

# The Effect of Anti-nutrition and Bioavailability of Iron of Moringa Oleifera on Rat Hematology Model Iron Deficiency Anemia. Review Article

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**Abstract** Anemia is one of the current health problems, about 50% of anemia is caused by dengue fever so this situation has become a global problem that harms human health, and the social and economic development of the country. The purpose of this review is to examine the implications of drying and fermentation processing methods, anti-nutrients and iron bioavailability of Moringa leaves on parameters of iron deficiency anemia. The narrative method describes research results based on inclusion criteria through article searches in the Google Scholar, PubMed, and Garuda databases. 54 articles corroborate the claims of the effectiveness of Moringa leaves in the treatment of iron deficiency anemia. consists of 34 preclinical research articles using mouse models. Moringa leaf supplementation as an anti-anemia in iron deficiency anemia rat model can be in the form of extract or dry powder and obtained from different processing methods. Moringa leaves are processed into a dry extract and/or dry powder capable of ameliorating hematological and biochemical parameters in preclinical research. The processing method has an impact on changes in anti-nutritional and iron bioavailability of Moringa leaves which have a positive impact on indicators of iron deficiency anemia.

**Keywords:** anemia, Moringa oleifera, processing, anti-nutrition, iron bioavailability, hematology, biochemistry

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## 1. Introduction

Anemia is one of the current global health problems, the prevalence in developing countries is 3-4 times higher than in developed countries. [1] Very risky for pregnant women and their fetuses, menstruating women, adolescents of childbearing age, and preschool children with varying prevalence in different countries. [2,3] About 50% of anemia comes from iron deficiency. Furthermore, it harms the growth and development of infants and children's cognitive development, reducing the movement activity of adolescents, fertile mothers, pregnant women, and the elderly. [4,5] thus having a great impact on human health, social and economic development of the country

Iron deficiency anemia occurs when there is not enough iron entering the bone marrow, causing hemoglobin levels

to fall steadily, transferrin saturations to increase beyond normal limits, and protoporphyrin failing to form heme, thereby interfering with hemoglobin synthesis. If there is a deficiency or excess of the standard value of iron in the body, it can cause serious problems. [6,7]

Oral iron supplementation is one of the recommended interventions to prevent iron deficiency anemia. [8] in the form of tablets or syrups with high concentrations, consisting of one or several nutrients, and can work quickly in a short time. [9] Among those commonly used in women is ferrous sulfate, [10] in Indonesia through a program of giving iron tablets where each tablet contains 200 mg of iron sulfate or the equivalent of 60 mg of elemental iron and 0.400 mg of folic acid. [11] However, ferrous sulfate has side effects, namely increasing systemic infection and epithelial inflammation, reacting with superoxide and hydrogen peroxide which further results in the Fenton reaction. [10] In addition, it causes

discomfort such as heartburn, nausea, stomach cramps, constipation, black stools, and diarrhea. [12,13] This makes patients reduce adherence to consuming regularly [14]

Another alternative to prevent iron deficiency anemia is to take advantage of the iron content found in dark leafy plants which have been shown to have a haematinic effect [15,16], and are rich in nutrients including calcium, iron, beta carotene, vitamin C, crude fiber and other important elements where this is a habit of the local community and has been strengthened through research publications whose utilization is through finished products or extracts. [17]. One that is spread outside and consumed is Moringa, a plant that grows well in humid tropics or hot dry soils and can survive in less fertile soils and dry soils. [18,19] Known by various names depending on the country of origin, generally the 'drumstick tree' comes from the Moringaceae family and there are 13 species scattered in various countries. [20]. Moringa leaves are part of this plant that can be used in various countries as food that functions as a drug that can fight diseases caused by nutritional deficiencies or as food that can enrich other foods' nutrients. [21]

There have been many studies on the effectiveness of Moringa, especially in the health and pharmacology fields, including having anti-oxidant, anticonvulsant, trypanosomal, anti-hyperglycemic, anti-free radical, anti-inflammatory, anti-diabetic, cardiovascular protection, brain health, hepatoprotective, antimicrobial and anti-diabetic activities. anti-bacterial helps the wound healing process, prevents malnutrition, and has nutraceutical, anticancer, and bioactive properties for drug development and phytonanoparticle development. [22,23]. Recently, there has been a lot of research interest in the role of Moringa in preventing iron deficiency anemia. Among them are basic ingredients for supplements to prevent iron deficiency anemia in pregnant women [24,25], postpartum mothers [26], and young women [27,28] as well as local food fortification based on flour and cereals in several countries.

Moringa leaves have a type of non-heme iron that is difficult to absorb and has low bioavailability. This is related to several things, including the negative interaction between anti-nutrient factors in Moringa leaves and several other nutrients that inhibit iron absorption. [7,29] But on the other hand the presence of anti-nutrients also has a positive impact as an antioxidant, anticancer, lowering cholesterol, lowering blood sugar, etc. as anti-bacterial, and inhibits tumor invasion [30,31] so the presence of anti-nutritional factors here is still needed. The relationship between anemia and anti-nutrition as stated by Ali et al (2010) that phytate contributes to anemia [32], but there is no explanation as to how the processing mechanism causes changes in anti-nutrients and iron bioavailability so that it affects the parameters of iron deficiency anemia either intervention in humans or animal models.

