

The Potency of Typical Plants of Central Sulawesi Province to Prevent Iron Deficiency Anemia: Experimental Study

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Abstract

Background: Central Sulawesi falls within the Wallacea region, making it home to various endemic flora and fauna. One distinctive plant in Central Sulawesi is the Moringa tree. The Moringa tree, especially its leaves, was often used as a culinary ingredient known as sayur kelor in the traditional cuisine of the Kaili ethnic group in Central Sulawesi Province.

Objective: This research aimed to determine the effectiveness of moringa leaf extract in increasing hemoglobin levels and erythrocyte count.

Method: Twenty-four male Wistar rats strain (*Rattus norvegicus*) 9–10 weeks old and 200–250 grams were divided into four groups (n=6), normal as a no treatment, negative control, ethanol extract, and positive control during the 15th day. Blood samples on the 15th day were administered to determine blood count.

Result: This study showed differences in hemoglobin and erythrocyte levels in the four experimental groups. The significant difference in average hemoglobin levels between Group 2 and Group 3 with a P-value <0.05 (P value 0.00). The difference between Group 2 and Group 4 is significant with a P-value <0.05 (P value 0.00), and the difference between Group 3 and Group 4 is significant with a P-value <0.05 (P value 0.03). the significant difference in the average erythrocyte counts between Group 2 and Group 3 with a P-value <0.05 (P value 0.00). The difference between Group 2 and Group 4 is not significant with a P-value >0.05 (P value 0.07). The difference between Group 3 and Group 4 has a P-value >0.05 (P value 0.63).

Conclusion: The research results indicate that pregnant Wistar rats experienced hemodilution characterized by a decrease in hemoglobin and erythrocyte levels during pregnancy. However, after being given ethanol extract from moringa leaves, there was an increase in hemoglobin and erythrocyte levels.

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Background

Anemia due to iron deficiency is a disorder caused by insufficient iron intake, low iron reserves, and the loss of iron in the body, resulting in a reduction in the formation of red blood cells (Benson et al., 2021). The etiology was diverse and caused by various risk factors that could decrease iron intake and absorption or increase iron requirements and loss (Cappellini et al., 2020). This type of anemia was a common form that affected more than 1.2 billion people worldwide, with a higher frequency in developing

countries (Camaschella, 2019). Women were more likely to experience iron deficiency anemia compared to men because women undergo menstruation, pregnancy, and breastfeeding (Petraglia & Dolmans, 2022). There was a tendency for fatigue to occur (König et al., 2020), and an increase in the occurrence of infections (Lee et al., 2022) were common symptoms found in iron deficiency anemia, while if anemia occurred during pregnancy, there was a risk of premature birth and pre-eclampsia (Detlefs et al., 2022).

During pregnancy, erythropoiesis process occurred, leading to an increased demand for iron (Guo et al., 2019). The body cannot synthesize iron, so the absorption of iron from food is a way to obtain iron (Moustarah & Daley, 2023). Excess iron is stored as a reserve in the form of ferritin (Gao et al., 2019). Iron reserves can be utilized in case of iron deficiency (Pandurangi et al., 2022). In Indonesia, preventing iron deficiency anemia is recommended by consuming blood supplement tablets for women of childbearing age and pregnant women with a dosage of 60 mg of elemental iron and 0.400 mg of folic acid (Kementerian Kesehatan Republik Indonesia, 2014). However, the administration of oral iron supplements with incorrect dosage and timing could irritate the stomach (Stoffel et al., 2020). Thus, one alternative solution used to prevent iron deficiency anemia is through specific nutritional interventions (da Silva Lopes et al., 2021).

Central Sulawesi is the largest province located on the island of Sulawesi, Indonesia. Geographically, the land area of Central Sulawesi province is 61.841.29 km², and the sea area is 189.480 km², covering the eastern and northern parts of the peninsula, including the Togeian Islands in Tomini Bay and the Banggai Islands in Tolo Bay. The population of Central Sulawesi Province is approximately 3.120,863 people. One of the ethnic groups in Central Sulawesi is the Kaili ethnic group (Dinas Kesehatan Provinsi Sulawesi Tengah, 2021). The Kaili ethnic group, located in Central Sulawesi Province, is spread across several regencies and cities, such as in Palu City, Sigi Regency, Parigi Regency, and Donggala Regency (Saleh, 2013). Central Sulawesi falls within the Wallacea region, making it home to various endemic flora and fauna. One distinctive plant in Central Sulawesi is the Moringa tree (Pitopang et al., 2014). The Moringa tree, especially its leaves, was often used as a culinary ingredient known as sayur kelor in the traditional cuisine of the Kaili ethnic group in Central Sulawesi Province (Prayugi et al., 2015).

