J Young Pharm, 2017; 9(1)Suppl: s79-s84
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org | www.phcog.net

Original Article

Effect of *Moringa oleifera* Leaves Extract Against Hematology and Blood Biochemical Value of Patients with Iron Deficiency Anemia

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ABSTRACT

Introduction: Approximately (24.8%) the world's population suffers from anemia and 50% of an anemic case is due to iron deficiency. This study investigated the efficacy of *Moringa oleifera* L leaves to extract as an iron booster and supplement to help overcome anemia in the community. **Methods:** It was a randomized, double-blind, placebo-controlled study in anemic women (hemoglobin 8-12g/dL), in which the water extract of moringa leaves was examined as an add-on therapy in the subject treated with ferrous sulfate (200mg/tablet). Thirty-five women subject of 16-49 years old were divided into 17 of moringa leaves and 18 of control. The extract of moringa leaves of 1400 mg was formulated in capsules and was administrated daily for 3 weeks. **Results:** The result showed there were significantly increase of mean of hemoglobin (0.794±0.81 g/dL), ferritin (29.378±42.48 ng/mL), MCHC (Mean Corpuscular Haemoglobin Concentration) (2.459±2.86 g/dL), RDW (Red Distribution Wide) (1.4±2.07 %) and decreased of platelets (36529.41±59024.48 /uL). The control groups were significantly increased of mean of the hemoglobin (0.644±0.83g/dL), erythrocytes (0.475±0.523 Tpt/L), hematocrit (2.189±l4.08 %), MCV (Mean Corpuscular Volume)(4.756±8.91 fL), MCH (2.183±2.47 pg) dan RDW (2.844±2.80%).The hematocrit (3.14±1.47%), MCH (Mean Corpuscular Hemoglobin) (3.495±1.33 pg), MCHC (3.264±0.96 g/dL) values of moringa leaves were significantly higher whereas the platelets count (55251.63±23404/uL) of moringa leaves were significantly lower ($p<0.05$) than those of control group. **Conclusion:** It can be concluded that moringa leaves extract could improve iron deficiency anemia in women.

Key words: Anemia, Ferrous sulfate, Ferritin, Hematology, *Moringa oleifera*.

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DOI: 10.5530jyp.2017.1s.20

INTRODUCTION

Approximately 162 billion people (24.8%) the world's population suffer from anemia[1] and 50% of an anemic case is due to iron deficiency.[2] According to data from Department of Health of the Republic of Indonesia (2013) prevalence of anemia is about 21.7%, and the prevalence of iron deficiency anemia in women aged 15-50 years in Indonesia is about 33.1% and young women is about 28%.

Iron deficiency anemia can cause both growth disruption and disruption of physical and mental development, lower intellectual, ability to learn, ability to exercise, impaired cognitive development and behavior. A weakened immune system, susceptibility to infection would lower health status thereby reducing the capacity, productivity, and creativity of adolescents[3], and during pregnancy can increase the complications, the risk of maternal mortality, the rate of prematurity, low birth weight and perinatal mortality rate.[4,5]

Moringa oleifera L (family: Moringaceae) is a kind of herbaceous plant used since ancient African communities to overcome malnutrition. It is known that moringa's fresh leaves 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 protein two times more than yogurts.[6] It is traditionally used for anemia, anxiety, asthma, blackheads, bronchitis, catarrh, cholera, conjunctivitis, cough, diarrhea, eye and ear infections, fever, swollen glands, headache, abnormal blood pressure, hysteria, pain in the joints, acne, and psoriasis.[6] In the previous study, *moringa* leaves extract demonstrated anti-anemia activity in rats induced aniline.[8]

Chemical constituents of moringa leaves have been reported to have antihypertensive effects, anticancer, and antibacterial activity, namely 4 (4'-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin A and B pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate.[6] Moringa has been reported to have antimicrobial activity / biocidal, an analgesic, anti-inflammatory, antidiabetic, antispasmodic, and antiulcer.[8] Until now, there is no clinical studies using moringa leaves extract as an anti-anemia.

METHODS

Leaves extract of *Moringa oleifera* were prepared by maceration methods and continued with a dry vacuum system, maltodextrin filler addition on extracts which may increase the mass. Each capsule contains 700 mg moringa leaves extract. The nutrient screening was conducted for the extract, including protein and vitamin C.

