EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title:EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya

Volume-2 | Issue-3 | May-June; 2020 | DOI:10.36349/easjnfs.2020.v02i03.015

OPEN ACCESS

Research Article

Evaluation of *Moringa Oleifera* Lam Leaves (Moringaceae) Diets Against Induced Anemia in Wistar Rats

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Article History
Received: 14.04.2020
Accepted: 25.05.2020
Published: 30.05.2020

Journal homepage:
https://www.easpublisher.com/easjnfs

Quick Response Code

Abstract: The effect of oleifera leaves diets against anemia was investigated in Wistar rats. Three groups of six rats each were formed. Moringa Hemolytic anemia was induced in rats by phenylhydrazine at a dose of 40 mg/kg body weight intraperitoneally for two successive days. The rats, which received phenylhydrazine were subjected to Ranferon® (Reference substance) and two régimes food P50% and P100% contained respectively 50% and 100% of sheet Moringa oleifera from day 2 (D2) until the end of the experiment to day 28 (D28). Blood was taken from all the rats to days D0, D2, D5, D14 and D28. Diets made from leaves of Moringa oleifera (P50% and P100%) and Ranferon® have significantly restored the red blood cell parameters changed by phenylhydrazine. In addition, P50% provided better values of erythrocyte parameters than P100%. However, no significant change in body weight was observed. This study revealed that these diets based on Moringa oleifera leaves has excellent therapeutic efficacy, thus confirming the use of this dietary supplement in the treatment of anemia.

Keywords: Moringa oleifera, Diets, Anemia, Erythrocyte parameters.

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 consequences on human health as well as on social and economic development. This pathology affects all ages and both sexes in general, but, in particular children, women of reproductive age, especially pregnant women (Bavhure *et al*., 2014). Anemia prevalence is from 3 to 4 times higher in developing countries than in developed countries (Zangeneh *et al*., 2017). Anemia has many causes such as nutritional deficiencies, severe bleeding, genetic defects, diseases infectious, prolonged use of nonsteroidal drugs and exposure to toxic substances such as phenylhydrazine that reduce the amount and quality of red blood cells (Assobayire *et al*., 2001; Tripathi, 2003; Vamsee *et al*., 2015). Several treatments are used to treat anemia. It involves oral iron, vitamin B9 or B12 supplementation, an injection of erythropoietin, a blood transfusion and a bone marrow transplant (Movaffaghi & Hasanpoor, 2006).

The valorization of rich plant resources in protein and micronutrients, accessible at low cost is a strategy to fight effectively against anemia (Anwar *et al*., 2007). It is in this context that a study was conducted on *Moringa oleifera* Lam., plant species belonging the family of Moringaceae which contains about 13 species (Olson, 2002). It is a tree that can reach 7 to 12 meters in height. This plant, native to India and Arabia, is widely cultivated in tropical and subtropical regions (Ramachandran *et al*., 1980). Many therapeutic properties are attributed to *Moringa oleifera* which is used in traditional medicine for the treatment of metabolic, inflammatory, infectious, parasitic diseases, cancer and also for the purification of water (Luqman *et al*., 2012; Sy-Ndiaye *et al*., 2016). Several studies have highlighted the exceptional nutritional qualities of *Moringa oleifera* Lam leaves (Moringaceae). Indeed, studies have shown the effectiveness of these leaves in preventing, correcting

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malnutrition and associated diseases, although they contain anti-nutritional factors such as phytates and oxalates (Thurber & Fahey, 2009). They can therefore constitute a food supplement for malnourished subjects due to its richness in proteins, vitamins (A, B, C, E) and mineral salts (Ca, K, Mg, P, Iron, Zn, Se, Cu, Mn , Na, Cl). In addition, the leaves of this plant are positioned as a tonic, strengthening and stimulating immune system for people living with HIV/AIDS (Girija *et al*., 1982; Thurber & Fahey, 2009).

The aim of this study is to investigate the effect of *Moringa oleifera* leaves powder diets on erythrocyte parameters in phenylhydrazine-induced anemic Wistar rats.

