Journal of Blood Medicine

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ORIGINAL RESEARCH

Effect of *Moringa oleifera* Leaf Powder on Hematological Profile of Male Wistar Rats

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Background: Indonesia is a country with high biodiversity of more than 20,000 plant species, and 35% of them are identified as having health benefits. *Moringa oleifera* is one plant that almost all of its parts have been used as nutritional supplements and traditional medicines. Moringa leaves contain nutrients, antioxidants, and bioactive substances that have anti-inflammatory, wound healing, and anti-anemia properties.

Purpose: This study aimed to investigate the hematological effect of Moringa leaf powder in male Wistar rats under normal conditions.

Methods: Twenty-four male Wistar rats strain (*Rattus norvegicus*) 9−10 weeks old and 250−275 grams were divided into four groups (n=6), normal as a control group and three other groups were given Moringa leaf powder at doses 200, 400, and 800 mg/kgBW during 12 weeks. Blood samples at week 12 were administered to determine blood count.

Results: The results of this study showed differences between the various doses of Moringa leaf powder for each hematological profile. These differences were more significant for MCH parameters that indicated a decrease in the D800 group compared with the control group.

Conclusion: In conclusion, this study revealed that the consumption of Moringa leaf powder for 12 weeks did not have a significant change in the hematological profile, except for the MCH value that revealed a modification.

Keywords: *Moringa oleifera*, hematological profile, Wistar rats, normal conditions

Introduction

Natural products from medicinal plants and their constituents have been used for the prevention and treatment of various diseases.[1] Nowadays, there is an increase in research focused on traditional medicine: herbs and medicinal plants.[2] Indonesia is a tropical country with high biodiversity of more than 20,000 plant species, and 35% of them are identified as having health benefits.[3,4] In Indonesia, consuming herbal plants as traditional medicine is still used to prevent and overcome various health problems.[5,6] Traditional herbal medicine is most frequently used because it is less expensive, avoids concerns about the side effects of chemical medications, and more closely corresponds with the patient's beliefs.[7] For example, research on the Meniran plant (*Phyllanthus niruri*) has the potential to function as an immune simulator via blood investigations.[8] One of the herbal plants in Indonesia that potentially affects blood is Kelor (*Moringa oleifera*).[6]

Moringa oleifera is the most widely cultivated species of a monogeneric family.[9] This plant can grow in tropical and subtropical regions.[10] In Indonesia, Moringa is known as kelor, also called a Miracle Tree because almost every part of this plant, such as leaves, bark, seeds, flowers, and roots, can be used as nutritional supplements, traditional medicines, and for other industrial purposes.[11–13] The leaves of *M. oleifera* contain nutrients, antioxidants, chemicals, and bioactive substances.[14] *Moringa oleifera* leaves are an excellent source of iron (28.2 mg/100g), calcium (2003.0 mg/100g), vitamins A (16.3 mg/100g), B-complex, and C as well as minerals.[15] This plant has been recommended by the World Health Organization (WHO) and the United Nations (UN) as an alternative to dietary supplementation in meeting

Received: 10 February 2023
Accepted: 11 August 2023
Published: 23 August 2023

nutritional requirements.[16,17] Several studies have been conducted to prove the efficacy of Moringa leaves such as anti-inflammatory, analgesic, antipyretic, antihypertensives, wound healing, anti-diabetic, antiasthma, anticancer, anti-arthritis, anti-epileptic, antiviral, antianemia, and many more.[14,18–28] Previous study also showed an increased level of hemoglobin, red blood cell count, hematocrit, and total iron content in Sprague Dawley female rat blood after 6 days received Moringa leaves extract.[14] Recent studies of Moringa diet also reported similar results in erythrocyte parameters in Wistar rats.[29] Other study stated that Moringa leaves extract can increase the level of hemoglobin, hematocrit, red blood cell, and total white blood cell count on days 8 and 15 of Wistar rats at normal conditions.[30]