Iron and screening of phytochemical constituent.

The iron content in the extract was analyzed using atomic absorption spectrophotometer according to the previous method.[7] Phytochemical constituent such as alkaloid, flavonoid, terpene, saponin, glycoside and

anthraquinone in the extracts was performed by according to the previous method.[7]

Research Design

Single-center, randomized, placebo-controlled trial, parallel design, enrolling anemic women 16-49 years olds. Patients were randomized into 2 groups within which they were received moringa leaves extract 1400 mg/ day with 200 mg ferrous sulfate or placebo consist of ferrous sulfate 300 mg. Inclusion criteria was women that selected by anamnesis and initial screening with women's hemoglobin levels (8-12g/dL) and not taking NSAIDs, aspirin, and corticosteroids in the long term. Exclusion criteria were pregnant or breastfeeding women, suffer from malabsorption syndrome, history of sickle cell anemia or thalassemia, chronic bleeding, have an anti-anemia drugs before and during the study, and suffering from severe constipation and allergies to *moringa* leaf.

Research protocol has been reviewed and approved by The Ethics Committee of Faculty of Medicine, Universitas Indonesia (No. 625/H2.F1/ETIK/2013). Patients 16 years or older (42 patients) from senior high school in Depok fulfilling the anamnesis and hematology examination. All patients were gave written informed consent for participation and randomized into 2 groups which had three weeks treatment, followed by hematology test. Seven patients had drop out criteria during research because of non-adherence and side effect of the extract.

Data Analysis

The tests conducted were Pair sample *t*-test or Wilcoxon signed rank test to determine differences in mean statistically significant from the two treatment groups of interconnected (before and after the test on each group). While the independent sample *t*-test or Mann-Whitney test was performed to determine the mean difference was statistically significant between the two treatment groups were not interconnected (the mean before and after group's *moringa* with the mean before and after control group).

RESULTS AND DISCUSSION

Analysis of nutrient and phytochemical screening of moringa leaves extract

The extract contained 27.33% protein using Kjeldahl method. Total iron content in *Moringa* extract of the averages 14.67mg/ 100g extract. The extract contained vitamin C was 759.05 mg / 100 g with HPLC. Analysis levels of nutrients in the capsules used in the research was calculated based on the amount of iron substance in 100 g of extract. If one capsule with 700 mg of the extract the contained iron levels are 0.103 mg of iron/ capsules, vitamin C 5.313 mg/capsule and protein levels of 39.043 mg in one capsule. The result of phytochemical screening can be seen in Table 1.

Comparing the nutrient level of vegetables (spinach and cinnamon leaf) that are often used to overcome the problem of malnutrition and nutrition world.[9] Spirulina is blue algae from marine natural products that contain the highest protein and iron.[10] In 1 tablet (500 mg) spirulina contains 0.9 to 3 mg of iron and 300 mg protein/ tablet. Compared to cinnamon leaf and spinach, *moringa* leaves have a protein content almost twice and four times higher than that cinnamon leaf and spinach, iron in *moringa* leaves is four times higher than spinach and five times higher than cinnamon leaf while the vitamin C of *moringa* leaves are 12 times higher than cinnamon leaf and 15 times higher than spinach.

Protein and iron levels were lower in research of Nweze, Onyekwere & Felix (2014), which are proteins (18.92%), and iron (3.66%), and in research of Triandita (2013) levels of iron in the water extract of *moringa* leaves obtained 12.87 mg / 100g, by using the method of MAE (Microwave Assisted Extraction) Vitamin C test using titration method while iron content analysis using atomic absorption spectrometry method (AAS). In the extraction simplest process, we used a method of maceration, percolation, filtration, evaporation that terminated by using a vacuum drying system for drying the extract, maltodextrin filler addition on extracts which may increase the mass and reduce the levels of nutrients extracts.