MATERIALS AND METHODS

Plant material

The plant material used in this study consisted of leaves of *Moringa oleifera* Lam (Moringaceae). These leaves have been identified by us. They have been thoroughly cleaned and dried in the laboratory of Physiology, Pharmacology and Pharmacopoeia at room temperature of 25 ± 2 ° C for two weeks. Then, the dried leaves were reduced to powder using an electric grinder (CULATTI, France). This powder was used to make food formulations.

Animal material

The animals used were adult albino rats (*Rattus norvegicus*) of both sexes with a mean body weight of 177.66 ± 25 g. Animals had free access to water and food. They have been acclimated to the animal husbandry conditions of the Animal Physiology Laboratory of Pharmacology and Pharmacopoeia, Training Unit of Science and Research of Nature Sciences (Nangui Abrogoua University, Abidjan, Côte d'Ivoire).

Foods and food trials

The experimental foods consisted in part of a food commonly used for breeding rats (*Rattus norvegicus*) in the laboratory of Physiology, Pharmacology and Pharmacopoeia, UFR Sciences de la Nature (Nangui Abrogoua University) and powdered leaves of *Moringa oleifera*. From food regime control (T), 2 diets food experimented (P50% and P100%) tested on rats were formulated. In these schemes, the flour powder soy was replaced e by the leaves of *Moringa oleifera*. The T regime not contained *M. oleifera* leaves. But, food regimes P50% and P100% contained respectively 50% and 100 %. The composition of food regime per 100g is reported in Table 1.

A total of three groups of six wistar rats were made up in this study. Each group included three males and three females. The s groups 1 and 2 received, respectively, a diet food area the proportions of 50% and 100% *Moringa oleifera* leaves powder *ad libitum* during 26 days. The group 3 received the reference molecule (Ranferon®) at a dose of 5 mg/kg of body weight for the same duration of experimentation.

Table 1: Composition of diets

Food ingredients	Composition (%) of diet		
	T	P50%	P100%
bakery dry bread spray (%)	44.5	44.5	44.5
crushed yellow corn spray (%)	25	25	25
dry fish spray (%)	16	16	16
soy flour (%)	14	7	0
Moringa oleifera leaf powder (%)	0	7	14
cooking salt (%)	0.5	0.5	0.5
Total	100	100	100

T: Basic food; P50%: Food T with half the soybean meal being replaced by *M. oleifera* leaf powder; P100%: Food T with half the soybean meal being replaced by *M. oleifera* leaf powder.

Induction of Anemia and Blood Sample and Analysis of Erythrocyte Parameters

Anemia was experimentally induced intraperitoneally in rats by a phenylhydrazine solution at a dose of 40 mg/kg of body weight for two successive days (D0) and (D1) according to the method of Naughton *et al*. (1995).

Venous blood from rats was collected using a sterilized Pasteur pipette from the retro-orbital sinus of the rat eye after an inhalation anesthesia with ethyl ether solution. The blood samples were collected in tubes containing an anticoagulant (EDTA) on days 0 (D0), 2 (D2), 5 (D5), 14 (D14), 28 (D28). These blood samples were immediately used for the ' analysis of red blood cell parameters such as erythrocytes, hematocrit (Ht), hemoglobin (HGB), mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). The determination of these parameters was made using an automatic counter (Sysmex XT-2000 i, Japan).

Statistical Analysis

The results were expressed as means followed by standard error on means (Mean±SEM). Changes in hematological parameters during each selected period

of rats growth were revealed using one factor (ANOVA1) associated with the *post-hoc*, test Tukey. The different statistical tests were considered significant at $p < 0.05$. The statistical analysis of the values and the graphical representations of the data were carried out using the GraphPad Prism software version 5.01 for Windows **(**GraphPad Software Inc., San Diego, MO, Califormia/USA, 2007).

RESULTS

Diets on body weight

The intraperitoneal injection of phenylhydrazine has induced a non-significant decrease ($p > 0.05$) in the body weight of the rats in the different groups on D2 compared to D0. The groups of rats treated with diets P50% and P100%; Ranferon® (5 mg/kg b.w.) have caused no significant variation ($p > 0.05$) body weight to D5, D14 and D28 compared to D2 (Table 2).