This review was written to examine the implications of anti-nutritional processing methods and iron bioavailability of Moringa leaves on parameters of iron deficiency anemia. The results of this review become our recommendations for conducting further research.

## 2. Method

### 2.1. Search Strategy

Data searches were carried out through providers of related research articles, namely: Google Scholar, Pubmed, and Garuda. Search data using keywords with two language versions, namely English "moringa oleifera", "anemia", "processing method", "anti-nutrient", "iron bioavailability" and Indonesian "kelor", "metode pengolahan", "anti nutrisi", "bioavailabilitas besi" respectively without narrowing or limiting the search using "or" and "and" to represent the research problem. The research problem that wants to be studied is how the influence of processing methods, anti-nutrients, and iron bioavailability of Moringa leaves on the parameters of iron deficiency anemia. The articles that become the criteria are journals, proceedings, gray literature, and hand searching.

### 2.2. Article Selection

Articles were selected based on inclusion and exclusion criteria. The inclusion criteria set by the researcher are (1) downloadable and complete manuscripts, (2) in the form of published articles/journals/proceedings, (3) in English and or Indonesian, (4) relating to the topics to be discussed, namely anemia, iron deficiency, moringa oleifera, processing methods, anti-nutrients, and mineral bioavailability, especially in iron, then matters relating to anemia parameter indicators after intervention in both animals and humans. (5) articles and/or journals published in 2010-2021, (8) research with animal studies and/or human studies. While the exclusion criteria set by the researcher are: (1) review of articles, abstracts, research reports, and proceedings that are not related to the inclusion criteria. If duplicate articles are found, only one will be presented. Unsuitable articles are not included in this review article. Based on these criteria, identification of data searches is carried out as shown in Table 1.

**Table 1. Identification of search data**

Number of articles through the initial search	445 articles
Number of articles that meet the inclusion criteria	308 articles
Number of articles used as review material	54 articles

### 2.3. Data Extraction

It begins with a screening process and data quality assessment until data that match the inclusion criteria are obtained. Then the researcher and the team extracted data using a form containing the year of publication, country of origin, and type of document. After the form was filled in, the researcher collected the required information based on the research questions. The scope of the information includes changes in anemia parameters after intervention in humans and animals, types of products (extracts and powders) and their applications, dietary formulations, types of induction and dosage, processing techniques, and their effects on anti-nutrients and iron bioavailability. Validation The data with the research team were then analyzed descriptively and presented through flow diagrams, charts, tables, and pictures as needed. The research flow is presented in Figure 1.

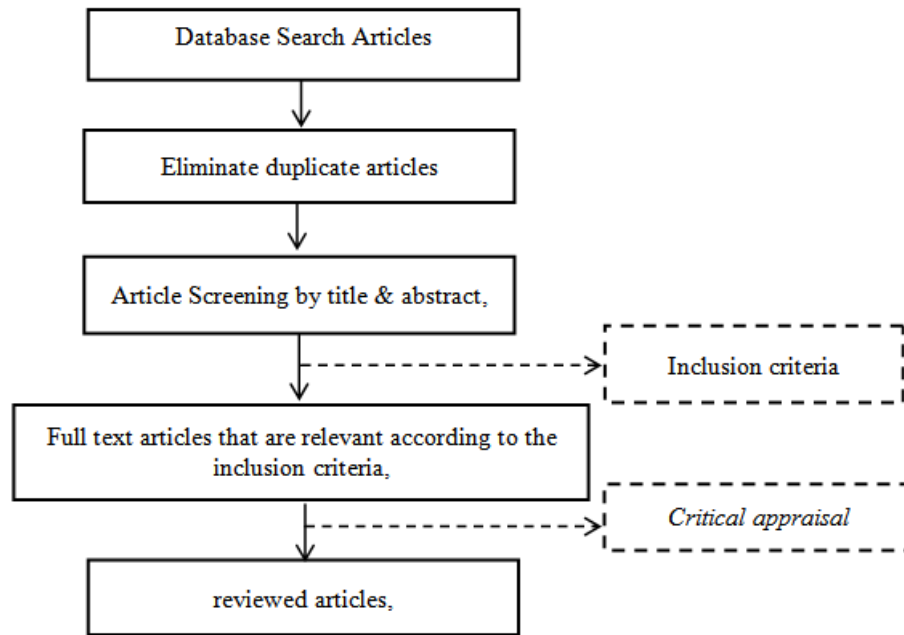


Figure 1. Research Flowchart

### 3. Result

#### 3.1. Effect of Anti-nutrients and Iron Bioavailability of Moringa Leaves and Their Impact on Indicators of Iron Deficiency Anemia

Many studies have found that have explained the impact of drying, heating, and fermentation on anti-nutritional Moringa leaves, including phytate, saponins, oxalates, tannins, alkaloids, flavonoids, phenols, cyanides, and trypsin. In general, the three methods can reduce anti-nutrients to a certain extent. The effect of heating sequentially from the most influential to cyanide, oxalate, and phytate, namely boiling > simmering > blanching. [33] Variations in temperature and duration of roasting have a major impact on reducing phytate and oxalate levels in dried Moringa leaves. [34]. Effect of drying on anti-nutritional Moringa

leaves sequentially from lowest to highest, namely oven drying < sun drying < air drying < freeze drying. A similar pattern also occurs in a study conducted by Umerah et al (2019) that the phytate levels, respectively, from the lowest results, were found in fresh leaves (without treatment), sun-dried < shade dried < fresh. [35] Meanwhile, saponins, oxalates, and tannins are sequentially shade dried < sun-dried < fresh [36]. While the effects of fermentation include reducing water content, increasing ash content, crude protein, crude fat, crude fiber, and nitrogen content. In addition, it reduces anti-nutritional saponins, oxalate, tannin, and phytate, increases iron, copper, and potassium, and decreases calcium, magnesium, manganese and zinc. In the study, fermented Moringa seeds were able to improve PCV, RBC and Hemoglobin and form the immune system of rat models, and this difference was significant compared to unfermented Moringa leaves [37]. The synergy between antinutrients and nutrient bioavailability of Moringa leaves is presented in Figure 2.