One type of plant that was beneficial both as a food source and medicine was moringa (Su et al., 2023). Moringa (*Moringa Oleifera* [L]) was a plant rich in nutrients such as vitamins, minerals, oleic acid, and essential amino acids, making it widely used as a food supplement and therapeutic therapy (Xiao et al., 2020). The part of the moringa plant with the highest nutrient content was the leaves, making it widely utilized as a nutraceutical (Kashyap et al., 2022). The literature review found that more than a hundred chemical compounds were discovered in all parts of moringa, such as alkaloids, flavonoids, anthraquinones, vitamins, glycosides, terpenes, and unique isolated components (Pareek, Pant, Gupta, Kashania, & Ratan, 2023). Furthermore, dried moringa leaves also contained 97.9 µg/g of iron, higher than beets and spinach (Rotella et al., 2023). However, in moringa, there were also antinutrients such as phytates, tannins, oxalates, alkaloids, and hydrogen cyanide, reducing the bioavailability of iron from moringa leaves (Auwal et al., 2019; Devisetti et al., 2016). Appropriate processing of moringa leaves can enhance the bioavailability of iron in moringa leaves (Mawouma et al., 2016). Moringa leaf extract is one form of processing moringa leaves that can enhance the bioavailability of iron (Mun'im et al., 2016; Segwatibe et al., 2023).

Method

Experimental design

This study employed a laboratory experimental design with a Completely Randomized Design (CRD).

Setting

This research was conducted in the laboratory of Padjajaran University, Bandung. From June to December 2022 using experimental animals which were divided into 4 groups as follows:

Group 1: This group was not mated and was given standard feed Cp551 ad libitum, then blood samples were taken on the 15th day.

Group 2: This group was mated using the method of 1 male and 1 female; the female was transferred to the male cage during proestrus. The next morning, the presence of a vaginal plug was checked, and a vaginal smear was examined to confirm copulation, marked by the presence of sperm. For seven days, the weight of the mice increased. After signs of possible pregnancy appeared, this group received standard feed Cp551 ad libitum, then blood samples were taken on the 15th day.

Group 3: This group was mated using the method of 1 male and 1 female; the female was transferred to the male cage during proestrus. The next morning, the presence of a vaginal plug was checked, and a

vaginal smear was examined to confirm copulation, marked by the presence of sperm. For seven days, the weight of the mice increased. After signs of possible pregnancy appeared, this group received standard feed Cp551 ad libitum and Maltofer syrup for seven days with a dose converted from human to mice using the Laurence and Bacharach conversion table, which is 3.6 mg/200 g, orally (p.o.) starting on the 8th day of pregnancy, and blood samples were taken on the 15th day.

Group 4: This group was mated using the method of 1 male and 1 female; the female was transferred to the male cage during proestrus. The next morning, the presence of a vaginal plug was checked, and a vaginal smear was examined to confirm copulation, marked by the presence of sperm. For seven days, the weight of the mice increased. After signs of possible pregnancy appeared, this group received standard feed Cp551 ad libitum and ethanol extract of moringa leaves for seven days with a dose of 150 mg/200 g, orally (p.o.) starting on the 8th day of pregnancy, and blood samples were taken on the 15th day.

Sample/Participants

The subjects used in the study were female Wistar strain white rats obtained from PT Kimia Farma (Persero). The rats were acclimated for seven days before being mated with male Wistar strain white rats using the 1 male 1 female method. Inclusion criteria for this study were approximately 10-12 weeks of age, weighing between 200-250 grams, and being healthy (actively moving, no fur loss or baldness). Exclusion criteria included a weight loss of more than 10% after the laboratory adaptation period. Based on the inclusion criteria, the researcher, using the Frederer formula, determined that there were 6 rats in each group, and there were 4 groups in this study, resulting in a total sample size of 24 rats.

Assessment Parameters

In this study, blood samples 3 mL were collected at weeks 12 from each rats using 3 cc syringe through left ventricle of heart after inhalation anesthesia with isoflurane solution. The blood samples were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) and analyzed using Sysmex hematology analyzer (VetScan HM5). These blood samples were immediately used for the analysis of hemoglobin (Hb), and erythrocytes (RBC).