Comparison nutrition levels of vegetables (spinach and katuk leaf (*Sauropus androgynous*)) that are often used to overcome the problem of malnutrition and nutrition world.[9] Spirulina is blue algae from marine natural products that contain the highest protein and iron.[10] In 1 tablet (500 mg) spirulina contains 0.9 to 3 mg of iron and 300 mg of protein/ tablet. When compared katuk leaf and, moringa leaves have a protein content almost twice and four times of the katuk leaf and spinach, iron in moringa leaves is four times higher than spinach and five times higher than katuk leaf while the vitamin C of moringa leaves are 12 times higher than katuk leaf and 15 times higher than spinach.

Patient Demographics Profile

Characteristics of the patient were assessed from age, occupation or education, weight, height, and body mass index (BMI). Educational characteristics of the subjects are 94.23% subjects are senior high school students (high school) and 5.77% work as employees and housewives. By the total number of subjects classified as mild anemia was 71.43% (25 people) and anemia was 28.57% (10 people).

Based on Table 2 anthropometric characteristics between moringa group and the control group have no significant difference. The average age of subjects is 17 years (control group) and 20 years in Moringa group. The height average of the two groups is 154 cm and the weight is 49 kg.

Parameters Measured in Research

The difference parameters average before and after the intervention.

To see the average difference before and after treatment each group tested by pair sample t test as shown in Table 3 and Table 4. In the Extract group (Table 3) showed significant differences in the increase in the value of hemoglobin ($p = 0.001$), ferritin ($p = 0.012$), MCHC ($p = 0.003$), RDW (0.013) and a decrease in platelets ($p = 0.021$). Significant increase in the control group parameters obtained in the hemoglobin value ($p = 0.004$), erythrocytes ($p = 0.001$), hematocrit ($p = 0.036$) and RDW ($p = 0.00$). While at MCV ($p = 0.037$) and MCH ($p = 0.002$) decreased significantly. The change in the value of other parameters is not significant ($p > 0.05$), probably due to inadequacy of the data to prove that there is a significant difference

During the supplementation period obtained an increase average in hemoglobin significant in both groups (0.794 ± 0.81 g / dl, $p = 0.001$) for moringa group and the control group (0.644 ± 0.83 g / dl, $p = 0.004$). In the literature described an increase in hemoglobin levels with consumption ferrous sulfate 300 mg (60 mg of iron) 1-2 times daily for 3-4 weeks will increase the hemoglobin 1-2 g / dl.[3] The mean increase in hemoglobin of non-pregnant women aged 15-49 years in developing countries is 0.864 g / dL, it is higher than that obtained by the researcher. However, when compared with studies of Idohou-Dossou et.al (2011) with an increase in the difference in mean hemoglobin (0.40 g / dl) in the treatment group, the results obtained by researchers is higher.

The significant increase can be seen in ferritin serum levels in the moringa group of 29.378 ± 42.48 ng/ml ($p = 0.012$). The results obtained are higher when compared to a research of Idohou-Dossou *et al* (2011) with no significant increase in 12 ± 0.4 ng/ml ($p = 0.601$) in the group of moringa [12]. The results of ferritin obtained are lower when compared with

spirulina research by Ramesh *et al* in 2013 showed a highly significant increase in the 58 728 ± 10 170 (p = 0.000).

Spirulina iron levels are 15-30 times than iron in moringa leaves and the duration of drug administration is a major factor in the blood ferritin serum improvement. According to the literature an increase in ferritin serum in 1-month consumption of 3-6 mg of Fe / kg body weight per day or for 12 weeks of ferrous sulfate 300 mg once daily consumption is more than 50 ng / dL.11 It takes a minimum of 12 weeks in the treatment of iron deficiency anemia in order to fill the void of iron reserves in the bone marrow, liver and macrophages and pregnant women need a longer time during the 9 months of pregnancy and during lactation.[12] For treatment with moringa leaves consumed to achieve optimal results for 3 months and preferably in the form of extracts because smaller than doses used.