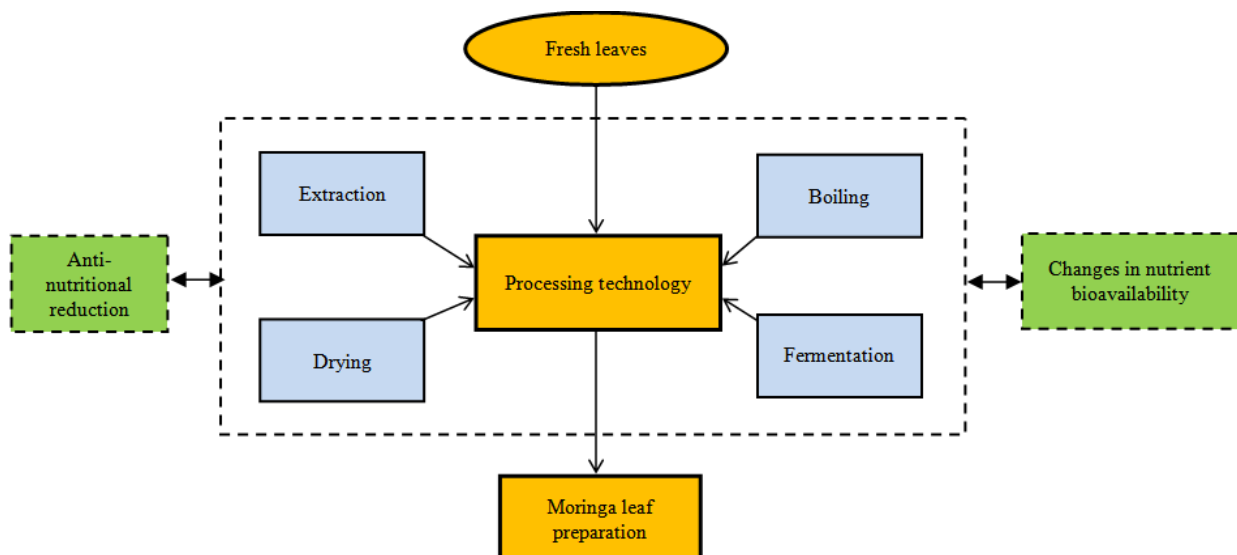


Figure 2. The synergy of anti-nutritional and nutrient bioavailability of Moringa leaves

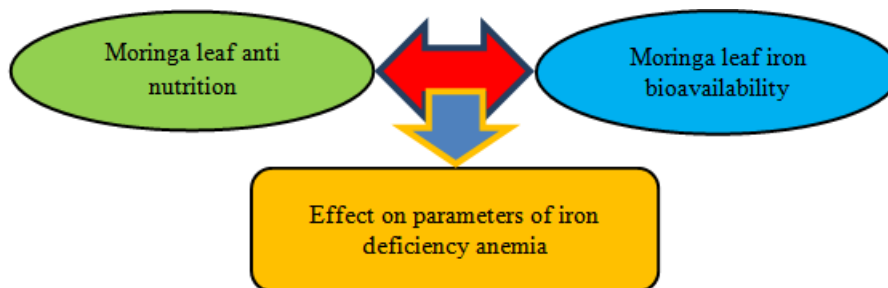


Figure 3. Correlation between anti-nutrients and iron bioavailability of Moringa leaves

Gallaheer et al (2017) reported that the absorption of iron from Moringa leaf powder mixed with typical Ugandan foods was low because iron absorption was inhibited by the activity of naturally occurring phytochemical components in these foods. It can be seen from the phytic acid content of the food at 63.97 mg/g bk with a ratio of phytate/Fe 10.5 [38]. The low iron absorption factor is also related to the agro-ecological zone [39], interactions between food nutrients including protein, fat, and mineral elements with anti-nutrients and antioxidants [40,41] Furthermore, Mongwaketse (2014) explained that the bioaccessibility of iron in dark leafy plants in Africa was significantly negatively related to phytate and tannin levels, and there was a positive relationship with the phenol component [42]. How the effect of changes in anti-nutritional and iron bioavailability of Moringa leaves on parameters of the rat model of iron deficiency anemia is our concern in future

research. The correlation between anti-nutrients and iron bioavailability of Moringa leaves on parameters of iron deficiency anemia is presented in Figure 3.

### 3.2. Moringa Leaf Extract and Powder Supplementation in Preventing Iron Deficiency Anemia Rat Model

It was started by giving induction to the rat model (Table 2) then continued with dietary intervention and observing the parameters of iron deficiency anemia for a certain period. Dietary interventions are processed and prepared by different methods including extraction, drying, fermentation, and/or a combination. From these different preparations, it is possible to have an impact on changes in nutritional composition, anti-nutrients, phytochemical content, and iron bioavailability of Moringa leaves.