Table 2: Influence of diets and Ranferon® on body weight of rats induced anemia

Duration (Days)	Groups of rats		
	P50%	P100%	Ranferon®
0 (D0)	234.5 ± 6.67	232 ± 6.25	233.8± 8.41
2 (D2)	228.3 ± 5.78 aNS	231.7 ± 5.93 aNS	229.3 ± 16.52 aNS
5 (D5)	231.2 ± 7.72 bNS	230.3 ± 11.02 bNS	233 ± 13.44 bNS
14 (D14)	253.2 ± 10.22 bNS	250.2 ± 14.63 bNS	256 ± 22.20 bNS
28 (D28)	265.7 ± 14.20 bNS	268.7 ± 17.97 bNS	268.8± 22.85 bNS

Values expressed as mean ± ESM; n = 6 rats/group; a: Compared to D0; b: compared to D2; NS: Not significant ($p > 0.05$); P50%: Food T with half the soybean meal being replaced by M. oleifera leaf powder; P100%: Food T with half the soybean meal being replaced by *M. oleifera* leaf powder.

Observed Anemia and Consequences on Red Cells Parameters

In groups of rats made (P50% P100% and Ranferon®, the mean values of erythrocyte parameters before the administration of phenylhydrazine (D0) are 7.55 ± 0.14 x10^6/µL; 12.50 ± 0.24 g/L; 38.43 ± 0.58 %; 50.79 ± 0.75 fL; 16.63 ± 0.37 pg; 32.95 ± 0.37 g/dL, respectively, for the number of erythrocytes, the concentration of hemoglobin, the level of hematocrit, MCV, MCH and MCHC. The mean values of the various parameters studied on D0 are normal compared to the reference values (Table 3). Two days after the administration of phenylhydrazine (D2), the results revealed, on the one hand, a significant decrease ($p < 0.001$) in erythrocytes, hemoglobin and hematocrit and, on the other hand, a significant increase ($p < 0.001$) of MCV, MCH and MCHC to D2 compared D0 among groups rats P50%, P100 % and Ranferon® (Table 3).

Table 3: Mean values of the erythrocyte parameters before and after induction of anemia

Normal values	D0	D2	Normal values
Erythrocytes (10^6/µL)	7.55 ± 0.14	3.57 ± 0.17***	5-12,5
Hemoglobin (g/dL)	12.50 ± 0.24	8.30 ± 0.29***	11-18
Hématocrit (%)	38.3 ± 0.58	22.05 ± 0.98***	36-52
MCV (fL)	50.79 ± 0.75	62.06 ± 1.32***	44.4-69
MCH (pg)	16.63 ± 0.37	23.53± 0.92***	12-24.50
MCHC (g/dL)	32.95± 0.37	37.58 ± 0.68***	21.6-42

Values expressed as mean ± SEM; n=18 rats/group. D0: Day before the administration of phenylhydrazine; D2: Two (2) days after the administration of phenylhydrazine; *** $p < 0.001$ D0 compared to D2. MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin

Diets on erythrocyte parameters

Effects of diets and Ranferon® on erythrocyte parameters in rats were shown by Figure 1 and 2. Results have indicated an increase significantly ($p < 0.001$) in number of erythrocytes (Figure 1A), hemoglobin concentration (Figure 1B) and hematocrit (Figure 1C) in groups of rats subjected to the P50% and P100% diet and the group of rats treated with Ranferon® on D5, D14 and D28 in comparison to D2. Moreover, the rats treated with the Ranferon®® and rats fed with food P50% and P100% showed a decrease significantly ($p < 0.001$) of MCHC to D5, D14 and D28 compared to D2. Regarding MCV, a significant decrease ($p < 0.001$) to D5 observed only in the groups of P50%, P100% and Ranferon®, then a significant increase ($p < 0.05$) only in the P100% group and ($p < 0.001$) in the Ranferon® group on D14 compared to D2. However, no significant variation ($p < 0.0\ 5$) observed on D28 (Figure 2). As for the MCH, P50%, P100% and Ranferon® have induced a significant decrease ($p < 0.001$) at D5 and D28 compared to D2. On the other hand, at D14, a significant decrease ($p < 0.001$) in MCH noted only in rats subjected to the P50% diet compared to D2 (Figure 2B).

