Nat. Volatiles & Essent. Oils, 2021; 8(5): 13276-13287

NVEO
Natural Volatiles &
Essential Oils

Effect Of Feeding Moringa Oleifera (Moringaceae) Leaves Extract On Rats With Induced Iron Deficiency Anemia

Naeem M. Rabeh[1] , Khaled A. El. Kady[1] , Hany G. Elmasry[1] , Basma I. Abdelhafez[1]

1-Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

Abstract

Iron deficiency anemia is the most common type of anemia related to malnutrition worldwide. It represents a major problem in developing countries, especially in Egypt. Moringa oleifera Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. The aim of this study was conducted to investigate the effect of Moringa oleifera leaves extract on anemic rats. Thirty five adult female Sprague-Dawley rats, were fed on basal diet for one week for adaptation, then divided into two main the first main group (n= 7); were fed on basal diet only and served as negative control group, second main group (n=28); were fed on basal diet deficiency in iron and add (10 g tannic acid/kg diet) for 3 weeks to induce anemia, then were divided into 4 subgroups, one of them was fed on basal diet only and kept as positive control group, while the other three subgroup were fed on basal diet and given orally 1 ml% moringa extract at the concentration of 5,10,15% respectively. The results indicated that, supplementation with Moringa leaf water extract can help overcome iron deficiency anemia. Due to improvement in mean hematocrit, hemoglobin, red blood cells and platelets. As compared to the positive control group. The obtained results revealed an improvement in their functions and liver profile due to moringa supplementation at the 3 tested levels. So, Moringa leaf is recommended to anemic patients.

Keywords: Anemia, Moringa oleifera, rats, Hemoglobin level.

Introduction

Anemia is a public health problem that affects populations in rich and poor countries **(Benoist et al., 2008).** However, the incidence of this organ dysfunction is greater in developing countries than in developed countries **(Ogbe et al., 2010).** Anemia has major outcomes on human health along with social and economic development. This pathology affects all ages and both sexes in general, but, in particular children, and pregnant women **(Bavhure et al., 2014).**

Anemia decreases the oxygen-carrying capacity of the blood due to reduction in circulating hemoglobin. The normal quantity of hemoglobin in humans is greater than 13 g/dl for males and 12 g/dl for females **(Okochi et al., 2013)**. According to the World Health Organization (WHO), about two billion people, representing 30% of the world's population were anemic **(Adusi- poku et al., 2008)**. Many factors including nutritional deficiencies, severe

bleeding, genetic defects, diseases infectious, prolonged use of nonsteroidal drugs and exposure to toxic substances caused anemia **(Vamsee et al., 2015)**.

These days, therapeutic drugs caused many side effects while, plants and traditional medicine are being increasingly recommended and the benefits of different plants are inviting more attention **(Ebrahimie et al., 2015)** .

Moringa oleifera (Moringaceae) is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics'. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa **(Anwar et al., 2005)**. For centuries, M. oleifera has been cultivated for its nutritional values **(Verma et al., 2009 and Sasikala et al., 2010).**

Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids **(Siddhuraju and Becker, 2003**). The leaves of this plant are positioned as a tonic, strengthening and stimulating immune system for people living with HIV/AIDS **(Thurber and Fahey, 2009).** In addition, M. oleifera is very rich in iron and it was estimated that, the dried 100 g leaf powder to contain about 28.29 mg **(Kasolo et al., 2012)**.

Therefore, this study was performed to assess the effect of moringa oleifera leaves extract on anemic female rats.

Materials and Methods

Materials

Chemicals: Casein, vitamins, minerals and cellulose were purchased from El-Gomhoria Company, Cairo, Egypt. Tannic acid was purchased from local distributer of (Sigma Chemical Co) Cairo, Egypt. **Kits**: for blood analysis was purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt. **Plant :**Fresh leaves of Moringa were obtained from National Research Institute. **Rats**: Adult female albino rats (Sprague- Dawley strain) (n=35 rat) weighing approximately (160 ±5 g.) were purchased from Helwan Experimental Animals Farm.