Intervention

Moringa leaf Extract: Moringa leaves were obtained around residential areas. They were then cleaned from the main veins and branch bones, washed thoroughly, and dried at room temperature (spread out) to obtain dried raw material. The next step involved adding a solvent (96% ethanol), soaking it for 1-3 days (1-3 times 24 hours) at room temperature. Subsequently, the next step was to filter the mixture and evaporate the solvent through evaporation to obtain a concentrated ethanol extract of moringa leaves (*Moringaoleifera* [L]); Maltofer (*iron polymaltose complex*): Maltofer syrup, every 5 mg, contains 50 mg of iron as iron (III)-hydroxide polymaltose complex (IPC), 0.41% alcohol, flavoring agents, sucrose, preservatives (E 216, E 218), and additional substances for liquid preparation. Obtained from Bio Farma pharmacy, Bandung. The dosage used was 3.6 mg/200 g; CMC (*Carboksil Metil Celulosa*) : The carrier solution for moringa leaf ethanol extract. CMC 0.05% was used to maintain the stability and homogeneity of the dissolved material; Cp551 standard feed : the standard feed produced by PT Charoen Popkhand Indonesia Tbk was in the form of pellets.

Data Analysis

The data were presented as mean with standard deviation (SD). The statistical significance of hematological parameter changes was evaluated by analysis of variance (ANOVA) and associated with post hoc (Bonferroni). Differences at p value

Ethical consideration

The research was conducted in accordance with ethical guidelines and obtained ethical clearance with the approval number 026/STIKes-DHB/SKet/PSKBS2/V/2022. After receiving the ethical clearance letter, this research was considered appropriate to be conducted by the Ethics Committee of Stikes Darma Husada Bandung, in Bandung. The study was carried out adhering to the applicable ethical guidelines for laboratory animals

Results

Table 1 shows the distribution of the sample numbers, where each group consists of 6 Wistar strain rats. There are 4 groups (no treatment, negative control, ethanol extract, and positive control).

Table 1. Distribution of sample numbers

Group	n
Group 1	6
Group 2	6
Group 3	6
Group 4	6
total	24

Table 2 Distribution of hemoglobin and erythrocyte frequencies

	Group 1	Group 2	Group 3	Group 4
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Hemoglobin (g/dl)	15.53 \pm 0.67	12.12 \pm 1.28	16.62 \pm 1.03	14.78 \pm 0.9
Eritrosit ($\times 10^6/\text{mm}^3$)	6.85 \pm 0.75	5.77 \pm 0.35	7.47 \pm 0.75	6.82 \pm 0.72

Table 2 shows the distribution of hemoglobin and erythrocyte frequencies in the four research groups. The group divisions are as follows: Group 1: This group consisted of rats that were not mated and were given standard feed Cp 551 ad libitum. In this group, the average hemoglobin level of Wistar strain white rats was 15.53 \pm 0.67 g/dl, and the average erythrocyte count was 6.85 \pm 0.75 ($\times 10^6/\text{mm}^3$). Group 2: This group consisted of rats that were mated to induce pregnancy and were then given standard feed Cp 551 ad libitum. In this group, the average hemoglobin level of pregnant Wistar strain white rats was 12.12 \pm 1.28 g/dl, and the erythrocyte count was 5.77 \pm 0.35 ($\times 10^6/\text{mm}^3$). Group 3: This group consisted of rats that were mated to induce pregnancy and were then given Maltofer syrup with a dose of 3.6 mg/200 g body weight orally for seven days. In this group, the average hemoglobin level of pregnant Wistar strain white rats was 16.62 \pm 1.03 g/dl, and the average erythrocyte count was 7.47 \pm 0.75 ($\times 10^6/\text{mm}^3$). Group 4: This group consisted of rats that were mated to induce pregnancy and were then given ethanol extract of moringa leaves with a dose of 150 mg/200 g body weight orally for seven days. In this group, the average hemoglobin level of pregnant Wistar strain white rats was 14.78 \pm 0.9 g/dl, and the average erythrocyte count was 6.82 \pm 0.72 ($\times 10^6/\text{mm}^3$).

Table 3. The difference in the average hemoglobin levels among the groups

P (Bonferroni)	Group 2	Group 3	Group 4
Group 2		0.00	
Group 3			0.03
Group 4	0.00		

Table 3 shows the significant difference in average hemoglobin levels between Group 2 and Group 3 with a P-value < 0.05 (P value 0.00). The difference between Group 2 and Group 4 is significant with a P-value < 0.05 (Pvalue 0.00), and the difference between Group 3 and Group 4 is significant with a P-value < 0.05 (P value 0.03).