Blood consists of important components that maintain and regulate the body's physiological regulations.[31] Components of blood consist of plasma and blood cells such as red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).[32] Red blood cells play an important function in circulating substances that enter the body, such as oxygen, from the lungs to all the body's cells to carry out metabolic processes.[33] Because of its important function, now blood has become one of the main parameters in preclinical, clinical, and biomedical research in both humans and animals.[34] Hematological profiles serve as a tool for evaluating and interpreting health conditions as well as a reference for initial values or controls in a study.[35] The presence of disease, metabolic disorders, organ or tissue injury, stress, and the influence of drugs can be seen from changes in hematological profiles.[36] For example, high hemoglobin levels and hematocrit values can indicate high feed conversion efficiency.[37] According to Takako Fujii's research, resistance training can also improve hemoglobin levels via increasing heme synthesis in the bone marrow.[38] For those reasons, authors decided to choose normal conditions rats without induction of chemical substances or exercise for this study.

There is a paucity of data on the hematological effects of Moringa leaf powder in the long term in normal conditions. Knowing the typical physiological values under normal conditions is desirable for the effective application of traditional herbal medicine.[37] Thus, this study aimed to investigate the effect of *Moringa oleifera* leaf powder consumption on hematological profile changes (hemoglobin, erythrocytes, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], leukocytes, and thrombocytes) in male Wistar rats under normal conditions.

Materials and Methods

Moringa oleifera Powder and Dose Selection

Moringa leaf powder was obtained from PT. Moringa Organik Indonesia, Blora, Central Java, Indonesia. It has been certified by the Indonesian Food and Drug Authority with a distribution permit BPOM RI MD 619111001777.[39]

We gave a dose of Moringa leaf powder once to each rat in the intervention group, per oral, once per day at 8 o'clock, for 12 weeks. At the end of this study, the rats were terminated. The blood was collected from cardiac puncture for hematological examination using a Sysmex hematology analyzer (VetScan HM5).

Ethical Statement

The procedures for treatment of the animals were performed according to the guidelines and were approved by the Research Ethics Committee of Universitas Padjadjaran with approval number 910/UN6.KEP/EC/2022.

Animal Model

We obtained 24 male Wistar rats (9–10 weeks old, with body weights within the range from 250 to 275 grams) from PT. Biofarma, Bandung, Indonesia. The rats were acclimatized for 2 weeks and had free access to food and water (ad libitum). The dark and light cycle environments were maintained within 12 hours with stable humidity and temperature around ± 22–24 °C. They have been acclimated to the animal husbandry conditions of the Animal Physiology Laboratory of Faculty of Medicine, Universitas Padjadjaran (Figure 1).

Experimental Design

The experiments were carried out in 24 male Wistar rats of the *Rattus norvegicus* with body weight 250−275 grams. The rats were acclimatized for 2 weeks under laboratory conditions. Completely randomized design (CRD) is used in this study. The rats were divided into four groups with six animals in each groups (n=6). The first group control (K) received standardized food and

Figure 1 Wistar rats after 12 weeks' administration of *Moringa oleifera* powder.

water p.o. for 12 weeks. The second group (D200) received Moringa leaf powder at doses 200 mg/kgBW/day p.o. for 12 weeks. The third group (D400) received Moringa leaf powder at doses 400 mg/kgBW/day p.o. for 12 weeks. The fourth group (D800) received Moringa leaf powder at doses 800 mg/kgBW/day p.o. for 12 weeks. The Moringa leaf powder is dissolved with the same volume of water (2 mL of aquades) for each group.

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), erythrocytes (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes (WBC), and thrombocytes (PLT).

Statistical Analysis

The data were analyzed by Statistical Package for Social Science (SPSS) software version 26 and 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 Tukey's test. Differences at p value <0.05 was considered statistically significant.