Table 2. Types of induction of anemia rat model with Moringa leaf diet intervention

Induction	Dose	Period	Injection	Reference
Aniline	0.003 m./g/days	2 days	intraperitoneal	[43]
Aluminum	0.5 mg/kgbw	21 days	Oral	[44]
Aluminum chloride	0.5 mg/kgbw	28 days	Oral	[45]
Aluminum sulfate	67.5 mg/kgbw	7 days	Intramuscularly	[46]
Phenylhydrazine	40 mg/kbbw	2 days	intraperitoneal	[47]
Phenylhydrazine	10 mg/kbbw	8-10 days	Oral	[37]
Phenylhydrazine	40 mg/kbbw	2 days	intraperitoneal	[48]
Phenylhydrazine	40 mg/kbbb	2 days	intraperitoneal	[49]

#### 3.2.1. Extraction

The dry extract obtained from the extraction method was then intervened in anemic rats. The type of solvent used varies and confirms that different types of solvent affect the hemoglobin level of rats with iron deficiency anemia models, namely n-hexane and ethyl acetate which are effective in increasing hemoglobin levels by 60% compared to ferrous fumarate. There are differences of opinion about the effect of the type of solvent on the hemoglobin level of rats which is thought to be due to the influence of additional materials used including Vitamin C, and similar plants that are hemolytic. The effect of the dry extract of Moringa leaves on Hemoglobin is presented in Table 3.

Table 3. The extraction method and rat model hemoglobin value

Method	The final product	Hemoglobin (gr/dl)		Reference
		positive control	Treatment	
ethanol 70%	Dried extract	14,03	Min. 14,45. Max 14,64	[50]
	Dried Extract (+ Vitamin C)	12,78	Min. 13,80 Max. 15,21	[51]
ethanol 70%	Dry extract (+ Sauropus androgynous dry extract)	13,7	12,81	[52]
	Dried Extract	13	Min. 15 Max. 17	[53]
Hexane 1%	Dried Extract	11,60	16,10	[46]
Ethyl acetate 1%	Dried Extract	11,48	16,46	[46]
Ethanol 1%	Dried Extract	11,63	15,18	[46]

### 3.2.2. Dry Combined Extraction

Then there is a slightly different method, namely the extraction method combined with drying. In general, it is confirmed that the combination technique is also able to increase hemoglobin levels and percentages. The higher the dose given, the better the hemoglobin level of the rat model. Additional ingredients used are soybean flour and growers mash. The effect of the dry extract of Moringa leaves from the combination of extraction and drying on Hemoglobin is presented in [Table 4](#).

**Table 4. The combination method of extraction and drying on the Hemoglobin Value of the rat model**

Stages	Intervention	Hemoglobin (gr/dl)		Reference
		positive control	Treatment	
Moringa leaves were dried at room temperature, blended until smooth, macerated with water, evaporated to dryness, and crushed again until smooth.	Dried extract (the result of shade dry drying and extraction) + (dietary formulations containing varying doses of soy flour)	7,72	Min.12.75 Max.14.53	[49]
	Dried extract (the result of shade dry drying and extraction) + (fed standard with growers mash)	9,8	13.8	[47]
Moringa leaves were dried at room temperature, blended until smooth, extracted 70% ethanol, evaporated to dryness, and crushed again until smooth.	Dried extract ((the result of shade dry drying and extraction) + (standard feed)	12,2	13,3	[44]
	Dried extract (the result of shade dry drying and extraction) + (standard feed)	Percentage increase (%) 1.98	Min. 25,26 Max. 34.97	[54]

### 3.2.3. Fermented Extract

there is an article that confirms that the standard feed intervention containing dried leaf extract fermented by lactic acid bacteria increased hemoglobin and red blood cell levels in anemic rats. Moringa leaf preparation method also uses the dry extraction method, adding probiotic lactic acid bacteria to the dry extract. The effect of supplementation with a dry extract of Moringa leaves fermented by lactic acid bacteria on hemoglobin is presented in [Table 5](#).

**Table 5. Fermentation method on rat hemoglobin values model**

Stages	Intervention	Hemoglobin (gr/dl)		Reference
		positive control	positive control	
Moringa leaves were washed with NaCl for 5 minutes, dried at room temperature, blended until smooth, macerated with water, evaporated to dryness, and crushed again until smooth. Dry extract added 2% E. durans, incubation 37oC 72 hours	Standard feed + Dried Moringa Leaf Extract + lactic acid bacteria probiotics	14,2	14,9	[37]

### 3.2.4. Drying

Diet intervention for anemia model mice using dried Moringa leaf powder resulted from the drying method and had a positive impact on anemia parameters. The procedure for drying Moringa leaves is that the harvested leaves are dried in the shade in a room between 4-14 days until the leaf weight is stable or marked by dry leaves, then the Moringa leaves are blended until smooth. Dried Moringa leaves are rich in nutrients so they are used as supplements to improve the nutritional status of individuals and communities, especially vulnerable groups. Dried Moringa leaf powder as much as 5% mixed in commercial feed can improve PCV, Hemoglobin, RBC, and body weight of adult anemic rats. so this confirms the statement that Moringa leaf powder is effective as a treatment for anemia. The effect of Moringa leaf powder on rat hemoglobin is presented in [Table 6](#).