Methods

Chemical analysis of moringa leaves: Proximate analysis involving moisture, protein, fat, ash and crude fiber were achieved according to the methods of **AOAC, (2005)**. Carbohydrates content was calculated by difference. Antioxidant activity in moringa leaves estimated according to **Burda and Oleszek, (2001)**.

The scientific identification of this plant was carried out at the National Scientific Research: **Kingdom**: plants, **Upper band**: terrestrial plants, **Section**: Vascular plants, **Phylum**: Eukaryotes, **Subphylum**: Seeds, **Class**: Dicotyledons, **Order**: Brassicales cabbage, **Family**: Albanian Moringaceae, **Gender**: Pan Moringa.

Preparation of the water extract of moringa:
The plant material was shade-dried for 4 days then they were milled into fine powder by electric blender**.** Then three samples were prepared by dissolving 5, 10, and 15 gram of dried Moringa leaves in 100 ml water respectively.

Induction of anemia: rats were fed on basal diet with (10 mg tannic acid/kg diet) for 3 weeks **(Afsana et al., 2004)** at the same time, iron was removed from the mineral mixture to induce anemia. Blood samples were be obtained after 3 weeks from tail vein to determine Hb concentration.

Biological study: This study was carried out at the Postgraduate Lab of Home Economic Faculty, Helwan University. Thirty-five adult female Sprague-Dawley rats were fed on standard diet for one week for adaptation. Rats then were randomly divided into two main groups as follow:
The first main group (n= 7) was fed on basal diet only and served as control negative group. The second main group (n=28) was fed on basal diet deficient in iron and added with (10 g tannic acid/kg diet) for 3 weeks to induce anemia, then was divided into four subgroups, the first subgroup was fed on basal diet with tannic acid and served as positive control group, the other three subgroups were fed on basal diet with tannic acid and given orally 1 ml/rat of Moringa extract at concentration of 5%, 10% and 15%, respectively.

At the end of the experimental period (6 weeks), rats were fasted over night before sacrificing, two blood samples were collected, the first sample will be collected into a tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and was used for assessment of the following erythrocytes indices. The second blood sample was collected into a centrifuge tube without any anticoagulant and was centrifuged to obtain serum which was stored at- 20°C until used for subsequent analysis.

Biological Evaluation : feed intake (FI) , feed efficiency ratio (FER) and body weight gain percent (BWG%) were determined according to **(Chapman et al., 1959)** using the following equation:

$$\text{BWG\%} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

FER= Body weight gain (g)/ Feed intake (g)

Biochemical analysis: Total red blood cell counts determined according to (**Dacie and Lewis,1991).** Hematocrit and hemoglobin concentration (**Alexander and Grifiths, 1993**) were determined. Serum Aspartate amino transferase (AST) and alanine amino transferase (ALT) **(Bergmeyer et al., 1978)** were determined**.**
Statistical Analysis:- The obtained results were analyzed according to SPSS program. ANOVA test was used to compare results among groups and $P<0.05$ was considered to be significant **(Snedecor and cochron, 1989).**

Results

Chemical composition of moringa (Moringa oleifera) leaves powder was presented in table (1). The results were determined for moisture, crude protein, total lipids, total carbohydrate, ash, crude fiber and total antioxidant activity, the ratios were 6.92g, 26.95g, 2.85g, 37.19g, 7.98g, 18.11g, 15.97 µg, respectively.

Table (1): Chemical composition and total antioxidant activity of moringa leaves powder (g / 100g)

Nutrients	Fresh leaves
Moisture (g)	6.92
Crude Protein (g)	26.95
Total Lipids (g)	2.85
Total Carbohydrate (g)	37.19
Crude Fibre (g)	18.11
Ash (g)	7.98
Total antioxidant activity (µg)	15.97

The effect of moringa on body weight of anemic rats are recorded in Table (2). There were no significant changes in the initial body weight of all groups of rats. The statistical analysis showed that the mean values of the final body weight, BWG%, and FER of positive anemic control group were significantly (P<0.05) decreased, compared to the negative control group. Anemic rats treated with different concentrations of moringa extract had significant (P<0.05) increase in FBW, BWG% and FER compared to the positive group (anemic rats). There were non-significant (p<0.05) changes in FBW, BWG%, FER between anemic rats treated with (5 or 10 %) moringa extract. Moreover, anemic rats treated with 15% moringa extract caused significant(P<0.05) increase in FBW, BWG% and FER compared to other anemic rats treated with 5 or10 moringa extract. The highest increase in BWG% was observed at the groups given 15% moringa extract.