Results

After 12 weeks, the results were analyzed using the licensed version of SPSS statistics 26. Mean ± SD was calculated to examine the effect of Moringa leaf powder on the hematological profiles. This study performed normality and homogeneity test on the data. We discovered that all groups' data are normally distributed and homogeneous. In the differences of hematological profiles means, we used the one-way ANOVA test and found significant differences in the MCH value of the D800 group than the control group ($p = 0.04$). Meanwhile, in other hematological profiles, there were differences but not statistically significant (Table 1). Based on further analysis using post hoc Tukey's test to know the differences

Table 1 Hematological Profiles of Male Wistar Rats with Moringa Leaf Powder During 12 Weeks

Hematological Profiles	International Unit	Groups				p value
		K	D200	D400	D800	
Hemoglobin	(g/dL)	14.02 ± 1.04	13.20 ± 0.81	14.5 ± 1.75	13.72 ± 0.92	0.38
Erythrocyte	(10^{12}/L)	9.00 ± 0.68	8.64 ± 0.69	9.57 ± 1.06	9.35 ± 0.58	0.27
Hematocrit	(%)	51.73 ± 3.39	49.73 ± 2.03	53.46 ± 5.18	49.15 ± 2.29	0.21
MCV	(fL)	55.80 ± 3.27	57.60 ± 3.78	56.17 ± 1.83	52.6 ± 2.41	0.07
MCH	(pg)	15.58 ± 0.54	15.32 ± 0.58	15.15 ± 0.37	14.68 ± 0.33	0.04*
MCHC	(g/dL)	27.12 ± 0.40	26.54 ± 0.94	27.07 ± 1.05	27.92 ± 0.91	0.13
Leukocyte	(10^9/L)	7.39 ± 2.53	8.24 ± 2.04	9.51 ± 1.40	8.12 ± 3.98	0.60
Thrombocyte	(10^9/L)	585.00 ± 48.16	540.60 ± 304.29	499.67 ± 109.66	603.80 ± 144.97	0.76

Notes: Values are expressed in means ± standard deviation; *Significant ($p<0.05$).
Abbreviations: K, control group; D200, Moringa leaf powder at the dose of 200 mg/kg BW; D400, Moringa leaf powder at the dose of 400 mg/kg BW; D800, Moringa leaf powder at the dose of 800 mg/kg BW.

among the groups of MCH value. We found that the mean MCH value of the D800 group was significantly lower than the control group (Table 2).

Hemoglobin is the main component of erythrocytes which is composed of heme (nonprotein) and globin (protein) parts.[40] Mean of hemoglobin level obtained in the control group was 14.02 g/dL, D200 group was 13.2 g/dL, D400 group was 14.5 g/dL, and D800 was 13.72 g/dL (Table 1). From these results, it can be seen that mean hemoglobin level was slightly higher in the D400 group compared to the other groups. The high levels of hemoglobin in the D400 group were related to the high content of Moringa leaves in protein and amino acids which act as hematopoietic growth factors, as well as vitamin C which plays a role in increasing the absorption of iron into the body.[41] However, the obtained data ranges were not significantly different from the acceptable ranges set above 12 g/dL in rats.[42,43]

Erythrocytes are hemoglobin-containing blood cells with a biconcave disc shape and no nuclei.[43] Mean of erythrocytes level obtained in the control group was 9×10^{12}/L, D200 group was 8.64×10^{12}/L, D400 group was 9.57×10^{12}/L, and D800 was 9.35×10^{12}/L (Table 1). From these results, it can be seen that mean erythrocyte level was slightly higher in the D400 group compared to the other groups. The high levels of erythrocytes in the D400 group were related to the content of hematinic agents such as folic acid, vitamin B6, and iron which can stimulate the erythropoietic pathway.[44]

Table 2 Result of Post Hoc Tukey's Test of MCH Value in Different Groups

Groups	Mean Difference	p values
K vs D200	0.26	0.81
K vs D400	0.43	0.44
K vs D800	0.90	0.03*
D200 vs D400	0.17	0.92
D200 vs D800	0.64	0.16
D400 vs D800	0.47	0.36

Note: *Significant ($p<0.05$).
Abbreviations: K, control group; D200, Moringa leaf powder at the dose of 200 mg/kg BW; D400, Moringa leaf powder at the dose of 400 mg/kg BW; D800, Moringa leaf powder at the dose of 800 mg/kg BW.