**Table 6. Drying method and rat model hemoglobin value**

Stages	Intervention	Hemoglobin (gr/dl)		Reference
		positive control	positive control	
Shade dried for 4 days and then blended until smooth	Dry powder	6.73	Min. 13.00 Max. 10.38	[55]
		8.30	14.02	[48]

## 4. Discussion

### 4.1. Effect of Processing on Changes in Anti-nutritional Levels of Moringa Leaves

The effect of food plant processing technology on anti-nutrition has been studied by several researchers. [56,57], and its use in humans and livestock. [58] and strategies to reduce it [59]. Affirmed that the anti-nutrients

of Moringa leaves depend on the processing method used. The existence of anti-nutrients is still contradictory, depending on the achievement targets of each researcher.

Anti-nutrients is a general term for various substances in feed ingredients that can interfere with the process of nutrient utilization in the digestive tract in both livestock and humans. Most of the anti-nutritional components are secondary metabolites of plants. Therefore, the anti-nutritional component becomes inseparable from the term plant secondary metabolite compounds and/or

phytochemicals. Anti-nutritional factors are one of the causes of reduced bioavailability of various nutritional components, and plant minerals that can cause health problems, namely deficiency of macronutrients and micronutrients. [59]

Moringa leaf heating technique by boiling is known to be the most significant in reducing anti-nutrient levels compared to simmering and blanching. In boiling, Moringa leaves are soaked in boiling water for a certain period, this breaks the chemical bonds in the vegetable structure and enzymatic activity. [33]. With the penetration of heat, the enzyme structure in the leaf tissue will be damaged and its activity will stop the strength of the cell wall in binding water will weaken, [60] making the drying time of the leaves will be faster, the bitter and sour taste will disappear [61] Heat treatment, including blanching, is usually used as a pre-heat treatment for vegetables before further processing or consumption which results in the reduction of water-soluble minerals, vitamins, and sugars. Phenol levels in some types of vegetables increase due to blanching (cooking or wet heating), [62]

Generally, drying vegetables uses sun drying and air drying at room temperature, while freeze drying and oven drying are rarely used. [63] The drying technique significantly affects the bioavailability and quantity of important components of food including phenolics, flavonoids, vitamin C, tannins, saponins, phytates, oxalates, alkaloids, cardenolides, and glycosides. Among the phytochemical components, only tannins were not affected by sun drying, room temperature air, freeze-drying, and oven drying techniques. If the ranking of the anti-nutritional results obtained against the drying variation, from the highest to the lowest, namely freeze-drying > air drying > sun drying > oven drying. This is because these components have a positive impact on temperature [36].

Roasting can reduce levels of oxalate, and phytate to the lowest level with a temperature setting of 60oC - 90oC for 100 minutes [34]. Roasting causes the leaves to be overexposed to heat for a long time. Several researchers have described the impact that occurs due to the type of drying on the structure and physicochemical, it is very possible that the changes in the structure and physicochemical of the leaves during drying also reduce the anti-nutritional levels of Moringa leaves that are not resistant to heat. The drying process removes water content because the heat and mass transfer processes simultaneously deactivate enzymes and deactivate food microbes to avoid food spoilage, and change food raw materials from solid, liquid, or semi-solid to become denser by evaporating the water content due to the sublimation process. structural and physicochemical changes are also modified to affect the quality of the final product, reducing damage by microorganisms to a minimum. [64]

Fermentation causes chemical and biochemical changes in macro and micro components to improve nutrients which have an impact on increasing bioavailability and digestibility. [65]. Microbes make Moringa leaves as a source of nutrients, decompose them into other substances, citric acid is converted to lactic acid and acetic acid and several other organic acids are also produced. [66] Among

the mechanisms, microbes release bioactive compounds from fiber by degrading them, metabolizing them, and releasing smaller molecules or their metabolites so that they are more easily absorbed in the intestines. This has an impact on the degradation of bonds between nutrients, enzymes, mineral components, and other components. In addition, low pH conditions greatly help the availability of iron [67]. This is followed by changes in anti-nutritional levels. Among them, total phenol increased during fermentation due to enzyme activity in *R. oligosporus* which was able to increase the phenolic compounds of Moringa [68]. One of the enzymes that play a strong role during the bioconversion process is -glucosidase, while its function is to break glycoside bonds which have an impact on the release of phenol bonds in the fruit matrix during fermentation. [69]

Other enzymes are polyphenol oxidases, peroxidases and lactases are also enzymes that are naturally present in foodstuffs and/or produced by microbes during fermentation. [70] The activity of glycoside enzymes may also have an impact on the anti-nutritional properties of Moringa leaves. The concentration of flavonols changes during the fermentation process of *R. Oligosporus*, flavonols are degraded to free forms or converted to other glycosides [66]. It was different when fermented by mixed cultures, namely *Aspergillus niger*, *Candida utilize*, and *Bacillus subtilis* at the end of the period of phenol and flavonoid fermentation, although at the beginning of the fermentation the phenol and flavonoid levels seemed to increase [71]. During fermentation, the mixed culture activity produces cellulase, pectinase, and xylanase enzymes and then destroys the structure of intact plant cell walls, then releasing flavonoids and phenols that are bound to plant cell walls [72]. Tannins, phytic acid, and glucosinolates are also reduced due to the secretion of tannase enzymes and phytase enzymes which can break down tannins, phytic acid, and glucosinolates. Over time, phytate is reduced by the phytase enzyme. Phytase enzymes can be produced from bacteria and fungi, plants, or from the intestinal mucosa. Phytase and tannase are sources of nutrition for *Aspergillus* to degrade tannins, phytates, and glucosinolates. [71]

#### 4.2. Effect of Changes in Anti-nutritional Factors and Fe Bioavailability of Moringa Leaves on ADB Parameters

Several research results suggest that processing technology participates in reducing anti-nutrients in Moringa leaves, then these changes also have an impact on hematological and biochemical parameters. According to Ali et al (2010) changes in phytic acid levels are closely related to anti-nutrients that lead to anemia. [32]. However, the mechanism has not been explained in detail.