Table (2): Effect of moringa leaves extract on body weight status of anemic rats .

Parameters / Groups	IBW (g)	FBW (g)	BWG%	FI (g/d/rat)	FER
Control –ve	164.75±2.05^{a}	201.00±1.95^{a}	22.06±2.11^{a}	15.00	0.040±0.03^{a}
Control +ve	167.61±1.25^{a}	156.50±1.65^{c}	-6.70±0.57^{d}	11.50	-0.016±0.01^{c}
5%	169.50±1.26^{a}	189.00±0.91^{b}	11.51±0.68^{c}	13.00	0.025±0.01^{b}
10%	169.75±2.39^{a}	190.50±2.95^{b}	12.22±0.75^{c}	13.40	0.026±0.01^{b}
15%	168.50±0.64^{a}	198.00±2.73^{a}	17.50±1.54^{b}	14.20	0.035±0.03^{a}

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

The effect of moringa on Hemoglobin (Hb) and Hematocrit (HCT) levels of anemic rats are recorded in Table (3). The obtained results showed that rats fed basal diet deficient of iron and added with tannic acid revealed a significant decrease (p<0.05) in the mean value of Hb and HCT as compared with those of negative control group with mean value. The anemic rats fed on moringa leaves extract at the different concentrations had significantly (P<0.05) increase in Hb and HCT as compared with that of positive control group. It was observed that,

there was significant difference in Hb and HCT among all the experimental groups. Moringa extract at (15%) had highly significant increment in Hb and HCT than the other groups.

The effect of moringa on red blood cells count, white blood cell levels and platelet of anemic female rats are recorded in Table (4). The results show that RBCs and platelet concentrations were significantly decreased (P<0.05) while WBCs was significantly decreased at the positive control group as compared with the negative control group. Treating anemic rats with moringa extract at the different tested levels caused a significant decreased (p<0.05) in WBCs and a significant increase in RBCs and platelet concentrations as compared with positive group. The highest increase in RBC and platelet as well as the highest reduction in WBCs were observed at the groups given moringa extract at 15%.

Table (3): Effect of moringa leaves extract on Hemoglobin and Hematocrit levels of anemic rats.

Parameters / Groups	Hb(g/dl)	HCT(%)
Control –ve	14.43±0.10^{a}	51.30±0.88^{a}
Control +ve	3.037±0.26^{e}	29.56±0.94^{e}
5%	11.15±0.18^{d}	38.90±1.16^{d}
10%	12.48±0.18^{c}	43.45±0.56^{c}
15%	13.05±0.13^{b}	47.63±0.69^{b}

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Table (4): Effect of moringa on red blood cells count and white blood cell levels of rats.

Parameters / Groups	RBCs (ml/cmm)	WBCs (th/cmm)	Platelet (Th/cmm)
Control –ve	9.04±0.36^{a}	5.04±0.12^{d}	171.20±1.97^{a}
Control +ve	4.33±0.14^{e}	9.09±0.16^{a}	89.53±1.98^{e}
5%	5.40±0.12^{d}	7.83±0.20^{b}	131.80±1.17^{d}
10%	6.35±0.13^{c}	7.37±0.15^{b}	146.80±1.05^{c}
15%	7.63±0.26^{b}	6.73±0.10^{c}	159.83±2.98^{b}

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

The Effect of moringa extract on mean corpuscular volume, mean corpuscular hemoglobin and lymphocytes concentration of rats recorded in Table (5). The results show that positive control group had significant (P<0.05) decrease in MCV, MCHC and lymphocytes compared to the negative control group. Data show that rats given orally 1 ml of moringa extract at (5%, 10% or 15%) had significant increase in MCV, MCHC and lymphocytes compared with positive control group. Moreover, there was no significant changes among the three tested levels. The highest increase in MCV, MCHC and lymphocytes was observed at the groups given moringa extract at 15%.