Figure 1 Illustrates the average hemoglobin levels that occurred in each treatment group

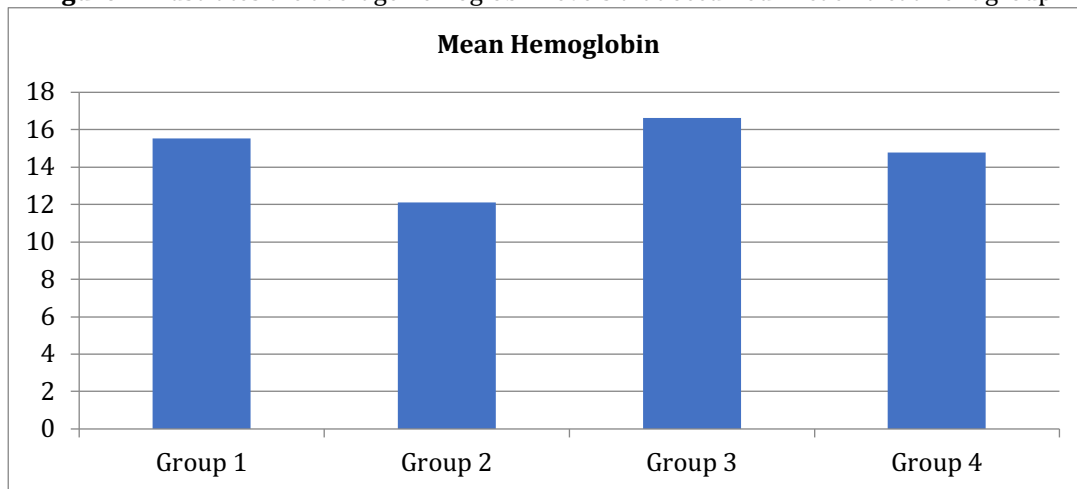


Figure 1 Average hemoglobin levels among the groups.

Figure 1 illustrates the difference in average hemoglobin levels among the four groups. Group 1 is higher compared to Group 2, and Group 2 is lower compared to Group 3 and Group 4, but Group 3 is higher compared to Group 4.

Table 4. The Differences in the average erythrocyte counts among the groups

P (Bonferroni)	Group 2	Group 3	Group 4
Group 2		0.00	
Group 3			0.63
Group 4	0.07		

Table 4 indicates a significant difference in the average erythrocyte counts between Group 2 and Group 3 with a P-value <0.05 (P value 0.00). The difference between Group 2 and Group 4 is not significant with a P-value >0.05 (P value 0.07). The difference between Group 3 and Group 4 has a P-value >0.05 (P value 0.63).

Figure 2 illustrates the average erythrocyte counts that occurred in each treatment group

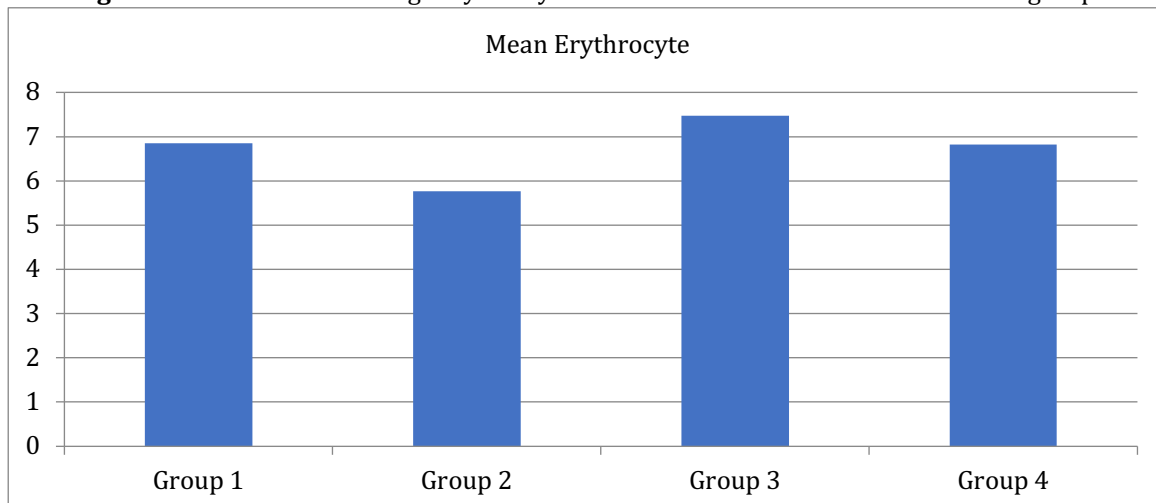


Figure 2 Average erythrocyte levels among the groups

Figure 2 shows the average difference in erythrocyte counts among the four groups. Group 1 is higher compared to Group 2, and Group 2 is lower compared to Group 3 and Group 4, but Group 3 is higher compared to Group 4.

