

Original article:

The Effects Comparisons of *Sauropus androgynous*, *Moringa oleiefera* alone and in combination on iron deficiency in anemia rats

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Abstract

Background: Leaves extract of *Sauropus androgynous* (Sa) or *Moringaoleiefera*(Mo) alone has been shown to increase hemoglobin level in iron deficiency anemia. The combination of the two extracts have been known to potentially have a synergistic effect on anemia. The objective of this study is to compare the anti-anemic effect of Sa, Mo alone and their combination on iron deficiency anemia in rats. **Methods:** Thirty female wistarstrain rats were randomly divided into 5 groups: normal, iron deficiency anemia, Sa, Mo and the combination of Sa and Mo. Beside the normal group, rats were fed Fe-deficient diet to induce iron-deficiency anemia. Level of hemoglobin, ferritin and plasma MDA were measured on day 8 (after induction of anemia) and day 29. Data were analyzed using One Way ANOVA followed by LSD post hoc test. **Result:** The rats fed with Fe-deficient diet for 7 days shown significantly had a lower plasma Hb and ferritin levels and higher plasma MDA levels compared to the normal group (p <0.05) After the treatment, the rats given the extracts significantly had a higher mean of Hb and Ferritin and a lower mean of MDA level compared to anemic control. The mean Hb, ferritin and MDA level of the combination group were close to normal group. **Conclusion:** The combination of the leaves extract of *Sauropus androgynous* or *Moringaoleiefera* is more effective against iron deficiency anemia compared to extract alone in rats.

Keyword: *Sauropus androgynous* extract; *Moringaoleiefera* extract; Combination extract; antianemia

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Introduction

Anemia is still become health concern in Indonesia¹⁻³. A study conducted by National Health survey (RISKESDAS) in 2013 showed that anemia in children under five years of age, pregnant women and the elderly in Indonesia were 28.1%; 37.1% and 44.2%, respectively⁴. The proportion of iron deficiency anemia in pre-school age children and women at reproductive age ranged from 25% and 37% respectively⁵. Several studies have shown that untreated iron deficiency anemia can have a serious impact on health⁶⁻¹¹.

The standard therapy for anemia is iron tablet supplements^{12,13}. Nevertheless, some studies suggested that iron tablet supplementations may cause gastrointestinal complaints such as nausea, constipation or significant diarrhea^{12,14}. Deficiency and excess of Fe can cause cell damage through oxidative stress¹⁵⁻¹⁸. The medium and high dose Fe supplementation for 4 weeks has been shown to increase in oxidative stress characterized by elevated levels of MDA in anemia-induced mice¹⁵. Antioxidant supplementation of vitamin C and E in patients with iron deficiency anemia for 15 days

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decreases plasma MDA¹⁷.

Indonesia has a lot of plants with potential for antianemia and antioxidants therapy, such as *Sauropus androgynous* and *Moringa oleifera*¹⁹⁻²¹. *Sauropus androgynous* and *Moringa oleifera* leaves are commonly consumed as leafy vegetable. These plants are easy to be found and used. *Sauropus androgynous* leaves contain higher Fe than *Moringa oleifera*. However, the utilization of *Sauropus androgynous* leaves as antianemia therapy should consider the safety range of the dose. There was a report on that outbreak of bronchiolitis obliterans after the consumption of *Sauropus androgynous* leaf juice²².

Sauropus androgynous and *Moringa oleifera* leaf have a complementary macronutrient and micronutrient compositions. The composition of minerals and vitamins per 100 g of fresh leaf of *Sauropus androgynous* contains 8.8 mg Fe, 543 mg P, 771 mg Ca 5600 mg carotene, 0.50 mg thiamine, 244 mg vitamin C and 0.21 mg riboflavin while 100 g *Moringa oleifera* fresh leaves contains 7 mg Fe, 70 mg P, 440 mg Ca, 6780 mg carotene, 0.07 mg thiamine, 240 mg vitamin C and 0.39 mg riboflavin⁽²²⁾. *Moringa oleifera* leaves also contain a higher total phenolic, flavonoid and ascorbic acid than its fruit and seeds. *Moringa oleifera*, respectively of 105.04 mg galicequivalen acid (GAE) / g 31.28 quercetin equivalent (QE) / g and 106.95 mg / 100 g²³. Some studies have proven antioxidant potential of *Moringa oleifera* leaves against free radicals and prevent oxidative stress^{24,25}. The combination of *Sauropus androgynous* leaf extract and *Moringa oleifera* leaves may have a synergistic effect on anemia and become an alternative to Fe supplementation. This study was aimed to compare the antianemic effect of *Moringa oleifera* and *Sauropus androgynous* leaf extract alone and their combination.

Methods

Induction of iron deficiency anemia in rats

Thirty female wistar strain rats weighed 190-220 g were adapted for 7 days then randomly divided into 5 groups: normal, iron deficiency anemia (IDA), *Sauropus androgynous* (Sa), *Moringa oleifera* (Mo) and combination of *Sauropus androgynous* and *Moringa oleifera* (SaMo). Each group consisted of 6 rats. The rats were fed with iron-deficiency diet (AIN-93 M) for 7 days¹¹ while 6 rats in the normal group were given a standard diet.