Table (5): Effect of moringa extract on mean corpuscular volume, corpuscular hemoglobin concentration and lymphocytes of rats.

Parameters / Groups	MCV (mm^3)	MCHC (g/dl)	Lymphocytes (%)
Control -ve	84.10±1.09[a]	46.86±1.05[a]	61.56±1.38[a]
Control +ve	55.16±1.50[e]	25.03±0.70[d]	37.70±1.24[d]
5%	64.96±0.98[d]	32.93±0.72[c]	43.10±1.33[c]
10%	71.66±1.35[c]	39.10±0.96[b]	50.00±0.89[b]
15%	75.76±0.49[b]	45.36±0.86[a]	58.60±0.92[a]

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Table (6) showed the effect of moringa extract on of liver functions of anemic female rats. Rats fed basal diet (positive control group) revealed a significant increase (p<0.05) in the mean value (167.00) of AST and ALT as compared with those of negative control group. Supplementation with moringa extract at the tested levels significantly decreased (P<0.05) serum AST and ALT as compared to the positive control group.There was a significant difference in AST and ALT among all the groups treated with moringa extract. The highest reduction in AST and ALT was observed at the groups given moringa extract at 15%.

Table (6): Effect of moringa extract on of liver functions of anemic rats.

Parameters / Groups	AST (µ/dl)	ALT(µ/dl)
Control –ve	86.70±1.56[e]	27.63±0.53[e]
Control +ve	167.00±2.67[a]	59.23±2.27[a]
5%	135.70±1.81[b]	43.80±1.02[b]
10%	122.10±2.04[c]	37.30±0.81[c]
15%	111.53±1.45[d]	32.67±0.62[d]

Discussion

Egypt is one of the developing countries that are facing the double burden of malnutrition; nutritional anemia is the most common type of it **(Hassan et al., 2007).** Iron deficiency anemia can cause both growth disruption and disruption of physical and mental development, lower intellectual, ability to learn, ability to exercise, impaired cognitive development and behavior **(Pivina et al., 2019).**

It is known that fresh leaves of moringa contain vitamin C seven times more than oranges, vitamin A four times more than carrots, calcium four times more than milk, potassium three times more than bananas and protein two times more than yogurts **(Kumssa et al., 2017).**

The present results are in the same line with (**EL-Bushuty and Shanshan, 2020; Aberra, 2011** and **Dubey et al., 2013)** who declared that moringa leaves powder had high content of protein, ash and fiber. Furthermore, antioxidant activity from leaves of moringa was high because of the increase in the concentration of polyphenolics **(Sreelatha and Padma, 2009)**. Moringa leaves powder contains considerable amount of polyphenolic compounds, benzoic and caffeine (**Halaby and Emara, 2015).**

The present results are in the same line with **Ameh et al., (2018)** who reported that the normal control group and the extract group (rats administered only M. oleifera extract at 500mg/kg body weight) had progressive increase in body weight. Treatment with an ethanolic extract of Moringa oleifera leaves at doses of 300 and 600 mg/kg of rats received PHZ caused an increase in body weight compared to negative control. (**Sarkiyayi and Abubakar, 2018)**.

Suzana et al., (2017) described a significant increase in hemoglobin levels in anemic patients by administering ferrous sulfas preparations 200 mg/day plus moringa leaf extract 1400 mg/day. **Yulianti, (2016)** also stated a significant increase in adolescent girls' haemoglobin levels after being given Moringa leaf extract. Meanwhile, **Abdul Mun'im et al., (2016)** reported that giving 792 mg / 200 g BW / day of Moringa leaf extract can increase hemoglobin levels and significantly improve the morphology of aniline induced rat erythrocytes. **Osman, (2012)** reported a significant increase in albino rats' haemoglobin levels induced with aluminium (AlCl3) and given 300 mg/kg moringa leaf extract for 21 days. These results are agreed with the obtained results of the current work.