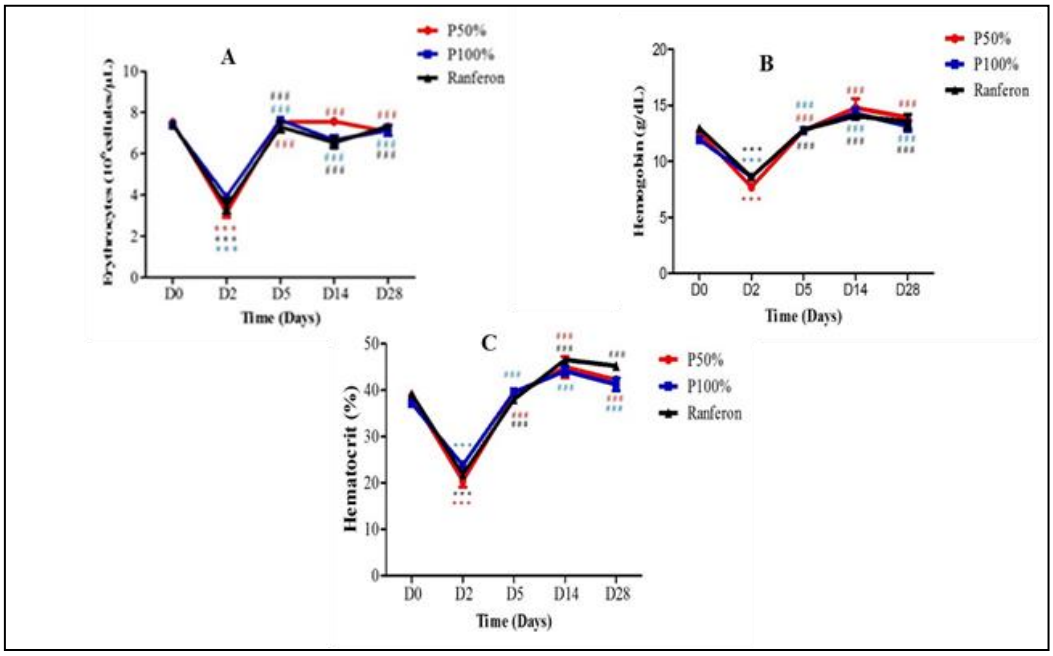

Figure 1: Effects of treatments on red cells count in rats

Values expressed as mean ± SEM; n = 6 rats/group; *** P <0.001; D2 vs D0 in each group; # # # P <0.001; # P <0.05; D5, D14 and D28 vs D2 in each group; P50%: Food T with half the soybean meal being replaced by *Moringa oleifera* leaf powder; P100%: Food T with half the soybean meal being replaced by *Moringa oleifera* leaf powder.