Fermentation as a processing technology that can reduce anti-nutrients, and at the same time essential elements such as potassium, magnesium, iron, and copper are increased, while the levels of sodium, calcium, manganese, zinc, cadmium, and lead are reduced [73]. Saponins, tannins, oxalates, and lectins trigger antibodies to elicit an abnormal immune response and simultaneously bind to erythrocytes to produce hemagglutination and anemia. [74]. Oxalate reacts with elements (Na<sup>+</sup>, Ca<sup>2+</sup>,

Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup>). [75] which the reaction has a negative impact, including Cd reacting negatively with erythrocytes, immune cells, and lymphocytes. [76]. Ca is correlated with hyperlipidemia, cardiovascular dysfunction, and hypertension when triggered by oxidative stress. [77] Pb lowers Hemoglobin accompanied by prolonged hemoglobinopathy, Na can trigger high blood pressure with heavy thrombosis. [78] The interaction between inorganic substances (Ca<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup>) with nutritional and anti-nutritional components in fermented supplements has an impact on changes in RBC and Hemoglobin in animal models, these interactions can be additive, competitive or antagonistic which can eventually interfere with hematopoiesis. [73,79]

The hematinic activity of probiotic moringa leaf extract was found to be superior to that of moringa leaf extract (without probiotics), probiotic bacteria, and commercial iron supplements. The combination of Moringa leaf extract with probiotic bacteria can act as an iron supplement to cure anemia. This combination can increase hemoglobin to normal in the fourth week after PHZ induction [37], improve blood cell production, and can treat hemolytic and hemorrhagic anemia [80]. *E. durans* as a probiotic maintained its immune-modulating properties and hence the lymphocyte count was slightly higher in the mice fed the combination diet [81], blood biochemical parameters are normal and show no adverse effect on biochemical response. [37,82]. and no damage to markers of hypocholesterolemia was found in experimental animals that were given dietary supplements of moringa seeds fermented by monoculture *R. Stolonifer* [73].

The bioavailability of food iron can be increased through fermentation technology, phytic acid is reduced due to fermentation because this phytic acid can bind food cations, and form an insoluble complex so that hydrolyzed phytic acid will release the bound ions so that the availability of minerals including iron is reduced. increase [83]. This theory contradicts the research results of Thierry et al (2013) that there is a negative correlation between phytic acid levels and iron bioavailability. [84]. The bioavailability of Fe (in vitro) of shade-dried Moringa leaf powder is low, possibly because Fe absorption is inhibited by the interaction of phytochemical components in food mixed with Moringa leaf powder [38]. This shows that the interaction between plants and/or foods that have high iron content when mixed and made into a processed product does not necessarily result in high iron bioavailability of Moringa leaves, although the addition of enhancers can increase iron levels to a certain level. In addition, interactions between phytochemical components in Moringa leaves including phytic acid, tannic acid, and calcium can suppress iron bioavailability. [85]. This makes the absorption of Moringa leaves Fe when in the duodenum inhibited. So in this condition, it is necessary to remove the dominant phytochemical components of Moringa leaves [38].

Polyphenols interact with non-heme iron resulting in impaired non-heme iron bioavailability [86]. In line with Armel, Edith, and Moses (2020) that high iron content is negatively correlated with free iron content which indicates low iron bioavailability, while the low iron bioavailability is due to phytate, and polyphenols chelate

iron so that the presence of phytate is important. The more mature the Moringa leaves, the higher the level of insoluble phytate, but it causes the iron content of Moringa leaves to be lower, which means that the presence of this phytate reduces the availability of Moringa leaf iron levels. [39] Contradictory to Kim et al (2008) reported that the polyphenol group i.e. epicatechin, kaempferol increased iron bioavailability, increased hepcidin expression (i.e. quercetin, genistein), while myricetin had the opposite action. [87]

The chelation formed between iron and phytate, fiber, and polyphenols causes the solubility of iron to decrease so that iron bioavailability is low, [42]. while another-iron chelation with amino acids, peptides, and polypeptides is possible to help the process of reducing Fe<sup>3+</sup> to Fe<sup>2+</sup> and will help the process of transporting cell membranes into the intestine. [88,89]. This insoluble phytate binds to minerals, classifies them, and forms bonds with minerals. This binding is due to the phosphate group carried by myoinositol [90].