However, the obtained data ranges were not significantly different from the acceptable ranges set from 8.32×10^{12}/L to 8.88×10^{12}/L in male rats.[43]

Hematocrit is the ratio of erythrocytes to total blood volume expressed as a percentage.[45] Mean of hematocrit level obtained in the control group was 51.73%, D200 group was 49.73%, D400 group was 53.46%, and D800 was 49.15% (Table 1). From these results, it can be seen that mean hematocrit level was slightly higher in the D400 group compared to the other groups. This result is positively correlated with changes in the number of erythrocytes.[46] However, the obtained data ranges were not significantly different from the acceptable ranges set from 40% to 50% in rats.[43,45]

Mean Corpuscular Volume (MCV) is one of the erythrocyte indices used to estimate the average size of erythrocytes.[47] MCV is obtained by dividing the hematocrit by the number of erythrocytes.[47] Mean of MCV value obtained in the control group was 55.8 fL, D200 group was 57.6 fL, D400 group was 56.17 fL, and D800 was 52.6 fL (Table 1). From these results, it can be seen that mean MCV value was slightly higher in the D200 group compared to the other groups. However, the obtained data ranges were not significantly different from the acceptable ranges set from 45.9 to 59.9 fL in male rats.[43]

Mean corpuscular hemoglobin (MCH) is an erythrocyte index that measures the average amount of hemoglobin in red blood cells, expressed in picograms (pg).[48] This parameter is obtained by calculating the level of hemoglobin divided by the number of erythrocytes per cubic millimeter of blood.[47] Mean of MCH value obtained in the control group was 15.58 pg, D200 group was 15.32 pg, D400 group was 15.15 pg, and D800 was 14.68 pg (Table 1). From these results, it can be seen that there was a decrease in the mean MCH value for increasing the dose of Moringa leaf powder. However, the value is still within normal limits from 14.6 to 21.3 pg in male rats.[8,43]

Mean corpuscular hemoglobin is in line with the changes that occur in the level of hemoglobin or the number of erythrocytes. In addition, this parameter also depends on the size of the erythrocytes, because smaller cells cannot contain as much hemoglobin as larger cells.[49]

Mean corpuscular hemoglobin concentration (MCHC) is an erythrocyte index used to determine the erythrocyte saturation index in the blood.[43] This examination is obtained by calculating the amount of hemoglobin in 100 erythrocytes and then dividing it by the hematocrit and the results are expressed in grams per deciliter (g/dL).[47] Mean of MCHC value obtained in the control group was 27.12 g/dL, D200 group was 26.54 g/dL, D400 group was 27.07 g/dL, and D800 was 27.92 g/dL (Table 1). From these results, it can be seen that mean MCHC value was slightly higher in the D800 group compared to the other groups. However, the obtained data ranges were not significantly different from the mean acceptable ranges set at 31.88 ± 1.11 g/dL in male rats.[8,43]

Leukocytes are cells that function as a defense system or body immunity against pathogens or infectious agent.[50] Mean of leukocytes level obtained in the control group was 7.39×10^9/L, D200 group was 8.24×10^9/L, D400 group was 9.51×10^9/L, and D800 was 8.11×10^9/L (Table 1). From these results, it can be seen that mean leukocyte level was slightly higher in the D400 group compared to the other groups. The high levels of leukocytes in the D400 group were related to the hematopoietic factor of Moringa leaves which has a direct influence on blood production in the bone marrow.[51,52] However, the obtained data ranges were not significantly different from the mean acceptable ranges set at $7.19 \pm 1.93 \times 10^9$/L in male rats.[43]