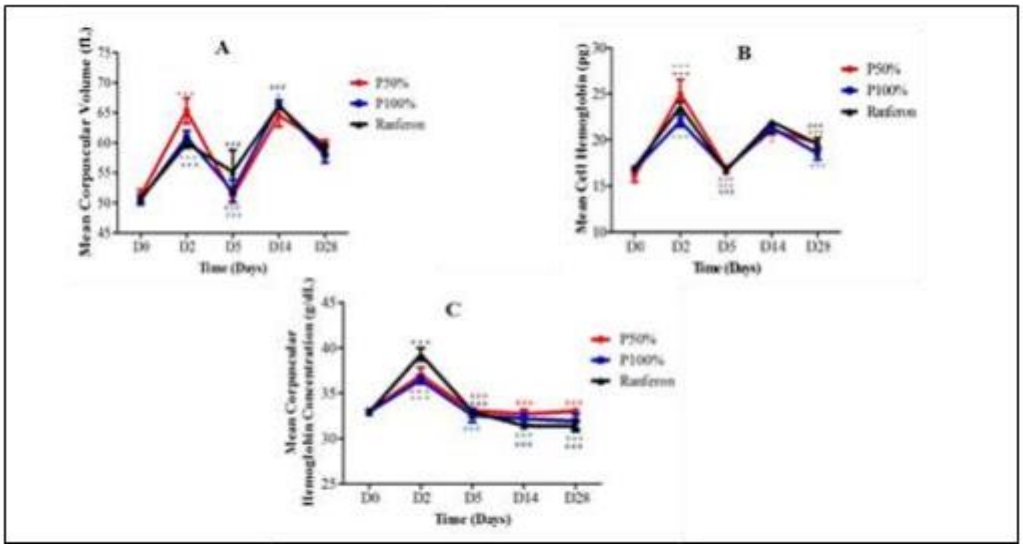

Figure 2: Effects of treatments on erythrocyte indices in rats

Values expressed as mean ± S M; n = 6 rats/group. *** P < 0.001; D2 vs D0 in each group; # # # P < 0.001; # P <0.05; D5, D14 and D28 vs D2 in each group; P50%: Food T with half the soybean meal being replaced by *Moringa oleifera* leaf powder; P100%: Food T with half the soybean meal being replaced by *Moringa oleifera* leaf powder.

DISCUSSION

The intraperitoneal administration of phenylhydrazine (PHZ) in rats for two successive days resulted in a non-significant reduction in body weight compared to the initial value (D0). Moreover, treatments did not increased significantly the body weight of rats throughout the study period after the induction of anemia. It appears that this diet would have no influence on the body weight of rats. Our results are contrary to those of Nku-Ekpang *et al*. (2015). These authors obtained a significant reduction in body weight of rats after administration of the PHZ compared to control normal rats. In addition, treatment of rats which received PHZ associeted with an ethanolic extract of *Moringa oleifera* leaves at doses of 300 and 600 mg/kg caused an increase in body weight compared to negative control (PHZ without treatment). According to these authors, the loss of body weight is one of the anemia symptoms. This change could be due a lack of appetite in rats. The observed difference in our study would be

justified by the interindividual variability of rats and their capacity to resist the linked effects of hemolytic anemia on body weight.

With regard to erythrocyte parameters, the mean values of erythrocytes number, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) obtained in rats before administration of phenylhydrazine were normal. These values are similar to reference data from Descat F (2002) work. The intraperitoneal administration on two consecutive days (D2) of PHZ reduced significantly the erythrocytes number, hemoglobin concentration, hematocrit level and a significant increase in MCV, MCH and MCHC compared to D0. According to Flanagan & Lessler (1970), PHZ induces hemolytic anemia characterized by a decrease in erythrocytes, hemoglobin, hematocrit level and an increase in MCV, MCH MCHC. In addition, authors have revealed that MCV, MCH and MCHC increase in pathological conditions such as cirrhosis and hemolytic anemia (Murakami *et al*., 1988; Sembulingam & Sembulingam, 2010). Our results are close to those obtained by Nku-Ekpang *et al*. (2015); Lavanya *et al*. (2018). However, they are different from those revealed by Ndem *et al*. (2013); Ponmozhi & Ramya (2015). These have shown a significant drop in MCV after intraperitoneal administration of PHZ for two successive days in rats. In our study, the PHZ induced a normocytic normochromic anemia due to the values of MCV, MCH and MCHC which are included within the rats Wistar reference values. The results obtained were similar to those of Kambou *et al*. (2015) and Umaru *et al*. (2018).

The groups of rats treated respectively with the P50% and P100% diets showed a restoration of the erythrocyte parameters modified after the induction of anemia by PHZ. The results revealed a significant increase in red cells, concentration of hemoglobin, hematocrit level on day D5 until the end of the experiment (D28). The improvement in hematopoiesis revealed by the significant increase in these parameters in all rats after the use of the P50% and P100% diets would be due to an intake of proteins, iron, B vitamins (thiamine, riboflavin and niacin), E (a-tocopherol) and nicotinamide contained in the leaves powder and demonstrated by several authors (Girija *et al*., 1982; Anwar *et al*., 2007; Thurber & Fahey, 2009). In fact, the amino acids of proteins, vitamins B, E and iron are involved in the synthesis of hemoglobin, the formation and maturation of red blood cells (Mathé *et al*., 1981). According to Ross (1999), the leaves of this plant are rich in phenolic compounds (flavonoids and tannins), saponins, triterpenes, sterols, alkaloids and has ther chemical constituents. The observed effects are justified by the presence of components phytochemicals, vitamins and minerals in the leaves of *Moringa oleifera* known to be involved in hematopoiesis influencing direct lies production of s cells blood uines at the marrow bone. Thus, the incorporation of *Moringa oleifera* leaves in rats food would oppose it to the effect of the PHZ.