Anti-nutrient interactions also occur between protein and mineral components, causing impaired nutrient bioavailability which leads to weight loss in model mice. Igwilo et al (2011) reported that after model rats consumed dried Moringa leaf powder which was added to commercial rat feed, it did not increase the weight of the rats, although it was known that Moringa leaf powder had high protein, the rats' body weight decreased during the treatment period. The result was because the presence of tannins prevented protein digestion by chelating and precipitation of protein, while oxalate reacted strongly through divalent ions such as calcium and zinc [91]. Furthermore, phytic and tannic acids did not affect the bioavailability of zinc and calcium. But the interaction between the two together with calcium can suppress the bioavailability of iron. [85]

By suppressing the levels of antinutrients to a certain level, the bioavailability of iron will increase, which is followed by improvements in the hematological and biochemical parameters of rat models with iron deficiency anemia. In the future, our research will examine the effect of oven drying combined with fermentation techniques on the hematological and biochemical parameters of rat models of iron deficiency anemia.

### **4.3. Effect of Moringa Leaf Extract and Powder Supplementation on Anemia Parameters**

The use of dried Moringa leaf extract or powder for the treatment of iron deficiency anemia in rat models has a significant effect on blood biochemical and hematological parameters. In pregnant women, adolescents of childbearing age, and children it is applied as a processed drink or processed food to improve the components of hemoglobin, hematocrit, MCV, MCH, MCHC, and other indicators. Some of the causative factors include additional ingredients used during treatment including vitamin C, blood-added tablets, royal jelly, and folic acid. In addition, because of the nutritional content of Moringa leaves and the function of the natural phytochemical components of Moringa leaves.

Moringa leaves have compounds that can help increase iron in the blood, including iron, vitamin C, vitamin A, and protein can increase hemoglobin, hematocrit, and erythrocyte levels. [18,92]. The presence of protein, iron, Vitamin A, and C from Moringa leaf powder has implications for nutrition so that it is bioavailable in mice, it is known that the stomach type of mice is the same as that of humans, namely monogastric, if it can occur in mice it is predicted to be the same in humans. [93] The addition of vitamin C to the dried Moringa leaf extract caused an increase in hemoglobin of anemia model rats by 55.7% while the remaining 44.3% was caused by external factors. Vitamin C can help make it easier to reduce ferrous iron (Fe<sup>3+</sup>) to iron (Fe<sup>2+</sup>) so that it is easily absorbed in the intestines. Vitamin C inhibits the formation of hemosiderin which is difficult to mobilize to liberate iron when needed. Therefore, vitamin C deficiency becomes more prone to anemia. [51,94]. The high content of iron, protein, and amino acids contained in Moringa leaves has been reported to have a role in the process of hematopoiesis, proliferation, and differentiation of blood cells. And the presence of vitamin C is very helpful in increasing iron absorption [95], Increased absorption of iron is caused by normal body processes, then increases the process of erythropoiesis when the body's cells are deprived of oxygen. [46].

The high vitamin A content of Moringa leaf powder contributes to suppressing anemia [96], Moringa leaf beta carotene is effective in increasing the availability of iron so that it has an impact on reducing moderate anemia rates by 10% in India [97] when supplementation is combined with vitamin A supplementation, the decrease in the prevalence of anemia is even greater to 98%. Furthermore, children who received combined vitamin A supplementation had higher hemoglobin levels than those who did not receive vitamin A supplementation. [98]. This confirms the results of the study that Vitamin A is associated with the production of erythrocytes, iron, and protein synthesis both in the mobilization and preparation of erythrocytes. Lack of vitamin A makes iron not stored and used in the process of erythropoiesis [99]. iron, vitamin B12, folic acid, vitamin B6, and protein are materials for the process of erythropoiesis in the bone marrow. A lack of one of these ingredients can result in decreased production of red blood cells or anemia [100].

Protein helps globin synthesis if low protein intake is caused by a low protein diet, it can inhibit the process of hemoglobin synthesis which leads to iron deficiency. One of the glycoproteins synthesized in the liver is transferrin. Transferrin plays a central role in iron metabolism in the body because transferrin that transports iron is circulated to the parts of the body that need it. Ferritin is a protein that can store iron and be reused according to the needs of the body. [101] The presence of protein also has an impact on the weight gain of rats [97]. Each hemoglobin molecule carries four oxygen molecules. If iron is reduced the blood cannot carry oxygen effectively. Oxygen is needed by the body for cells to function normally. Iron deficiency can cause anemia [102]. The process of iron absorption occurs in the proximal jejunum duodenum to carry out protein synthesis so that in blood plasma iron is oxidized to iron blood plasma then transferrin binds to transport iron into the bone marrow to combine to form red blood cells.

Hematocrit levels are highly dependent on the number of erythrocytes because they have the largest cell mass in the blood. Increased and decreased levels of hematocrit in the blood can have an impact on blood viscosity. The greater the percentage of the hematocrit level, the blood viscosity will increase. [103]

Supplementation in model rats improved the morphological composition of blood as a result of anemia-induced toxicity, which was characterized by increased hemoglobin levels and hematological parameters of model rats after the intervention. Although the time required varies, the supplementation of Moringa leaf extract and/or powder has been successfully claimed as an anti-anemia supplement. Moringa leaf extract plays a role in correcting the toxic effects caused by aluminum by facilitating the absorption of iron, if the amount is adequate it can be used for the synthesis of hemoglobin and animal muscle tissue including kidneys and bones to assist in the process of making RBC [44]. affect other hematological and biological activities, including iron contained from absorbed Moringa leaf extract did not affect WBC, PLT, and LYM. Furthermore, iron absorption also has no effect on electrolyte components including Na, K, Ca, and P either because of the presence of AlCl<sub>3</sub> or Moringa leaf extract. [44]. With the improvement in hematological parameters, it is evidence that Moringa leaf extract has an ameliorative effect on the impact of iron deficiency anemia caused by AlCl<sub>3</sub>. This is also reinforced by the indicator that there is no significant effect on the ALP activity of the rat model, which means that during AlCl<sub>3</sub> toxicity there is no disturbance/damage to the structure of the hepatocellular membrane, and the AST activity of the rat model which may be caused by the antioxidant activity of Moringa leaf extract. [45] and the ameliorative effect also has the same effect on anemic patients due to aluminum toxicity.