Ferritin is an acute phase protein that increases its value to the state of inflammation and infection, so if at the time of taking the blood patients experiencing cough, colds or other infections it will increase the value of ferritin. Measurements of different acute phase proteins can help to interpret the value of ferritin serum if the concentration of the acute phase proteins is increased it indicates encountered inflammation. Checking acute-phase protein CRP that often used is (C-reactive protein) and α1-acid glycoprotein (AGP), because it is increased quickly to the inflammation and also down quickly. The value may not reflect accurately the iron status in developing countries where malaria, HIV, seasonal infectious diseases, and tuberculosis are prevalent.[2]

The significant increase in erythrocyte can be seen in the control group with a mean difference of 0.475 ± 0.52 erythrocytes million/mL (p = 0.001), but not significantly in the moringa group with a mean difference of 0.482 ± 2.26 million/mL (p = 0.394). When compared with the research of Gunadi, (2008) the difference in the mean increase in erythrocyte 0:24 ± 0:57 million/uL (p = 0.03) in the consumption of Fe three times a day. Gunadi Research (2008) was conducted in children aged 9-12 years with hemoglobin <12g / dl was given a dose of 300 mg three times daily Fe and Fe second group was given once daily for 4 weeks.[13] It also should be considered the cause of secondary illnesses suffered by research subjects who have not been detected, diet and lifestyle subjects that cause the erythrocytes did not increase after administration of oral iron tablets regularly.

Hematocrit values were significantly increased in the control group with a mean difference 2.189 ± 4.08% (p = 0.036) while in the moringa leaf were decreased (4,558 ± 0.95%) (p = 0.404). According to research Idohou-Dossou *et al* in 2011, a significant hematocrit values also occurred in the control group with a mean of 1.6 ± 2.55% (p = 0.002) and in the group of *moringa* leaf non-significant increase in hematocrit, 0.1 ± 2.53% (p = 0.788).[14] There is some significant increase in hematocrit in line with the increase in the number of erythrocytes in the blood and marks the start of an improvement of anemia, but the decline does not mean worsening anemia. There are several factors that affect them, the number of erythrocytes, if in the state of anemia; hematocrit will fall, thus helping the mechanisms of anemia faster. Some factors affecting hematocrit value besides a disease is malnutrition, deficiency of vitamin B and C (lower) and improve the condition of dehydration and hypovolemia.[15]

The results showed MCV decrease significantly in the control group with a mean difference value (4,756 ± 8.91 fL) p = 0.036. In the moringa group (0.635 ± 11.22 fL) (p = 0818). While research Idohou-Dossou et al in 2011, MCV values increased but not significantly. In the Moringa group 0.4 ± 6.9fL and p = 0.443 and in control group 1.3 ± 5.6 fL, p = 0189.[14] The decrease of MCV is influenced by the number of the erythrocyte. MCV is a parameter that sensitive toward changes in erythrocyte when compared with MCH and MCHC to determine the possibility of iron deficiency. The possibility of impairment of MCV strongly supports the iron deficiency anemia treatment that has not been completed so it takes extra time 2-3 weeks. The purpose is so that microcytic anemia can be corrected to achieve normal values.

Mean erythrocyte hemoglobin or MCH acquired shown a significant reduction in the control group (2.183 ± 2.47pg) p = 0.002. This decrease is influenced by the value of the erythrocyte. While there is a significant increase in the moringa group MCH by 1.312 ± 4,936 pg, p = 0.028. When compared with research of Idohou-Dossou, *et al* 2011, a significant increase seen in the moringa group and control group with consecutive values (1.3 ± 2.4) pg, p = 0.001 and ((1.7 ± 2.05) pg, p = 0.001.[14]

Mean erythrocyte hemoglobin levels or MCHC for *Moringa* group increased significantly with a mean difference of 2.459 ± 2.86 g / dl, p = 0.003, while in the control group were not significantly decrease (0806 ± 2.81 g / dl) and p = 0.241. It happened due to the influence of MCV and MCH values were also decreased. The results obtained showed a significant reduction in MCHC indicating ADB but not yet corrected.

RDW value of the results showed a significant improvement in both groups, respectively 1.4 ± 2:07%, (p = 0.013) for moringa group and 2,844 ± 2.8%, (p = 0.000) for the control group. The increase of RDW indicates ADB has not reached normal values because it is necessary to increase the duration of therapy in order to return to normal RDW values along with increased MCV.