Thrombocytes are blood cells that play an important role in the process of hemostasis for wound healing.[53] Thrombocytes are also produced in the bone marrow and are derived from megakaryocyte whose cytoplasm is fragmented.[53] Mean of thrombocytes level obtained in the control group was 585×10^9/L, D200 group was 540.6×10^9/L, D400 group was 499.67×10^9/L, and D800 was 603.8×10^9/L (Table 1). From these results, it can be seen that mean thrombocyte level was slightly higher in the D800 group compared to the other groups. However, the obtained data ranges were not significantly different from the mean acceptable ranges set at $1041 \pm 144 \times 10^9$/L in male rats.[43]

Discussion

Every part of *Moringa oleifera* can be used as a nutritional supplement and traditional medicine.[12,13] One of them is the leaves part, which is rich in vitamins, minerals, and effective antioxidants.[54] Previous studies have proven that *Moringa oleifera* can be used an anti-inflammatory, analgesic, antipyretic, antihypertensive, antidiabetic, accelerating wound healing, anti-cancer, and increasing production of blood cells.[18–22,24] Despite the numerous studies that have been

conducted on *M. oleifera*, there is paucity of data on the hematological effects of Moringa leaf powder. During its normal condition, it may have good or adverse effects on hematological parameters. Hence, it becomes necessary to investigate hematological profile changes in given *M. oleifera* leaf powder in normal conditions of male Wistar rats.

The hematological profile is useful to assess the health condition and as a reference value of the initial (baseline) or control in a study.[55,56] The presence of metabolic disorders, diseases, damage to the structure and function organ, the influence of drugs, and stress can be seen from the changes in hematological profile.[36] With regard to erythrocyte parameters, the mean values of hemoglobin, erythrocytes number, and hematocrit obtained in rats after administration of Moringa leaf powder were correlated each other and within normal range values. These values are similar to reference data form Marlies de Kort's work.[43]

Table 1 shows that the rat group given 400 mg/kg BW Moringa leaf powder had higher hemoglobin levels, erythrocyte counts, and hematocrit values than the other groups. This shows that the greater number of erythrocytes in the blood, the greater hematocrit value in the blood.[46] Hematocrit levels can be decreased by several conditions that cause a decrease production, damage, or the number and size of erythrocytes.[57] Thus, the hematocrit value is very dependent on the number of erythrocytes because erythrocytes are the cells with the largest mass in the blood.[46,58] These results are also related to the components of Moringa leaves such as protein amino acids, vitamins B, E, and iron play a role in the synthesis of hemoglobin, the formation and maturation of red blood cells.[29,41,59]

Table 1 shows a decrease in MCH and MCV values at each increase in the dose of Moringa leaf powder simultaneously. Because, the interpretation of the MCH value must consider the cell volume or MCV value, but is not useful in hypochromic conditions where MCH is not accurately used.[49] It is possible that MCHC values are different to MCV and MCH values because this parameter is quite sensitive to test errors.[49]

The decrease in MCH values in this study could be influenced by several factors. First, the bioavailability of iron in Moringa leaves is low due to its high phytic acid content.[60] Phytic acid is the largest storage form of phosphorus in cereals and legumes. Phytic acid is considered an antinutritional compound in foodstuffs because under natural conditions, phytic acid will form bonds with divalent minerals (Ca, Mg, Fe) and proteins to become compounds that are difficult to dissolve.