Discussion

Moringaoleifera is a plant that has been traditionally used in herbal medicine and has several health benefits (Matic et al., 2018; Meireles et al., 2020). The entire plant, including its leaves, seeds, and pods, could be utilized in traditional medicine and as a nutritional supplement (Nurhayati et al., 2023). A number of studies show the pharmacological benefits of the Moringa plant (leaves, pods, bark, sap, flowers, seeds, seed oil and roots), all parts of this plant have potential as drugs used for many years as antihypertensive, anti-anxiety, anti-diarrheal and as diuretics (Pareek, Pant, Gupta, Kashania, Ratan, et al., 2023). The leaves are rich in amino acids and essential minerals such as calcium, iron, and zinc, as well as high in protein (Wardana et al., 2022).

Iron deficiency anemia most often occurs during pregnancy due to an imbalance between iron consumption and iron use. During pregnancy there is an increase in iron needs to meet iron needs for mother and baby. In addition, as compensation for the presence of blood discharge during the birth process (Ataide et al., 2023; Georgieff, 2020). Various interventions are carried out to overcome this problem, one of which is by taking oral iron supplementation, but there are gastrointestinal side effects such as nausea so that pregnant women are not obedient in consuming oral iron supplementation (Garzon et al., 2020). As an alternative solution to this problem, various studies were conducted to study traditional plants that contain iron. One plant that contains iron as well as vitamin C is *moringaoleifera* lam (Rotella et al., 2023; Sultana, 2020). Iron and vitamin C are important components in the treatment of iron deficiency anemia. Iron is an important part of hemoglobin, a protein in the blood that carries oxygen. While vitamin C plays the role of a facilitator that converts iron from ferric form (Fe^{3+}) to ferrous (Fe^{2+}) which is easily absorbed by the body. The body needs this convergence in order to use iron properly. In addition, vitamin C also affects the production of hepcidin, a hormone that controls iron homeostasis (Krisnanda, 2019; Li et al., 2020).

The results of this study are in line with previous research findings that state that moringa leaf extract at a dose of 792 mg/200 g body weight per day can improve erythrocyte morphology and increase hemoglobin and erythrocyte levels (Mun'im et al., 2016). For a certain population, moringa leaves have been proven to increase hemoglobin levels in women with iron-deficiency anemia (Suzana et al., 2017), adolescent girls (Khanam et al., 2022) and pregnant women (Derbo & Debelew, 2023). The consistency of the results of this study indicates that the consumption of moringa leaves is a viable intervention for preventing iron-deficiency anemia. This can occur because moringa leaves are rich in nutrients and beneficial for health in many ways (Gopalakrishnan et al., 2016). One of the nutrients contained in fresh moringa leaves is vitamin C (187.96 – 278.50 mg/100g) (Sultana, 2020). Vitamin C is beneficial for increasing the bioavailability of iron (Sabatier et al., 2020). Additionally, dried moringa leaves also contain 97.9 µg/g of iron, which is higher than beets and spinach (Rotella et al., 2023)

The increase in hemoglobin and erythrocytes in this study is attributed to the presence of iron found in moringa leaves. Moringa leaves are a source of non-heme iron (Ems et al., 2023). Approximately 90% of the iron absorbed through food is non-heme iron, which is in the form of a Fe^{3+} complex. This form will be reduced in the intestines to the Fe^{2+} form (Piskin et al., 2022). Afterward, the iron will bind with protoporphyrin and form hemoglobin (Farid et al., 2023). Furthermore, iron is needed for the synthesis of red blood cells. The stability of the red blood cell count is influenced by the balance of erythropoiesis processes in the bone marrow and erythrophagocytosis by lymph and liver macrophages, which recycle iron, proteins, and lipids from red blood cells (D'Alessandro et al., 2023). Therefore, moringa leaves can be used as an alternative to prevent iron-deficiency anemia, especially in pregnant women. Proper processing of moringa leaves can provide the necessary iron intake for pregnant women without any side effects.

Conclusion

The research results indicate that pregnant Wistar rat experienced hemodilution characterized by a decrease in hemoglobin and erythrocyte levels during pregnancy. However, after being given ethanol extract of moringa leaves, there was an increase in hemoglobin and erythrocyte levels. Further research is needed to determine the proper processing of moringa leaves that can provide a significant effect on humans

Declaration Conflicting Interest

All authors stated no conflict of interest in this research

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Author Contribution

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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