The effects of this food regime are similar to certain plants described by certain authors. Gbenou *et al*. (2008); Umaru *et al*. (2018) showed, respectively, the therapeutic properties of *Justicia secunda* leaves and *Solanum nigrum* leaves in resorption induced anemia PHZ. Our results revealed that Ranferon® effectively restored all of the erythrocyte parameters studied. The P50% and P100% diets practically have effects similar to those of Ranferon®, the reference anti-anemic substance. However, these blood biological parameters are significatively better in rats subjected to P50% than those P100%.

CONCLUSION

Injection of phenylhydrazine to rats caused anemia haemolytic normochromic normocytic characterized by a significant reduction of erythrocytes, hemoglobin and hematocrit below normal reference values. The treatment of the rats by diet constituted of 50% and 100% of powder leaves of *Moringa oleifera* normalized quickly modified erythrocyte parameters. The effect of food regime used is similar to that of Ranferon®®, reference antianemic substance. The ability of this plant to effectively fight against anemia is linked to the phytochemicals, vitamins and minerals present in the leaves. The results of this study confirm that the leaves of *Moringa oleifera* can be used as a dietary supplement in the treatment of anemia. Incorporating powdered *Moringa oleifera* leaves into the diet could permit to resorb of malnutrition and anemia. The diet with 50% complementary leaves of *Moringa oleifera* has better than that which does contain 100%. However, additional work would be possible to demonstrate the mechanisms of anemia treatment.

REFERENCES

1. Benoist, B., Mc Lean, E., Egli, I. & Cogswell, M. (2008). Worldwide prevalence of anemia 1993-2005- WHO Global Database on Anemia. WHO-CDC, p 48.
2. Ogbe, S. R. J., Adoga, G. I. & 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.
3. Bavhure, B., Borive, M. & Kadima, J. (2014). Haematic and hepatroprotective potentials of Hypoestes triflora aqueous leaf extract in guinea-pigs. Int J Pharm Sci Res, 5(9), 3726-3732.
4. Zangeneh, M. M., Pooyanmehr, M. & Zangeneh, A. (2017). Evaluation of the anti-anemic potential of Glycyrrhiza glabra aqueous extract in Phenylhydrazinetreated rats. Iranian J Pharmacol Ther, 15: 1-9.