Differences in the extraction method can also result in interactions between the solvent and dissolved compounds with the same polarity, where the polarity of the phytochemical compound is the same as that of the solvent. [104]. These differences will result in different content of compounds because they are related to the physical and chemical properties of the phytochemical compound group. Generally, after the natural ingredients have been extracted for a certain time, they are filtered to take the filtrate and separated from the solvent by evaporation using a rotary vacuum evaporator by setting a certain temperature, pressure, and rotation rate to obtain a thick extract, then the yield is calculated. [105]. From the different types of solvent fractions in the Moringa leaf extraction process, it is known that there are differences in hemoglobin levels of ADB rats, namely, hemoglobin increased during the intervention. According to Ricika (2020), the iron content obtained from ethanol extraction with the ethanol fraction is different, because these differences affect the formation of Ferrous which will bind to protoporphyrin to form heme and globin in a reaction catalyzed by the ferrochelatase enzyme so that perfect hemoglobin is formed. [46,106]

Moringa leaf extract combined with soy flour had an impact on increasing hemoglobin levels in rat models. Gnanngoran et al (2020) stated that the combination improved and increased hemoglobin levels after PHZ

induction on day 2, and exceeded the highest normal level on day 14. This increase was related to nutrients and biochemical components naturally contained in the diet. [49] As explained that Moringa leaves are rich in nutrients such as vitamins, minerals, proteins, carbohydrates, and lipids [92]. The improvement of hematopoiesis was shown by a significant increase in the number of erythrocytes after consuming *Moringa oleifera*, this was caused by the intake of protein, minerals, especially iron, vitamin B (thiamin), E ( $\alpha$ -tocopherol), and nicotinamide contained in *Moringa* leaf powder. [18,92]. Furthermore, amino acids (proteins), vitamins B, E, and iron are involved in the synthesis of hemoglobin and the formation and maturation of red blood cells. [49]. The combination of dry extract of *Moringa* leaves with grower's mash can increase WBC, RBC, Hemoglobin, and PCV of anemia model rats. The combination may also have an impact on humans when used for traditional medicine. [47] but this needs further tests

After getting the induction, the rat's hemoglobin level was lower than normal due to the metabolic activity of the inducer. By increasing the dose of leaf powder, the hemoglobin levels of the anemic rats gradually improved and returned to above normal within a certain period. The results of the study by Madukwe et al (2013) revealed that the addition of *Moringa* leaf powder in various doses to commercial feed was effective in increasing the hemoglobin of rats with anemia after induction of Cyclophosphamide. But what is of concern is the dose of protein contained in *Moringa* powder in his research, it is said that *Moringa* leaf powder with a dose of 5% protein is more effective than 10% protein. It is known that protein is a fundamental component in the blood so the increase in hemoglobin levels is an implication of the availability of nutrients including protein, as well as iron, and vitamins A and C. If these nutrients are biologically available in rats, the same indication occurs in humans. because it is known between humans and rats is monogastric type. [55]. In line with the study of Coulibaly et al (2020) that the use of higher doses of *Moringa* leaf powder, namely 7% and 14% in the diet formula for feeding anemic rats after PHZ was induced, was able to improve hematopoiesis activity which was characterized by significantly increasing anemia parameters, which means that increasing the dose had a significant effect. First, it occurs for several reasons: amino acids from protein, B vitamins, E, and iron are involved in the synthesis of hemoglobin, and the formation and maturation of red blood cells. Second, because of the effect of the phytochemical components of vitamins and minerals in *Moringa oleifera* leaves which are known to be involved in hematopoiesis which affects the direct production of blood cells in the bone marrow. [48]

The combination of royal jelly with *Moringa* leaf extract is claimed to be a non-pharmacological alternative supplement to prevent iron deficiency anemia in pregnant women, which has been proven to effectively increase the erythrocyte index (MCV, MCH, and MCHC). [107]. The opinion is in line with the same results in Wistar rats that received a combination of honey and royal jelly for 15 days giving significant changes in hematological parameters, hematocrit, MCV, and MCHC. [108]. The iron content in honey and royal jelly has been shown to

increase human blood erythrocytes and increase hemoglobin levels. In addition, Royal jelly acts as an energy recovery tonic, reduces pain, and increases appetite.

## 5. Conclusion

*Moringa* leaf supplementation as an anti-anemia in iron deficiency anemia rat model can be in the form of extract or dry powder and obtained from different processing methods. *Moringa* leaves are processed into a dry extract and/or dry powder capable of ameliorating hematological and biochemical parameters in preclinical research. The processing method has an impact on changes in anti-nutritional and iron bioavailability of *Moringa* leaves which have a positive impact on indicators of iron deficiency anemia.

## Conflict of Interest

None.

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