Based on the results of the statistical analysis there are no significant differences between the two groups, indicating moringa does not affect the increased production of leukocytes, although the contains of protein in moringa are quite high, about 27% of the nutrients of Moringa leaves. There is a non-significant decrease in moringa group in leukocytes and the control group actually increased, although non-significant. Leukocyte increased due to an external reaction in the bone marrow due to infection and inflammation, stress (spasms, anxiety) drugs. Leukocytes are physiologically affected by the physical activity of moderate-severe, emotional disturbances, seizures, paroxysmal tachycardia, and menstruation. Increased leukocytes can also be caused due to the occurrence of infection and acute inflammation, drugs, eg aspirin, procainamide, potassium iodide, sulfonamides, and others.[16] Leukopenia can also be caused by drug use, especially acetaminophen, sulfonamides, PTU, barbiturates, diazepam, diuretics, indomethacin, methyldopa, and others.[16]

Platelet decline significantly in the group of moringa (-36529.41 ± 59024.48 / uL) (p = 0.021) and control group (18722.22 ± 78553.6 / uL) (p = 0.326). The decrease is due to lack of iron in the bone marrow that helps the production of platelets, antibiotic drugs (chloromycetin, streptomycin), sulfonamides, aspirin (salicylates) and others. In addition, it is also influenced by strenuous physical activity.[16]

Comparison of the effectiveness of moringa group and control group

To determine the statistical tests that will be used to process the data comparison of the effectiveness of moringa group and control group, the Independent test T-test has been done. There are significant differences in hematocrit, MCH, MCHC and platelets (Table 5).

From the results of statistical analysis chi-square test was obtained factor that influence the body's iron status significantly with a p-value (p <0.05) was menstruating (affecting the value of ferritin, erythrocytes, and platelets) menstruating subjects in both groups. In the moringa group, the consumption of side dishes and fruit-vegetable affect hematocrit values. Whereas in the control group eating frequency affects the value of ferritin, the consumption of side dishes has a significant effect on the value of hemoglobin, ferritin, hematocrit and leukocyte and drinking affect the levels of ferritin.

Bioavailability and low iron levels in foods and types of foods typical of the population of Indonesia are known as one of the main causes of iron deficiency.[17] In addition, the compliance of women of reproductive

age to consume low iron supplements. In a study in lactating women in Senegal, only 88% of anemic women claimed to have consumed iron tablets but the number of tablets taken and the duration of treatment is unknown.[14]

Although the hemoglobin concentration increased significantly in Moringa and Moringa group ($p < 0.05$) after the third week 64.7% of the sample in the Moringa group and 61.1% in the control group had a hemoglobin concentration of less than 12g / dL. The possibility of the presence of intestinal parasites such as *Schistosoma, Ankylosotomia, Ascaris lumbricoides* and *Trichuris trichloro* affect the existence of iron with a comparison of several studies conducted in Indonesia showed the prevalence of 60-90%.[18]

Ferritin serum was also found to increase significantly in the moringa group and 54.7% of samples in the group of moringa have raised its iron reserves and 11.1% of samples in the control group. The increase of ferritin serum is in line with research Angeles-Agdeppa, *et al* 1997 in young Indonesian women who consumed iron supplements at a dose of 200 mg of the element iron weekly for 3 months.[2] In the control group no significant increase in ferritin serum and only 11.1% of the samples increased during the intervention. These results support the theory that the low bioavailability of iron in foods from plant sources.[19]

In another study on Moringa leaf, nutrient analysis and bioavailability of iron explain that the leaves contain protein and essential minerals but the iron levels that high enough in Moringa leaves have a low bioavailability (2.2%). This is due to the polyphenol content which is a potent inhibitor of iron bioavailability.[20]

The inhibitory effect is related to the structure of the phenolic and catechol galloyl that form chelating complex polyphenols with iron and non-heme iron.[19] So that the amount of iron consumed per day in the diet plus moringa leaf extract may be very low and not able to cover their daily needs. To improve the bioavailability of iron in the leaves of moringa according to Yang & Tsou (2006) by cooking, boiling, heating so that the interaction with polyphenols reduced so the iron can be free. Fresh and dried leaf powder, when boiled, will increase the bioavailability of iron respectively 3.5 and 3 times.[21]

