The Effectiveness Of Moringa oleifera L (Moringa oleifera L) Fraction On The Improvement Of Hemoglobine Levels Of Betinine Rat (Rattus norvegicus) Anemia Model Of Iron Deficiency

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A R T I C L E    I N F O

Keywords: Moringa leaf fraction, Fraction, Iron Deficiency Anemia, Hemoglobin grade

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A B S T R A C T

The iron deficiency anemia is kind of anemia because the less of iron that make the function to tie oxygen is down. Young girl is one of sufferer group who’s prone to get anemia. The long consequence of anemia for the young girl will disturb fatal function of some organ in their body. In general arise of headache symptoms and pain in the centre of head. And the in pregnancy will increase some complication risk such as maternal death. Premature born and perinatal death. One of nature ingredients (herbal) which is reported to have high iron content is kelor leaf (Moringa oleifera L) for about 28, 29 mg/100 g kelor. The purpose of this research is to know the effectiveness of Kelor leave fraction (Moringa oleifera L) to increase hemoglobin grade with preclinical. Experimental research with complete random design (RAL) has been done in February till April 2019 in medical faculty laboratory of universitas sriwijaya Palembang. Sample of the research is female rat that has inclusion criteria as much 30 rats. Divided into 5 group. That is negative control group. Positive control (ferrous fumarate 60%), n–hexan fraction, ethyl acetate, and 1% concentration of kelor leave ethanol, the data of Hb all of subject before and after treatment from subject of iron deficiency anemia has collected by using Easy Touch GC沪 tool. The analysis data of this research used SPSS 2.2 version. The result of the research said that the hemoglobin grade before and after treatment showed significant increasing(p<0,05) for n–hexane fraction and acetate ethyl >45%. While ethanol fraction only 32%.

1. Introduction

Anemia is one of the most common nutritional problems and difficult to treat globally. Anemia affects both developing and developed countries with major consequences for human health and social and economic development. Iron deficiency anemia occurs at all stages of the life cycle, but is more common in pregnant women and young children, adolescents, especially women, are particularly susceptible to iron deficiency.13 14

Based on the age group, anemia sufferers aged 5-14 years were 26.4% and 18.4% in the 15-24 years age group. The long-term impact of anemia in adolescent girls is that if later pregnant will increase the risk of complications, the risk of maternal death, premature birth and perinatal death.10

One of the treatments for iron deficiency anemia is by giving blood added tablets in the form of Fe tablets. Fe tablet is a tablet for nutritional anemia control supplementation, each tablet contains 200 mg of ferrous sulfate (equivalent to 60 mg of elemental iron) and 0.25 mg of folic acid.11 Taking blood booster tablets can cause side effects such as nausea, vomiting, epigastric pain and sometimes diarrhea or constipation, dark colored bowel movements. This can reduce compliance in consuming blood-added tablets.7

Moringa (Moringa oleifera L) is a local plant that has
been known for centuries as a multipurpose plant, nutrient dense and medicinal, containing more natural compounds and varieties than other types of plants. According to research results, Moringa leaves contain vitamin A, vitamin B, vitamin C, calcium, potassium, iron and protein in very high amounts easily digested by the human body. The high content of iron (Fe) in dry moringa leaves or in the form of moringa leaf flour, which is equivalent to 25 times higher than spinach, can be used as an alternative to treat anemia naturally. The content of moringa compounds has been researched which states that moringa leaves contain 28.29 mg of iron in 100 grams (DM)\(^9\).

Oral toxicity (LD50) of *Moringa oleifera* leaves with oral administration of up to 2000mg / kg shows no change in clinical or pathological signs.\(^8\) There has been no research on the effectiveness of Moringa leaves in increasing hemoglobin in anemia, so it is necessary to carry out a thorough study in vivo to prove the effectiveness of Moringa leaves in increasing hemoglobin.

In this study, a model of anemia was used, which was stimulated using aluminum sulfate. Aluminum sulfate is used because it can affect the process of forming red blood cells and inhibit iron absorption by reducing the amount of ferritin.\(^1\)

Based on the description above, this study aims to complement the data on the effectiveness of *Moringa oleifera* L leaf fraction in cases of iron deficiency anemia using mice, because from previous studies there has been no test using *Moringa oleifera* L leaf fraction.

### 2. Research Methods

This research was conducted at the Biomolecular Laboratory and Animal House of the Faculty of Medicine, Sriwijaya University, Palembang in February-April 2019. Ethical approval was obtained from the Health Research Ethics Committee of Muh. Hoesin and FK Sriwijaya University Palembang with Number: 014 / Kepkrsmhflansri / 2019. Moringa leaves are obtained from the Karyajaya Palembang area.