Plasma collection and analysis

The blood samples were obtained from blood orbital sinus on day 8 and day 29. Blood samples added with heparin were centrifuged and the plasma were stored at -20 ° C for further analysis. cyanmethemoglobin hemoglobin and ferritin concentration were evaluated with spectrophotometer. MDA level was evaluated with barbituric thio acid reactive system (TBARS) using a spectrophotometer. Measurements were conducted at the Center for Food and Nutrition Studies of Gadjah Mada University Yogyakarta.

Dose of Extract

Dosage used for leaf extract of *Sauropus androgynous* was 300mg/day, while the leaf extract of *Moringa oleifera* was 80 mg/day. The combination extract consists the combinations of *Sauropus androgynous* (150 mg/day) and *Moringa oleifera* (40mg/day). The extracts were prepared with a 70% ethanol using maceration method. The leaves were obtained from Sleman, Yogyakarta Indonesia.

Statistical analysis

Statistical analysis for mean differences of hemoglobin and MDA before and after the treatment were analyzed using One Way ANOVA test followed by LSD Post Hoc test with a significance value of $p < 0.005$ (table 1). The mean difference in hemoglobins serum, ferritin and MDA level prior to the treatment with extract were analyzed with Kruskal Wallis test followed by Mann Whitney test with significance value of $p < 0.005$ (table 1). The mean difference of hemoglobin, serum ferritin and MDA level in each group before extract and after administration of extract was analyzed using paired sample T-Test.

Ethical clearance

The ethical clearance for this research was obtained from Bioethics Commission of Medical / Health Research Faculty of Medicine Sultan Agung Islamic University Number 01/I/2018 / Bioethics Commission

Results

The mean of hemoglobin and ferritin level in iron deficiency anemia (IDA), *Sauropus androgynous* (Sa), *Moringa oleifera* (Mo) and combination of *Sauropus androgynous* and *Moringa oleifera* (SaMo) fed with iron deficient diet for 7 days was significantly lower than those of the normal group. In contrast, the mean MDA levels in the IDA, Sa, Mo and SaMo groups fed with iron

deficient diet for 7 days was higher and significantly different from those of normal group . The mean haemoglobin, ferritin serum and MDA level in IDA, Sa, M o and SaMo combination fed with iron deficient for 7 days did not show significant differences (Table 1)

After the treatment for 21 days, the mean of hemoglobin and ferritin levels in the 3 treatment groups were higher than IDA group and lower than the normal group. In contrast, the mean MDA in the treatment group showed a

lower concentration than the IDA group, but higher than normal group (Table 1). The mean hemoglobin, ferritin and MDA after administration of the combination SaMo extract has been shown to be comparable to that of normal group. Likewise, the highest mean difference of hemoglobin and ferritin was found in the group given the combination extract SaMo. The mean difference in hemoglobin and ferritin in *Sauropus androgynous* group was higher than that of in *Moringa oleiefera* .

Table 1 .Mean and Standard Deviation of Hemoglobin, Ferritin and MDA level

Variables	Time	Normal	IDA	Sa	Mo	Combination of SaMo	p
Hb (g/dl)	D-8	14 ± 0.14 ^a	9.2 ± 0.34 ^b	8.8 ± 0.39 ^b	9.15 ± 0.32 ^b	9.08 ± 0.16 ^b	0.180*
	D-29	13.7 ± 0.29 ^a	8.9 ± 0.27 ^b	12.02 ± 0.26 ^c	10.57 ± 0.33 ^d	12.81 ± 0.18 ^c	0.000*
	Δ	-0.29 ± 0.19 ^a	-0.25 ± 0.12 ^a	3.21 ± 0.58 ^b	1.42 ± 0.55 ^c	3.37 ± 0.24 ^b	0.000**
Ferritin (µg/l)	D-8	41.6 ± 0.93 ^a	9.3 ± 0.48 ^b	10.06 ± 0.20 ^b	9.73 ± 0.83 ^b	9.69 ± 0.83 ^b	0.212**
	D-29	40.73 ± 1.06 ^a	9.21 ± 0.48 ^b	35.39 ± 0.38 ^c	33.9 ± 0.3 ^d	39.15 ± 0.51 ^c	0.000*
	Δ	-0.87 ± 0.60 ^a	-0.16 ± 0.19 ^b	25.33 ± 0.52 ^c	24.17 ± 1.30 ^c	29.46 ± 0.94 ^d	0.000**
MDA (nmol/ml)	D-8	1.64 ± 0.13 ^a	8.1 ± 0.24 ^b	8.16 ± 0.46 ^b	8.1 ± 0.22 ^b	8.19 ± 0.33 ^b	0.961*
	D-29	1.83 ± 0.12 ^a	8.3 ± 0.21 ^b	2.92 ± 0.20 ^c	3.6 ± 0.15 ^d	2.3 ± 0.16 ^c	0.000*
	Δ	0.20 ± 0.11	0.20 ± 0.14	-5.23 ± 0.65	-4.50 ± 0.37	-5.82 ± 0.46	0.000*