5. Assobayire, F. S., Adou, P., Davidson, L. & Cook, J. D. (2001). Hurrel R. Prevalence of iron deficiency with and without concurrent anemia in population group with high prevalences of malaria and other infections: a study in Cote d'Ivoire. Am J Clin Nutr, 74: 776-782.
6. Tripathi, K. D. (2003). Essential of medical pharmacology. Jaypee brothers medical publishers (P) Ltd; 5th edition; pp167-184.
7. Vamsee, V. A., Jyothi, Y., Rina, P., Rajdwip, G., Ronak, P. & 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.*
8. Movaffaghi, Z. & Hasanpoor, M. (2006). Effect of therapeutic touch on blood hemoglobin and hematocrit level. J Holist Nurs, 24: 41-8.
9. Anwar, F., Latif, S., Ashraf, M. & Gilani, A. H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res, 21 (1),* 17-25.
10. Olson, M. E. (2002). Intergeneric Relationships within the Caricaceae-Moringaceae Clade (Brassicales) and Potential Morphological Synapomorphies of the Clade and Its Families. Systematic Botany, 27, 55-73.
11. Ramachandran, C., Peter, K.V. & Gopalakrishnan, P. K. (1980). Drumstick (*Moringa oleifera*): multipurpose Indian vegetable. *Economic Botany, (34),* 276-283.
12. Luqman, S., Srivastava, S., Kumar, R., Maurya, A. K. & Chanda, D. (2012). Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in vitro* and *in vivo* assays. *Evid. Based Complement.* Alternat Med, 32, 47-66.
13. Sy-Ndiaye, A., Fall, A.D., Ndiaye, M., Sall, A. O., Sy, G. Y., Bassène, E. & Dièye, A. M. (2016). Mise en évidence de l'activité antiinflammatoire des sous-fractions méthanoliques des feuilles de *Moringa oleifera* Lam. (*Moringaceae*) chez le rat. *Int J Biol Chem Sci, 10 (2),* 760-768.
14. Thurber, M. D. & 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.
15. Girija, V., Sharada, D. & Pushpamma, P. (1982). Bioavailability of Thiamine, Riboflavin and Niacin from Commonly Consumed Green Leafy Vegetables in the Rural Areas of Andhra Pradesh in India. Inter J Vitamin Nutr Res, 52, 9-13.
16. Naughton, B. A., Moore, E., Bush, M. E., Rabbit, D. M. & Domfest, B. (1995). Hemostatic alterations associated with phenylhydrazineinduced haemolytic anemia. Eur J Clin Invest, 25, 722-727.
17. Nku-Ekpang, O-A. T, Nwaehujor, C. O., Ofem, O. E. & Ezekiel, J. I. (2015). Effect of *Moringa oleifera* Lam. Ethanol Leaf Extract on Hematology in Phenylhydrazine-induced Anemic Albino Wistar Rats. *American J Pharm Sci, (3*), 67-73.
18. Descat, F. (2002). Hématologie du rat : hémogramme et myélogramme. Thèse médecine vétérinaire de Toulouse, Tou 3-4011, France. p 91.
19. Flanagan, J. P. & Lessler, M. A. (1970. Controlled phenylhydrazineinduced reticulocytosis in the rat. *The Ohio J Sci, 70 (5*), 300-304.
20. Murakami, A., Kitazono, Y., Jiwajinda, S., Koshimizu, K. & Ohigashi, H. (1998). Niaziminin, a thiocarbamate from the leaves of *Moringa oleifera*, holds a strict structural requirement for inhibition of tumor-promoter-induced Epstein-Barr virus activation. Planta Medica, 64, 319-323.
21. Sembulingam, K. & Sembulingam, P. (2010). Essentials of medical physiology. 5th ed. Jaypee Brothers Medical Limited, pp 85-89.
22. Lavanya, S., Swathi, K., Babu, V., Srivani, Metlakunta, A. S. & Manish, G. B. (2018). Evaluation of anti anaemic activity of aqueous *Piper betle* leaf extract against phenyl hydrazine induced anaemia in wistar rats. European J Pharm Med Res, 5 (12), 226-230.
23. Ndem, J. I., Otitoju, O., Akpanaiabiatu, M. I., Uboh, F. E., Uwah, A. F. & Edet, O. A. (2013). Haematoprotective property of *Eremomastax Speciosa* (Hochst.) on experimentally induced anaemic wistar rats. *Ann Biol Res, 4 (6),* 356-360.
24. Ponmozhi, E. & Ramya, B. (2015). Anti-anemic activity *Murraya koenigii* leaves on phenylhydrazine induced anemia in rats. World J Sci Res, 1 (1), 1-8.
25. Kambou, P. S., Bleyere, N. M., Attemene, D. S. D., Tiahou, G. G., Dembele, A. & Sess, E. D. (2015). Antianaemic effect of spirulina in rabbits (*Oryctolagus cuniculus*), a made and used food supplement in Côte d'Ivoire. Sch Acad J Biosci, 3 (9), 725-732.
26. Umaru, H. A., Moses, M. A. & Zailani, H. A. (2018). Effect of *Solanum nigrum* Methanol Leaf Extract on Phenylhydrazine Induced Anemia in Rats. *Jordan J Biol Sci, 11 (1),* 65-71.
27. Mathé, G., Boivin, P., Caen, J., Turpin, F., Griscelli, C., Florentin, I. & Subtil, E. (1981). Sang tissus hématopoïétique et lymphoïdes et leurs relations avec l'immunité. *in* : Sémiologie médicale Eds Flammarion Médecine-Sciences, Paris; France, pp 63-80.
28. Ross, I. A. (1999). Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses. Totowa: Humana Press. pp 234-235.
29. Gbenou, J. D., Tossou, R., Dansou, P., Fossou, M. & Moudachirou, M. (2006). Etude des propriétés antianémiques de *Justicia secunda* vahl (acanthaceae) chez des rats de souche wistar. *Pham Med Trad Afr*, 14, 45-54.