This causes minerals and proteins to not be absorbed by the body, or their digestibility is low.[60] Second, iron in Moringa leaves cannot be absorbed properly due to the presence of nonabsorbable complexes with phytic acid, tannins, and some dietary fiber which can bind with iron in the intestinal lumen.[61] In addition, the quercetin content of Moringa leaves can increase iron uptake in the apical enterocyte area but reduce iron output in the basolateral area and reduce the expression of ferroportin (FPN) in the intestine.[62] In the long term, quercetin and polyphenols can also act as iron chelation if too much.[62] Third, the environmental temperature can also cause unacceptable shifts in values.[63,64] MCH, MCV, hematocrit, and platelet parameters stored for less than 4 hours at 33°C exhibits unacceptable bias, so the recommended time limit from collection to processing of blood samples should be adapted to areas where ambient temperatures are high.[63] Therefore, the conditioning of rats and the components of Moringa leaf powder need to be further considered comprehensively in future studies.

The highest leukocyte levels appear in the rat group at a dose of 400 mg/kg BW Moringa leaf powder. This result is similar to the study conducted by Ufelle et al, which revealed an increase in total white blood cell count on days 8 and 15 at normal conditions with doses of 150 mg/kg BW and 300 mg/kg BW Moringa leaf powder.[30] Another study by Ajugwo et al concludes that Moringa leaves extract has the tendency to increase blood parameters such as white blood cells.[51]

According to several studies, rats fed Moringa leaves extract had a slight increase in total leukocyte levels. This can be attributed to the anti-bacterial effects, although the mechanism of action is yet unknown.

The highest thrombocyte levels appear in the rat group at a dose of 800 mg/kg BW Moringa leaf powder. These results are in line with the research study of Kodi et al, which shows that the mean value of total platelets count was increased in rats with a low protein diet added aqueous extract of *Moringa oleifera* for 0, 7, and 14 days.[65] Similar results were revealed by Amara et al.[66] The main function of platelets is to maintain hemostasis. *Moringa oleifera* can stimulate platelet proliferation and facilitates hemostasis based on these findings. However, further investigation is necessary to explain this mechanism.

This study had several limitations, including (i) the researchers used the whole compound of Moringa leaf powder so that there is a possibility that it contains anti-nutritional substances; (ii) the researchers did not re-examine the active substance content of the Moringa leaf powder before administering it to the rats; (iii) only one blood sample was taken at the end of the study; and (iv) the hematology profile was the one type of standardized examination used in this study. These limitations will be considered in future studies to improve the reliability of experimental research data.

Conclusion

The results showed that normal rats revealed no increases in hemoglobin, erythrocytes, hematocrit, MCV, MCHC, leukocytes, and thrombocytes cell count on 12 weeks given by Moringa leaf powder. However, Moringa leaf powder at the dose of 800 mg/kgBW/day can significantly decrease the MCH value of normal rats ($p<0.05$). So, daily administration of Moringa leaf powder (200 mg/kgBW and 400 mg/kgBW) over a period of 12 weeks to normal rats is harmless, and no adverse effects are demonstrated during hematological investigations, but at 800 mg/kgBW doses still need supervision.

However, further research can be conducted on the active substance that has the most effect on the hematological profile, so that only active substance isolates that affect hemoglobin or other parameters. Blood samples were taken that can be done periodically, and a blood smear is used or total iron concentration is calculated to confirm this harmless action.

Acknowledgments

The authors would like to thank to Putri Teesa Radhiyanti Santoso, dr., M.Kes, Ph.D, Hanna, dr., M.Kes, AIFO, Ph.D., Resti Gradia Dwiwina, dr., M.Kes for article reviewer and examiner, and Mr Gilang for assisting in animal treatment. We would also like to thank Universitas Padjadjaran, for funding this research through Riset Disertasi Dosen Universitas Padjadjaran (2203/UN6.3.1/PT.00/2022).

Author Contributions

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.

Funding

This research was funded by Universitas Padjadjaran, grant number 2203/UN6.3.1/PT.00/2022 Riset Disertasi Dosen Universitas Padjadjaran.

Disclosure

Mrs Vita Murniati Tarawan reported grants from Universitas Padjadjaran during the conduct of the study. The authors report no other conflicts of interest in this work.

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