can also cure anemia because of folic acid deficiency.[12]

Tables 3 and 5 found that the control group given the tablet Fe with doses 2× 250 mg also experienced a significant increase in hemoglobin levels.[13] This is in line with the theory that the treatment of a tablet Fe can increase hemoglobin level.[13] This result is also supported because the intake of vitamin C that respondents consumed in the control group increased by 38.1% although it is still under the standard of the axon so it can help the process of absorption of iron. This is in line with the theory that vitamin C can effectively aid the absorption of iron and play a role in iron metabolism.[12]

Effect of giving moringa leaf flour biscuit (cookies) to iron intake

Research results can seen in Tables 3 and 6. Finding the amount of iron intake underwent a significantly different change of improvement before and after treatment in the intervention group with a difference of mean 2.51 and *p*-value = 0.001 < 0.05, in the control group was also increased with a difference of mean 1.05 and

p value = 0.152 > 0.05 which means it does not differ significantly before to after treatment. Based on the data in Tables 3 and 6 test result analysis difference. The mean group intervention and control group get the value *p* = 0.116 > 0.05 which means there is no significant difference to the increase in iron intake among the two groups. This research is purely a effect of the intervention given can be seen the characteristics and iron intake before the treatment both groups homogeneous.

This outcome was not in line with the research conducted by Hadju and Bahar (2014) found that the results after the treatment of moringa extract, the amount of protein intake, iron, and fiber decreased in both groups although meaningless and the comparison between the two groups is also meaningless (*p* > 0.05). Results of recall at the beginning of food research found there are still many nutrients intake of pregnant women who do not meet the nutritional adequacy rate such as iron and zinc.[6]

A significant increase before and after the administration of the moringa leaves' flour biscuit in the treatment group is supported by considerable nutrient content, amino acids and various micronutrients, especially the high enough iron that is 28.29 mg in kelor leaves.[10]

In a given moringa leaf flour biscuit contains moringa leaf flour amounting to 2.8 g each piece. Increased iron intake between the two groups is not significantly different because of the lack of variation of food consumed and still many pregnant mothers who spend the morning meal due to nausea.

Effect of giving moringa leaf flour biscuit (cookies) to zinc intake

Zinc is an essential nutrient substance that must be fulfilled during the period of pregnancy. Zinc absorunic absorption is antagonistic to each other especially when found with high concentrations. The average zinc will be absorbed by about 20%–40% of the food intake and will increase when the level of zinc in the food

is low. The amount of zinc that can be absorbed by the body ranges from 15 to 40%. Zinc absorption is influenced by the body's zinc status. If more zinc is needed, more and more zinc will be absorbed. In addition, the type of food consumed also affects the absorption of zinc. Fibers and phytic acid will inhibit the bioavailability of zinc. The amount exceeding the body needs will also inhibit the absorption of zinc. The value of albumin in plasma is the main determinant of zinc absorption, albumin is the main transport tool of zinc. Zinc absorption will decrease when blood albumin decreases.[14]

The results of the study can be seen in Tables 3 and 6 found that the amount of zinc intake experienced an increased change although it did not differ significantly before and after treatment in both groups. In the interventional group the mean difference 0.14 with p value = 0.144 > 0.05, in the control group also experienced an increase with the difference of mean 0.03 and p value = 0.277 = >0.05. An analysis difference between the mean between the intervention group and the control group gets the p value = 0.878 > 0.05 which means there is no significant difference. Although the increase in the zinc intake in both groups is not significant but the average increase in the intervention group is higher than in the control group. These results proved purely the effect of after the intervention, can be seen from the characteristics of both groups that are already homogeneous before being given treatment so that it can be said no other bias affects.

Biscuits (cookies) of Moringa leaf flour is made by researchers using the formulation that has been made by Dewi (2018) that has been tested organoleptic, proximate level and Fe.[7] Zinc is a minor mineral that the body needs. Minor mineral deficiencies will cause health problems that are also serious. When our diet is healthy and varied, then the body will get enough minerals. However, if the diet is not balanced or the body has impaired mineral absorption, then the body can suffer from mineral deficiency. Moringa contains minerals of calcium, chromium, copper, fluorine, iron, manganese, Magnesium, Molybdenum, phosphorus, potassium, Sodium, Selenium, Sulphur, Zinc. Zinc content in the leaf powder of the moringa is 6 times more than almond.[15]

The results of this study were not in line with those done by Hadju & Bahar (2014) found that the results of moringa extract increased zinc intake in the intervention group and decreased in a meaningless control group. At the beginning of research found that food recall results are still many nutrients intake of pregnant women who do not meet the nutritional adequacy rate (AKG) mainly energy, vitamin B2, and vitamin C, even some other nutrients intake does not meet 50% of Standard of nutritional adequacy such as vitamin B1, D, E, folic acid, and mineral groups such as calcium, iron (Fe) protein intake, iron, and fiber also decreased in both groups but meaningless and the comparison between the two groups is also meaningless (p > 0.05).[6]

Table 3 shows that almost all nutrient intake (except protein and phosphorus) consumed by pregnant women in both groups after being given intervention still has not reached the nutritional adequacy rate in accordance with the provisions of Regulation of Health minister Republic Indonesia No. 28 year 2019. This is closely related to the respondent's gestational age, which is an average aged 4–8 weeks (trimestrer I). The theory says that pregnant women in the I nudge trimester are still experiencing nausea and vomiting, thus affecting the number and frequency of eating. In the first months of pregnancy expectant mothers often get sick due to increased estrogenic hormones and HCG increases, muscle tonus decreases so that motility is also reduced causing longer food in the stomach and that has been digested longer in the intestines.[16]

On characteristics of family income is known that in the two group have low income is under the regional minimum wage City of Makassar. Income is an indirect cause affecting the amount of zinc intake in pregnant women because it is related to the selection and quality of food ingredients. Poor quality of food will inhibit the process of absorption of zinc in the body. The mother with a income of <Regional minimum wage increases the incidence of anemia by 3.4 times compared to mothers with >Regional minimum wage.[17]

Conclusion

There is the effect of giving cookies of kelor leaf flour to hemoglobin level but has no effect on iron intake and zinc intake in pregnant women anemia.

Advice

It is recommended for pregnant women to make use of local crops especially kelor leaves to help fulfill the nutrient nutrients in pregnancy to avoid anemia.

Conflict of interest

The authors declare no conflict of interest.

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