* One Way Anova test, **Kruskal Walis test. At D-8 the value of p between the IDA, Sa, Mo and combination groups, when compared to the normal group then p = 0.000. Different letters indicatesignificant differences p <0.005

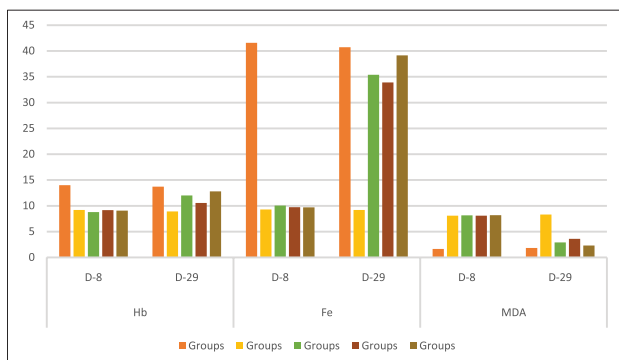


Figure 1. Mean hemoglobin h (g / dl), ferritin (µ g /l) and MDA (nmol / ml) before (D-8) and after (D-29) the administration of extract alone and in combination. Paired T-Test p <0.005

Discussion

Iron is a major component needed in the formation of hemoglobin and erythropoiesis. In this currentstudy, induction of AIN93 -M-MX deficiency diet for 7 days were success in making iron deficiency in anemia rats marked by decreased level of serum hemoglobin and ferritin concentration by 35% and 77% respectively compared to the normal group given standard diet . Serum ferritin is an indicator that leads to iron storage in the body, where 1 µ g/L of serum ferritin is equivalent to 120 µ g of iron deposits per kg body weight. The decrease of serum ferritin signifies an iron depletion.

Sauropus androgynous leaves containing chlorophyll

have been shown to have antiemetic effect in female rats induced by sodium dinitrate, while the administration of *Moringa oleifera* leaves also have been shown to have higher anti-anemic activity compared to iron tablet supplementation²¹. This may be due to the Fe content in fresh leaves of *Sauropus androgynous* higher than fresh leaves *Moringa oleifera*. In this study, the administration of combination leaf extract of *androgynous Sauropus* and *Moringa oleifera* for 21 days may increase mean haemoglobin and ferritin plasma in anemic rats. However, in this study, the number of Fe in each extract was not established. Interestingly, in the combination group of *Sauropus androgynous* leaves - *Moringa oleifera* was found to have the highest increase by 41.08% and 75.25% respectively. The combination of *Sauropus androgynous* leaves and *Moringa oleifera* may have a synergistic effect on anemia. The previous study showed that the 35 mg Fe diet from *Moringa oleifera* effect on gene expression of liver hepcidin, transferrin, TRF-2, and ceruloplasmin²¹.

Iron is required for the production of hemoglobin. The decrease of hemoglobin will cause the decrease of oxygen concentration in the body that can cause hypoxia. Hypoxia leads to oxidative stress. In iron deficiency anemia the concentration of SOD, CAT and GPx antioxidant enzymes are decreased^{17,26,27}. Hypoxia and decreased concentrations of antioxidant enzymes play a role in lipid peroxidation and oxidative stress in erythrocytes. Oxidative stress on the erythrocyte membrane can cause erythrocytes in iron deficiency anemia to have higher rigidity than normal cells²⁸ so that erythrocyte is prone to undergo eryptosis²⁹. The degree of oxidative stress in this study was measured from the concentration of malondialdehyde (MDA).

On the other hand, post-supplementation of Fe tablets indicated an increase in lipid peroxidation through fenton reactions, damaging proteins and

nucleic acids and causing oxidative stress^(15,30). Previous research shown that supplementation of Fe tablets at the dose of 4.8 mg/100g BW for 4 weeks increase MDA concentration of rat liver and decrease SOD concentration and GPx¹⁵. The addition of antioxidant vitamins A, C and E in Fe supplementations can decrease MDA concentration and increase the concentration of GPx enzyme¹⁷.

Supplementation of combination extract of *Sauropus androgynous-Moringa oleifera* leaves for 21 days can lowered the MDA concentration by 71.19%. These effects may be associated with a higher increase in Hb levels in the combination group so as to bind more oxygen than the extract alone. In addition, these effects may be correlated with antioxidant components of *Sauropus androgynous-Moringa oleifera* leaves extract combination.

Conclusion

The combination of the leaves extract of *Sauropus androgynous* and *Moringa oleifera* (SaMo) had increase hemoglobin and ferritin and lower MDA level compared to single extract in diet anemia-induced rats.

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Conflict of interest

The researcher has no conflict of interest in this publication

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