This study was a laboratory experimental study with a completely randomized design using 30 experimental animals Wistar rats (*Rattus norvegicus*) which were divided into 5 groups. The inclusion criteria were healthy mice, weighing 150–200 grams, and 7 weeks old before adaptation and exclusion criteria were weight reduction> 10% during the adaptation period and looked sick.

The five groups were adapted for 7 days and then given aluminum sulfate (Prakash Chemicals International) a dose of 67.5 mg / kgBW intramuscularly for 7 days to reduce hemoglobin levels.\(^6\) On the 8th day the treatment was started and lasted for 7 days according to group. Measurement of hemoglobin levels was carried out three times, namely on the 0th, 8th, and 14th day of treatment. Hemoglobin levels were measured using Easy Touch GCHb. Data on hemoglobin levels were tested descriptively, the mean difference test used the LSD (*Least Significant Difference*) test, and the increase in hemoglobin levels used the *Paired t* test.

### 3. Results

The treated mice showed the highest increase in hemoglobin levels in the P4 group, P3 group, P5 group, positive group, and negative group, respectively. Table 2 shows that the average hemoglobin level before being given treatment is lower than after being given treatment. This indicates that the hemoglobin level increased higher after being treated with n-hexane, ethyl acetate and ethanol fractions of Moringa leaves. Hemoglobin levels were lower in untreated negative controls. This is because there is more iron in the fraction of n-hexane, ethyl acetate and ethanol from Moringa leaves so that the formation of Ferrous binds to protoporphyrin IX to form heme and globin in a reaction catalyzed by FECH (*Ferrochelatase*) which will form complete hemoglobin. The results of this study indicated that the fraction of n-hexane, ethyl acetate and 1% ethanol of Moringa leaves were effective in increasing hemoglobin levels. In addition, from this table it can be seen that the increase in hemoglobin levels is in accordance with the results of the AAS
(Atomic Absorption Spectrophotometry) method, namely the large amount of iron (Fe) content in the n-hexane, ethyl acetate and ethanol fractions of Moringa leaves.

The results of the post hoc suitability test of the Benferoni test in table 3 show that the positive control group will be compared for its dose suitability in the negative control group and treatment group 1 (n-hexane fraction), treatment 2 (ethyl acetate fraction) and treatment 3 (ethanol fraction) leaves Moringa concentration of 1% p<0.05 was a significant difference (0.000). In addition, from the Benferoni test, it was seen that in the positive control group compared to all treatment groups of 1% Moringa leaves there was no significant difference, and the comparison between all treatment groups of 1% Moringa leaves also showed no significant difference.

**Table 1** Distribution of Treatment Groups

<table>
<thead>
<tr>
<th>Category</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Na CMC 1%</td>
</tr>
<tr>
<td>Positive</td>
<td>Ferrous Fumarate 1.08 mg / 200gBB</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>Moringa leaf n-hexane fraction 1%</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>Ethyl acetate fraction of Moringa leaves 1%</td>
</tr>
<tr>
<td>Treatment 5</td>
<td>Moringa leaves ethanol fraction 1%</td>
</tr>
</tbody>
</table>

**Table 2** Effectiveness in Groups on Hemoglobin Levels

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>N</th>
<th>Hb Pre (g%) Mean ± SD</th>
<th>Hb Post (g%) Mean ± SD</th>
<th>P</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>6</td>
<td>11.30 ± 0.25</td>
<td>11.46 ± 0.27</td>
<td>0.054</td>
<td>15 %</td>
</tr>
<tr>
<td>Positive control</td>
<td>6</td>
<td>11.60 ± 0.35</td>
<td>15.48 ± 1.20</td>
<td>0.000</td>
<td>35.3 %</td>
</tr>
<tr>
<td>Moringa leaf n-hexane fraction 1%</td>
<td>6</td>
<td>11.60 ± 0.42</td>
<td>16.10 ± 0.40</td>
<td>0.000</td>
<td>45 %</td>
</tr>
<tr>
<td>Ethyl Acetate Fraction of Moringa Leaves 1%</td>
<td>6</td>
<td>11.48 ± 0.58</td>
<td>16.46 ± 0.47</td>
<td>0.000</td>
<td>45.3 %</td>
</tr>
<tr>
<td>Ethanol Fraction of Moringa Leaves 1%</td>
<td>6</td>
<td>11.63 ± 0.53</td>
<td>15.18 ± 0.85</td>
<td>0.000</td>
<td>32.3 %</td>
</tr>
</tbody>
</table>