Moreover, dry M. oleifera leaves are rich in essential nutrients and might be used in food supplementation to improve the nutritional status of individuals and communities especially the vulnerable groups (**Madukwe et al.,2013). Dona et al., (2017)** concluded that moringa leaves extract could improve iron deficiency anemia in women. In addition, **Suzana et al., (2017)** showed a significant differences in the decrease in platelets. **Ameh et al., (2018)** found significant ($P>0.05$) decrease in RBCs and significantly increased WBCs compared with the normal control group.

On the other hand, **Hermayanti et al., (2020)** demonstrated that moringa leaf extract and vitamin C did not appear to affect the total iron binding capacity (TIBC), MCV and MCH. Oral administration of Moringa oleifera leaf extract irrespective of the dose has the tendency to increase blood parameters such as WBC, RBC, Hb and PCV in anaemic rats **(Anslem et**

al.,2017). **Also, Fatema et al., (2020)** reveals that Moringa oleifera leaf has preventive role against cyclophosphamide induced anemia.

The obtained results are agree with the results of **Ameh et al.,(2018) ; Negm, (2019) and Al-Gebily et al., (2019)** whom declared that Moringa oleifera extract has a hepato-nephro-protective therapeutic effect on obese rats; this was monitored from marked improvement in ALT, AST and ALP activities as well as urea and creatinine levels in serum; this result goes in parallel with that of **Fakurazi et al., (2012)** who attributed that to the antioxidant potential of Moringa oleifera extract included phytochemicals. Also, **Halaby et al. (2013)** stated that this effect might be due to the potent antioxidant property of Moringa oleifera extract contents (vitamins, a-tocopherol, ascorbic acid and 3-carotene, as well as glutathione) that act against oxidative stress. These findings are also in accordance with **(Toppo et al., 2015 and Habib and Al-Moalem 2018).**

Conclusions: It can be said that fortification with Moringa leaves has positive effects on anemic rats. Based on our results we recommend for further studies on larger patients and longer period of follow up to emphasize our conclusion.

References

Abdul Mun'im; Meidi U. P.; Santi P. Sari and Azizahwati (2016). Anti-anemia Effect of Standardized Extract of Moringa Oleifera Lamk. Leaves on Aniline Induced Rats. Pharmacognosy Journal, 8, (3).

Aberra, M. (2011): Comparative assessment on chemical compositions and feeding values of leaves of Moringa oleifera using in vitro gas production method. Ethiop. J. Appl. Sci. Technol., 2(2): 31-41.

Adusi-Poku Y.; Sittie A.; Mensah MLK.; Sarpong K.; Fleischer TC. and et al (2008). Effectiveness and safety assessment of mist tonica as herbal haematinic. Afr J Tradit Complement Altern Med 5: 115-119.

Afsana, K.; Shiga, K.; Ishizuka, S.; and Hara, H (2004): Reducing effect of Ingesting tannic acid on the absorption of iron but not of zinc, copper and manganese by rats. Biosci.Biotechnol.Biochem., 68(3):584-592.

Al-Gebily, M.; Morsy, F.; Elzawahry, E.; Ibrahim, D. and Abdel-Wahhab, K. (2019). Obesity Modulating Efficiency of Moringa oleifera Extract on Obese Modeled Rats. Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology, 11(1):75-91.

Alexander R.R. and Grifiths J.M. (1993): Basic biochemical methods (2nd ed.) (pp 186-7). New York: Wiley-Liss.

Ameh, S.S. and Alafi, O.F, (2018). Effect of Ethanol Extract of Moringa Oleifera Leaves in Protecting Anaemia Induced in Rats by Aluminium Chloride. IOSR J Biotechnol Biochem, 4(6): 34-52.

Anslem O.A.; Philippe E.M.; Tchounga S.K. and Vivian C.O. (2017): Effects of Moringa oleifera Leaves Extract on Haematological Parameters of Phenylhydrazine Anaemia Induced Wistar Rats. Int J Pub Health Safe 2: 139.

Anwar F.; Ashraf M. and Bhanger M.I. (2005): Interprovenance variation in the composition of Moringa oleifera oilseeds fromPakistan. J Am Oil Chem Soc **82**: 45–51.

AOAC, (2005): Official Methods of Analysis of Association of Official Analytical Chemists. In: Kenesseth Helrick, B.,15th Edition.