The effectiveness of moringa leaf water extract in Wistar rats at a dose of 400 mg/kg, showed an increase in hematocrit hematologic parameters significantly, but the value of hemoglobin, erythrocytes, MCV, MCH and MCHC showed no significant differences, and with higher doses actually reduce the value of hematological parameters of the mice.[22] In contrast to research Madukwe, *et al* 2013 on the effectiveness of moringa leaf dry powder treat anemia in mice, results in a dose of 5% protein in moringa leaf powder seen a significant increase in hemoglobin values, PVC, and erythrocytes. But at a dose of 10% of the hematologic values decreased significantly.[23]

Factors influencing changes in parameter values

Coffee, milk, tofu, and noodles are statistically having no effect on the value of the parameter subjects (Table 6. This may be due to lack of data (number of samples) were processed to prove the effect of food on the parameters significantly. Meat and fish contain heme iron that well absorbed (20-30%). The side dishes also increase the absorption of non-heme iron from foods consumed at the same time and dose dependent. Hallberg and Rossander (1984) found that the absorption of non-heme iron increased 2.5-fold ($p < 0.01$) after the addition of meat (75g) into a food derived from corn, rice, and black beans.[24]

Mechanisms of animal protein increase the absorption of non-heme iron is a cysteine-containing peptide and the active components in the meat is of L-α-glycerophosphocholine, hydrolytic products of phosphatidylcholine (lecithin) which occurs in tissue. Phosphatidylcholine is one of the major phospholipids in cell (mostly in animals meat), consisting of glycerophosphocholine and branch chain hydrocarbons having acyl, alkyl or alkenyl and associated with phospholipid Phosphatidylcholine is also available as a natural phospholipid precursor and metabolite derived from soy lecithin, which can be used to increase the absorption of non-heme iron from vegetarian food.[25]

Polyphenols are found mainly in tea and coffee has the effect of reducing the absorption of non-heme iron and coffee which has about half the inhibitory effect of the inhibitory effect of tea. Other drinks such as red wine, cocoa, and teugae herb has proven to inhibit the absorption of non-heme iron.[26] Black tea polyphenol is the most powerful potent inhibitor when compared green tea, cocoa or red wine, and there are reports that 20-50 mg of polyphenols reduce the amount of absorption of non-heme iron from white bread (50-70%), while 100-400 mg polyphenols (equivalent to one cup of tea / instant coffee) reduce iron absorption of non-heme bread for 60-90% polyphenols Although the strong binding proteins, the addition of milk to black tea or coffee does not reduce the inhibitory effect of iron.[24] Ascorbic acid has inhibitory effects against tannins and polyphenols on the absorption of non-heme iron which is about 50 mg of ascorbic acid required to counteract the effects of 100 mg of tannic acid.[24]

Calcium contained in foods or supplements have been reported to reduce the absorption of non-heme iron and heme iron.[24] Calcium and iron compete for binding with one or more substances that are important in the absorption of Fe lines. The addition of calcium (40-600mg) on cereal grain significantly reduces the absorption of iron and inhibition occurred at a dose level of 300 mg. Greater inhibiting effect when calcium is added to the dough because it reduces the degradation of phytate during fermentation. Consumption of milk or cheese (equivalent to 165 mg of calcium) to reduce the absorption of iron, respectively by 57% and 46%.[24]

Phytic acid (myoinositol hexaphosphate) is found in whole-grain cereals, nuts and seeds, soy protein (such as tofu and tempeh) have been proved to be inhibitors of iron absorption of non-heme [10]. The addition of 2 mg, 25 mg and 250 mg of phytate phosphorus (P-phytate) into bread significantly reduced iron absorption respectively 18%, 64% and 82%.[26] The inhibitory effect of phytate on iron absorption may be reduced by vitamin C and an estimated 80 mg of ascorbic acid required to counteract the inhibitory effect of 25 mg P-phytate, and in very large doses are needed to overcome the inhibitory effect of high phytate diet (250 mg P -phytate).[26]

The main mechanism of ascorbic acid increases the absorption of iron in the formation of the complex soluble iron that is easily absorbed and mechanisms that are able to reduce Fe3 to Fe^{2+}. Fe^{2+} is a form of iron that can enter into mucosa cell. The effect of ascorbic acid against the iron inhibitory effect of the most effective absorption of foods is containing

Table 1: Summary results of the qualitative phytochemical screening of water extracts of Moringa leaves compared with other studies