**Table 3** Dosage Suitability

<table>
<thead>
<tr>
<th>p value</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.000</td>
<td>1.000</td>
<td>0.274</td>
<td>1.000</td>
<td>0.385</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>0.000</td>
<td>0.274</td>
<td>1.000</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Treatment 3</td>
<td>0.000</td>
<td>1.000</td>
<td>0.385</td>
<td>0.052</td>
<td></td>
</tr>
</tbody>
</table>

Benferoni test, p = 0.05

4. **DISCUSSION**

In this study, it was assumed that the ethanol extract of Moringa leaves could increase hemoglobin levels in anemia model mice. Moringa leaves are a source of *non-heme* iron, so to determine the effectiveness of Moringa leaves in increasing the hemoglobin level of rats in this study, the hemoglobin levels were first decreased in experimental animals to make mice a model of iron deficiency anemia.

This aluminum sulfate inducing agent is used in the conditioning of experimental animals because the use of aluminum sulfate can affect the formation process of
red blood cells and inhibit iron absorption by reducing the amount of ferritin so that iron deficiency anemia occurs.1

The Benferoni test results showed that the hemoglobin levels in all treatment groups, namely the group of mice given the fraction of n-hexane, ethyl acetate and 1% moringa leaf ethanol were significantly different from the hemoglobin level in the negative control group, namely the group that was not given intervention, and Hemoglobin levels in all treatment groups given various doses of fractions did not differ significantly compared to hemoglobin levels in the positive control group, namely the group given blood-added tablets. These results indicate that the hemoglobin level in the group of rats given the fraction of n-hexane, ethyl acetate and ethanol from Moringa leaves at a dose of 1% is proportional to the administration of blood-added tablets. It can be said that giving Moringa leaves gives an effect comparable to standard treatment, namely blood booster tablets.

Seeing the difference in the increase in hemoglobin levels in the treatment group with a dose of 1% fraction of n-hexane, ethyl acetate and ethanol from Moringa leaves which was significantly different compared to the group of rats given blood-added tablets showed that the difference in the increase in hemoglobin between groups was different. The largest increase in hemoglobin was found in treatment group 2 (1% moringa leaf ethyl acetate fraction), which was 16.46 ± 0.47 g%, followed by treatment group 1 (1% moringa leaf n-hexane fraction) of 16.10 ± 0.40 g%, and treatment group 3, namely 15.18 ± 0.85 g%. The increase in hemoglobin in the positive control group was 15.48 ± 1.20 g%.

Based on the analysis, it is known that the fraction of n-hexane, ethyl acetate and 1% ethanol from Moringa leaves can increase hemoglobin levels greater than the group given standard anemia drugs, namely 60% ferrous fumarate blood supplement tablet. Of the three treatments with the same dose, the ethyl acetate fraction dose of 1% Moringa leaves was the greatest in increasing hemoglobin levels among fractions with the same dose in the other treatments. However, through the post hoc test to see the difference in the increase in hemoglobin levels in the treatment group showed that the doses of n-hexane, ethyl acetate and ethanol fractions of Moringa leaves did not provide significantly different hemoglobin levels so it could be said that the three fractions of n-hexane, ethyl acetate and ethanol of moringa leaves given the same effect. When referring to rational drug use, the smallest dose that gives a therapeutic effect is selected. The ethyl acetate fraction dose of 1% Moringa leaves was chosen as an effective dose to increase hemoglobin levels and needs to be tested further so that it can be used in humans.

The increase in hemoglobin levels in experimental animals given the fraction of n-hexane, ethyl acetate and ethanol from Moringa leaves occurred because the AAS test results reported that the Fe content in the ethanol fraction of Moringa leaves contained iron of 19.03 mg in 1 gram of ethanol fraction in the form of paste, whereas The content of moringa compounds has been researched and reported by Ibok Oduro et al in 2008 which states that the ethanol extract of Moringa leaves contains 28.29 mg of iron in 100 grams (DM).9

Vegetable food is a source of non-heme iron in the form of ferric bonds (Fe³⁺). Iron in the form of ferric will be reduced by gastric juice (HCl) to form ferrous (Fe²⁺) which is more easily absorbed in intestinal mucosal cells. Iron is absorbed in the duodenum and upper jejunum by a complex process. In the stomach, Fe³⁺ dissolves in stomach acid, is then bound by gastroferin, and is reduced to Fe²⁺. In the intestine, Fe²⁺ is oxidized to Fe³⁺ which then binds to apoferritin which is then transformed into ferritin, and liberates Fe²⁺ into blood plasma. In plasma, Fe²⁺ is oxidized to Fe³⁺ and binds to transferrin which transports Fe²⁺ into the bone marrow to form hemoglobin. Transferrin transports Fe²⁺ into iron stores in the liver, bone marrow, spleen, and reticuloendothelial system, where it is oxidized to Fe³⁺.11