Bavhure, B.; Borive, M. and Kadima, J. (2014): Haematic and hepatroprotective potentials of Hypoestes triflora aqueous leaf extract in guineapigs. Int J Pharm Sci Res, 5(9):3726-3732.

Bergmeyer H.U.; Schreiber P. and Wahlefeld A.W.(1978): Optimization of methods for aspartate and alanine aminotransferase. ClinChem; 24:58-61.

Benoist, B.; Mc Lean, E.; Egli, I. and Cogswell, M (2008): Worldwide prevalence of anemia 1993- 2005- WHO Global Database on Anemia. WHOCDC, p :48.

Burda, S. and Oleszek, W. (2001): Antioxidant and antiradical activities of flavonoids .Journal of Agriculture and Food Chemistry, 49 (6):2774–2779.

Chapman D.G.; Castilla R. and Campbell J. A. (1959): Evaluation of protein in food. LA. Method for the determination of protein efficiency ratio. Can. J. Biochem. Physiol., 37: 679 – 686.

Dacie J.V. and Lewis S.M. (1991): Practical haematology (7th ed.) (pp 37-85). Edinburgh:ELBS with Churchill Livingstone.

Dona, S.; Franciscus D. S.; Azizahwati; Retnosari A.; Santi P. S. and Abdul Mun'im (2017). Effect of Moringa oleifera Leaves Extract Against Hematology and Blood Biochemical Value of Patients with Iron Deficiency Anemia. Journal of Young Pharmacists, 9, Issue 1(Suppl).

Dubey, D.; Dora, J.; Kumar, A.; Gulsan, R. (2013): A Multipurpose tree- Moringa oleifera. International Journal of Pharmaceutical and Chemical Sciences, 2(1):415-423.

Ebrahimie M.; Bahmani M.; Shirzad H.; Rafieian-Kopaei M. and Saki K (2015): A review study on the effect of Iranian herbal medicines on opioid withdrawal syndrome, Journal of Evidence-Based Complementary and Alternative Medicine, 20(4): 302-309.

EL-Bushuty, D. and Shanshan, N. (2020): The Effect of Moringa Leaves Powder on Hypercholesterolemic Male Rats and the Possibility of Adding to some Vegetable Recipes. Journal of Research in the Fields of Specific Education, 6(29): 635-659.

Fakurazi S.; Sharifudin S.A. And Arulselvan P. (2012): Moringa oleifera hydroethanolic extracts effectively alleviate acetaminopheninduced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 17:8334-50.

Fatema, F.; Nahar, S. and Akhter, Q.S. (2020): Cyclophosphamide induced anemia in Wistar rats: can it be prevented by Sajna (Moringaoleifera)? Journal of Bangladesh Society of Physiologist, 15(1):11–16.

Habib, M.K. and Al-Moalem M.H. (2018): Effect of Moringa Leaves and Seeds on Osteoporosis in Rats. J. Food and Dairy Sci.9 (10): 129 – 135.

Halaby M.; Elmetwaly E. and Omar A. (2013): Effect of Moringa oleifera on serum lipids and kidney function of hyperlipidemic rats. Egyptian J. Appl. Sci. Res., 9(8): 5189 - 5198.

Halaby, M. and Emara, N. (2015): Influence of Moringa oleifera on non-alcoholic fatty liver in adult albino rats . Middle East Journal of Applied Sciences,5: 902-912.

Hassan H.; Moussa W. and Ismail I. (2007): Assessment of dietary changes and their health implications in countries facing the double burden of malnutrition: Egypt, 1980 : 2005.

Hermayanti, D.; HawinNurdiana, H.; Setiawan,W.; Refa Maulana and Azhar, R.(2020): The Effect Of Moringa Leaf (Moringa Oleifera Lam) And Vitamin C Combination Extract In Improving Anemia Indicators Deficiency Of White Rice Iron Male Diet Low Rice Iron. Jurnal Saintika Medika , 16 (2):104-114.

Kasolo J.N.; Bimenya G.S.; Ojok L. and Ogwal-Okeng, J.W. (2012): Sub-acute toxicity evaluation of Moringa oleifera aqueous and ethanol extracts in Swiss Albino Rats. Inter J Med Plant Res. 1:75-81.