Secondary Metabolite Identification	Results	The other Research	
		A	B
Alkaloid	+	+	+
Anthraquinone	+	+	ND
Glycoside	+	-	+
Saponin	+	+	+
Phenol	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+

Explanation: A) Nair, V.M, Roopalatha, U.C, (2013), B) Okwari, Emerole, Dasofunjo, Ezugwu, Obi, (2014).ND: Not Detected

Table 2: Anthropometric characteristics of the study patients

Parameters	Moringa		Control	
	Baseline	Sig(2-tailed)	Baseline	Sig(2-tailed)
Number of samples	17(80.95%)		18(85.71%)	
Mild anemia	11(64.7%)		14(77.77%)	
Moderate anemia	6(35.3%)		4(22.17%)	
Age	20.24±8.356	0.003	17±1.572	0.002
Weight	50±4.301	0.981*	48.33±2.88	0.586*
Height	155.12±4.78	0.639*	153.28±3.88	0.924*
Body Mass Index	20.81±2.01	0.328*	20.57± 1.46	0.835*

*) Normally distributed data if Sig. (2-tailed) / (p> 0.05), and showed no significant difference (homogeneous data)

Table 3: Mean difference parameters (before and after treatment) in the extract group

Parameters	Moringa group			
	Baseline	Post Intervention	Mean Difference	Sig(2-tailed)
Hemoglobin	10.58±1.36	11.37±1.46	-0.794±0.81	0.001*
Ferritin	11.90±22.14	41.27±37.15	-29.378±42.48	0.012*
Erythrocyte	4.40±0.58	4.70±1.39	-0.482±2.26	0.394
Hematocrit	33.64±3.90	32.68±4.47	0.947±4.56	0.404
MCV	77.16±11.97	76.52±12.63	0.635±11.22	0.818
MCH	24.62±4.84	25.93±4.6	-1.312±4.94	0.289
MCHC	31.62±2.21	34.08±2.31	-2.459±2.86	0.003*
RDW	15.18±3.12	16.58±3.45	-1.400±2.07	0.013*

*) Sig. (2-tailed) of <0.05, significantly different between before treatment (baseline) and after post-intervention

Table 4: Mean difference parameters (before and after therapy) in the control group

Parameters	Control group			
	Baseline	Post Intervention	Mean Difference	Sig(2-tailed)
Hemoglobin	10.93±1.02	11.57±1.12	-0.644± 0.83	0.004*
Ferritin	24.42±43.55	47.82±65.07	-23.407±58.60	0.108
Erythrocyte	3.95±0.49	4.43±0.48	-0.475±0.523	0.001*
Hematocrit	33.01±3.24	35.19±3.36	-2.189±4.08	0.036*
MCV	83.03±11.69	78.27±7.0	4.756±8.91	0.037*
MCH	28.06±4.30	25.87±2.6	2.183±2.47	0.002*
MCHC	33.53±2.34	32.72±1.59	0.806±2.81	0.241
RDW	13.36±2.12	16.20±3.47	-2.844±2.80	0.000*

*) Sig. (2-tailed) of <0.05, significantly different between before treatment (baseline) and after treatment (post-intervention)

Table 5: Comparison of the effectiveness of moringa leaves compared to the control

Parameters	Mean difference		Sig.(2-tailed)
	Moringa group	Control group	Equal variances assumed
Hemoglobin	-0.794±0.81	-0.644± 0.83	0.594
Ferritin	-29.378±42.48	-23.407±58.60	0.734
Erythrocyte	-0.482±2.26	-0.475±0.523	0.066
Hematocrit	0.947±4.56	-2.189±4.08	0.039*
MCV	0.635±11.22	4.756±8.91	0.212
MCH	-1.312±4.94	2.183±2.47	0.012*
MCHC	-2.459±2.86	0.806±2.81	0.002*
RDW	-1.400±2.07	-2.844±2.80	0.093

*) Sig. (2-tailed) of <0.05, significantly different between the Moringa group and control group

Table 6: Assessment of factors affecting the intake of iron status through changes in parameter values