Other substances besides iron contained in Moringa leaves which are thought to increase hemoglobin include vitamins A, C, B, calcium, potassium, iron and protein in very high amounts and are easily digested and assimilated by the human body and Moringa leaves.
contain a lot of various substances. Macro and micro nutrients as well as active ingredients that act as antioxidants and important nutrients such as iron (Fe) 28.2 mg, calcium (Ca) 2003.0 mg and vitamin A 16, 3 mg rich in β-carotene, protein, vitamin A, C, D, E, K and B (thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamins B6, B12 and folate and various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids.  

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transformed into ferritin, and liberates Fe\textsuperscript{2+} into blood plasma. In plasma, Fe\textsuperscript{2+} is oxidized to Fe\textsuperscript{3+} and binds to transferrin which transports Fe\textsuperscript{2+} into the bone marrow to form hemoglobin. Transferrin transports Fe\textsuperscript{2+} into iron stores in the liver, bone marrow, spleen, and reticuloendothelial system, where it is oxidized to Fe\textsuperscript{3+}.\textsuperscript{11}

Other substances besides iron contained in Moringa leaves which are thought to increase hemoglobin include vitamins A, C, B, calcium, potassium, iron and protein in very high amounts and are easily digested and assimilated by the human body and Moringa leaves contain a lot of various substances, macro and micro nutrients as well as active ingredients that act as antioxidants and important nutrients such as iron (Fe) 28.2 mg, calcium (Ca) 2033.0 mg and vitamin A 16.3 mg rich in β-carotene, protein, vitamin A, C, D, E, K and B (thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamins B6, B12 and folate and various types of antioxidant compounds such ascorbate acid, flavonoids, phenolics and carotenoids.\textsuperscript{3}

The iron content in Moringa leaves acts as a major nutrient in the process of hematopoiesis in the spinal cord, because Moringa leaves are reported to be rich in iron.\textsuperscript{4} In addition, the protein and amino acid content in Moringa leaves also acts as a hematopoietic growth factor. Moringa leaves are reported to contain high protein and amino acids, besides that they play an important role in managing the proliferation and differentiation of blood cells. The vitamin C content in Moringa leaf extract also increases the absorption of iron in the body.\textsuperscript{5}

This increase is caused by normal body processes, which will increase the erythropoiesis process (formation and maturation of erythrocytes) which occurs when the body's cells are deprived of oxygen.\textsuperscript{12} The increase in hemoglobin levels in the moringa leaf fraction treatment group was higher than that of giving blood added tablets.

![Figure 1. Effectiveness in Groups on Hemoglobin Levels](image-url)
### Figure 2. AAS examination on the three 1% Moringa leaf fractions

<table>
<thead>
<tr>
<th>No</th>
<th>Sample Code</th>
<th>Lab Code</th>
<th>Sample Conditions</th>
<th>Unit</th>
<th>Test parameters</th>
<th>Analysis Result</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol fraction of Moringa leaves</td>
<td>T008</td>
<td>Brown</td>
<td>Mg/kg</td>
<td>Fe</td>
<td>19.03</td>
<td>IK 05-LPT-FMIPA (AAS)</td>
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<tr>
<td>2</td>
<td>n hexane fraction of Moringa leaves</td>
<td>T009</td>
<td>Brown</td>
<td>Mg/kg</td>
<td>Fe</td>
<td>111.34</td>
<td>IK 05-LPT-FMIPA (AAS)</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate fraction of Moringa leaves</td>
<td>T010</td>
<td>Brown</td>
<td>Mg/kg</td>
<td>Fe</td>
<td>147.48</td>
<td>IK 05-LPT-FMIPA (AAS)</td>
</tr>
</tbody>
</table>

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- The results displayed relate only to the item tested
- The test report may not be reproduced except in its entirety, without the approval of the Integrated Testing Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, UNSRI
- Complaints against test results are only valid for 2 weeks from the date the test results report is published

### 5. CONCLUSION

That the n-hexane fraction, ethyl acetate fraction and 1% ethanol fraction of Moringa leaves differed in their effectiveness in increasing hemoglobin levels in iron deficiency anemia models compared to 60% ferrous fumarate. Meanwhile, of the three fractions, the n-hexane and ethyl acetate fractions were the most effective at increasing hemoglobin levels compared to 60% ferrous fumarate.

### 6. REFERENCES