Kumssa, D.B.; Joy, E.J.; Young, S. D.; Odee, D. W.; Ander, E. L. and Broadley, M.R. (2017): Variation in the mineral element concentration of Moringa oleifera Lam. and M. stenopetala (Bak. f.)Cuf.: Role in human nutrition. PloS one, 12(4): e0175503.

Madukwe E.U.; Ugwuoke A.L. and Ezeugwu J.O. (2013): Effectiveness of dry Moringa oleifera leaf powder in treatment of anaemia. International Journal of Medicine and Medical Sciences, 5(5):226-228.

Negm, S. H. (2019): Effect of Soybean, Virgin Coconut and Moringa oleifera Seed Oils on the Propylthiouracil Induced Hypothyroidism in Rats. Alexandria Science Exchange Journal, 40(OCTOBER-DECEMBER), 649-659.

Ogbe, S.R.; Adoga, G.I. and Abu, A.H. (2010): Anti anemic potentials of some plant extracts on phenyl hydrazine-induced anemia in rabbits. Journal of Medicinal Plant Research, 4: 680-684.

Okochi Y.I.; Akpotuzor J. and Alli L.A. (2013): Comparison of an African herbal formula with commercially available haematinics. African J Biotechnol 2: 219-227.

Osman H.M.; Shayoub M.E.; Babiker E.M. and et al., (2012): Effect of Ethanolic Leaf Extractnof Moringa Oleifera on Aluminium-induced Anemia in White albino Rats, Jordan Journal of Biological Science, 5: 255-260.

Pivina, L.; Semenova, Y.; Doşa, M.D.; Dauletyarova, M. and Bjørklund, G. (2019): Iron deficiency, cognitive functions, and neurobehavioral disorders in children. Journal of Molecular Neuroscience, 68(1):1-10.

Sarkiyayi, S. and Abubakar, M.A (2018): Effect of ethanol leaf extract of Acacia nilotica on phenyl hydrazine induced anemia in rats. IOSR J. Pharm. Biol. Sci, 13:01-10.

Sasikala V.; Rooban BN.; Priya S.G.; Sahasranamam V. and Abraham A. (2010): Moringa oleifera prevents selenite-induced cataractogenesis in rat pups. J Ocul Pharmacol Ther., 26(5): 441 - 447.

Senedecor,G and Cochran , W.(1989): statistical methods methods 7th ed, lowa state university press ,Ames,USA,90.

Siddhuraju P.and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (Moringa oleifera Lam.). J Agric Food Chem 15: 2144–2155.

Sreelatha, S. and Padma, P.R. (2009): Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods for Human Nutrition, 64: 303–311.

Suzana, D.; Suyatna, F.D.; Andrajati, R.; Santi, P. S. and Mun'im, A (2017): Effect of Moringa oleifera leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia. Journal of Young Pharmacists, 9(1):S79.

Thurber, M.D. and Fahey, J. W (2009): Adoption of Moringa oleifera to combat undernutrition viewed through the lens of the “Diffusion of Innovations” theory. Ecol Food Nutr, 48 (3):212-225.

Toppo, R.; Roy B.; Gora R.; Baxla S. and Kumar P. (2015): Hepato-protective activity of Moringa oleifera against cadmium toxicity in rats.Vet. World. 8(4): 537–540.

Vamsee, V.A.; Jyothi, Y.; Rina, P.; Rajdwip, G.; Ronak, P. and Vijay, Y. (2015): Comparative anti anemic activity of Azadirachta indica leaves and its combination with Emblica officinalis in phenylhydrazine induced anemia using rats. J Chem Pharm Res, 7 (8):1019-1022.

Verma A.R.; Vijayakumar M.; Mathela C.S. and Rao C.V. (2009): In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol J., 47(9): 2196 – 2201.

Yulianti H, Hadju V, Elma Alasiry. 2016. Pengaruh Ekstrak Daun Kelor Terhadap Peningkatan Kadar Hemoglobin Pada Remaja Putri di SMU Muhammadiyah Kupang. JST Kesehatan, 6:399 – 404.