Parameters	M ens	Animal Protein		Tea		Coffee		Milk		Fruits-Vegetables		Tofu-temp eh		Noodles	
	truation	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Hemoglobin	1.00	0.08	0.01*	0.10	0.33	1.00	0.53	0.54	1.00	0.25	1.00	0.43	0.53	1.00	1.00
Ferritin	0.01*	1.00	0.05*	1.00	0.00*	1.00	1.00	1.00	0.29	0.41	1.00	1.00	0.21	1.00	0.58
Erythrocyte	0.04*	0.06	0.25	1.00	1.00	0.08	0.44	0.58	0.17	0.33	1.00	0.47	1.00	1.00	1.00
Hematocrit	0.23	0.00*	0.04*	0.15	0.62	0.58	1.00	1.00	1.00	0.05*	1.00	0.47	0.25	0.62	1.00
MCV	0.63	0.35	0.33	0.64	1.00	0.29	1.00	021	0.25	0.64	0.27	0.47	0.52	0.13	0.53
MCH	0.11	0.34	0.33	1.00	1.00	0.24	1.00	0.51	1.00	0.30	0.27	0.51	0.52	0.60	1.00
MCHC	0.25	0.29	1.00	1.00	0.60	0.52	1.00	0.54	0.29	1.00	0.13	1.00	0.21	0.54	1.00
RDW	0.23	0.13	1.00	0.64	1.00	0.09	1.00	1.00	1.00	0.64	1.00	0.11	1.00	1.00	1.00
Leukocyte	0.69	0.64	0.01*	1.00	1.00	0.29	0.53	1.00	0.33	0.64	0.63	021	0.53	0.13	1.00
Platelets	0.02*	0.62	1.00	0.60	1.00	1.00	1.00	0.19	0.61	1.00	1.00	1.00	1.00	0.60	0.47
ESE.	0.69	0.33	0.63	1.00	0.62	0.24	1.00	1.00	1.00	1.00	0.34	0.51	0.51	1.00	1.00

(p<0.05), different significant, A= Moringa Group, B= Control Group

high phytate and polyphenols.[26] The effect of ascorbic acid towards meat in facilitating the absorption of non-heme iron, showed the addition of ascorbic acid (100g) for foods containing beef and the result of absorption increased 4-fold compared with the food without beef is only 1.67-fold increase iron.

A slight improvement in the hemoglobin concentration on moringa group can be explained by an increase in protein intake or in the presence of ascorbic acid and beta-carotene in the leaves of moringa powder, which is a non-heme iron enhancers. The content of protein (amino acids) can also contribute to the activity of erythropoietin by providing amino acids for porphyrin, globin and transferrin synthesis.[27]

CONCLUSION

Based on the analysis of the results of this study concluded that supplementation of Moringa leaf water extract as a natural supplement in addition to ferrous sulfate can help overcome iron deficiency anemia. Extract of moringa leaves as an add-on therapy showed improvement in mean hematocrit values, MCH, MCHC and platelet decrease and mean difference in the parameter values of Moringa group significantly on the value of hemoglobin, ferritin serum, MCHC, RDW, and platelets. However, in the control group also found an increase in hemoglobin, erythrocyte, hematocrit, MCV, MCH and RDW post-treatment compared before. Factors that affect the value of such parameters are menstruation (effect on the value of ferritin, erythrocytes, and platelets) in both groups. In moringa group, foods which affect the parameters are sided dishes and fruit-vegetable that affects hematocrit values, whereas, in the control group, the side dishes affect the value of hemoglobin, ferritin, hematocrit and leukocyte and tea consumption on ferritin serum values.

ACKNOWLEDGEMENT

This study financially was supported by Directorate of Higher Education, Ministry of National Education through Hibah PUPT 2014.

CONFLICT OF INTEREST

None.

ABBREVIATIONS USED

MCH: Mean corpuscular hemoglobin; **MCHC:** Mean corpuscluar hemoglobin concentration; **MCV:** Mean corpuscular volume; **RDW:** Red distribution wide.

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Article History: Submission Date: 21-12-16; Revision Date:07-01-17; Accepted Date:21-01-17.
Cite this article: Suzana D, Suyatna FD, Azizahwati, Andrajati R, Sari SP, Mun'im A. Effect of Moringa oleifera Leaves Extract Against Hematology and Blood Biochemical Value of Patients with Iron Deficiency Anemia. J Young Pharm. 2017;9(1)Suppl:s79-s84.