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Research Article

Antianemic Power of Aqueous Extract of *Moringa oleifera* (Moringaceae) Leaves in Phenylhydrazine Induced Anemic Wistar Rats (*Rattus norvegicus*)

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Abstract: The present study was to evaluate the anti-anemic power of total aqueous extract of *Moringa oleifera* leaves (TAEMo) on phenylhydrazine induced anemic Wistar rats. First, hemolytic anemia was induced in rats by intraperitoneal administration of phenylhydrazine (PHZ) at the dose of 40 mg/kg body weight for two consecutive days. Then, the anemic rats were orally treated daily for 26 days with aqueous extract of *Moringa oleifera* leaves (TAEMo) at doses of 400, 800 and 1600 mg/kg b w. and Ranferon®-12 (Reference substance). The rats were analyzed for erythrocyte parameters such as erythrocyte cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration on day 0, 2, 5, 14 and 28. The results revealed that the aqueous extract restored rapidly the modified erythrocyte parameters at the end of treatment. TAEMo at 1600 mg/kg showed an effect similar to the reference substance, Ranferon®-12. The study of reversibility after stopping treatment showed that not all erythrocyte parameters studied were significantly affected. This study found anti-anemic properties of *Moringa oleifera* leaves aqueous extract thus confirming the use of this plant in the treatment of anemia in the population.

Keywords: Rats, *Moringa oleifera*, Anemia, Erythrocyte parameters, Phenylhydrazine.

INTRODUCTION

Anemia is a widespread public health problem with serious human, social and economic consequences. It can lead to a higher risk of infection, a decline in cognitive function and a decrease in physical work capacity (Stevens, G.A. *et al.,* 2013). This disease affects people of all ages. The World Health Organization (WHO) estimates that worldwide anemia is as high as two billion (Erin, M. *et al.,* 2009). It has been classified by WHO as one of the ten most serious diseases of modern world (Agarwal, K.N. *et al.,* 1991). This condition is much more common in developing countries. The regions with higher prevalence of anemia are South Asia and Africa (Kalenga, M. K. *et al.,* 2003). The groups at high risk are infants, children with intensive growth and pregnant women. The causes and predisposing circumstances of anemia are multiple. The most important are nutritional deficiencies, parasitic infections, chronic infections as well as pregnancy and lactation (Asobayire, F. S. *et al.,* 2001).

Several modern medicines are offered to patients depending on the type of anemia. This is an additional iron, vitamin B9 or B12, immunosuppressants or corticosteroids, blood transfusion, bone marrow transplant and erythropoietin injections (Movaffaghi, Z., & Hasanpoor, M. 2006). Despite the progress in medical care, people are moving towards traditional medicine for their primary health care. Faced with this craze, World Health Organization recommends that health practitioners verify the effectiveness of natural

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substances through scientific studies (OMS 2002). It is in this context that a study is being conducted on a medicinal plant whose leaves with multiple virtues are used by population to treat several pathologies including anemia.

Moringa oleifera is a tree 10 to 12 meters tall (Gupta, R.K. 2010). This plant species has been introduced and naturalized around villages in northern Côte d'Ivoire (Bouquet, A., & Debray, M. 1974). Several pharmacological and nutritional studies have been carried out on the different parts of this plant (Ndabigengesere, A. *et al.*, 1995 ; Dehot, M.U. 1998; & Rao, A.V., & Rao, L.G. 2007). This study aims to assess the therapeutic efficacy of an aqueous total extract from the leaves of *Moringa oleifera* on an experimental model of anemia induced in rats Wistar.

MATERIAL AND METHODS

Plant material

The plant material used in this study consisted of leaves of *Moringa oleifera* and identified by us. The leaves were carefully cleaned and dried in the Physiology, Pharmacology and Pharmacopoeia laboratory at room temperature of 25 ± 2 °C for two weeks. Then, the dried leaves was reduced to powder using an electric grinder (CULATTI, France). This was used in an extraction of an aqueous extract.

Animal

Wistar rats adult albino (*Rattus norvegicus*) of weighing 177.66 ± 25 g were used for this study. The animals had free access to water and food. They had been maintained under standard conditions of the Laboratory of Physiology, Pharmacology and Pharmacopeia of the Training Unit of Science and Research of Nature Sciences (Nangui Abrogoua University, Abidjan, Côte d'Ivoire).

Extract preparation method

One hundred and fifty grams of *Moringa oleifera* leaves powder was macerated during 24 hours in 2500 mL of distilled water under magnetic stirrer (SB 162 STUART, United Kingdom). The maceration obtained was double-filtered on hydrophilic cotton and Whatman n°1 filter paper. The filtrates were evaporated and oven dried (JP SELECTA S, Spain) at 45 °C for 72 hours. The 30.16 g quantity of powder with a yield of 20.11% was obtained and stored in the refrigerator at 7 °C until use. This made powder was used to prepare the total aqueous extract of the leaves of *Moringa oleifera* (TAEMo).

Induction of anemia and treatment of rats

Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (PHZ) at a dose of 40 mg/kg body weight for two days (D0 and D1) (Naughton, B. A. *et al.*, 1995). Rats were homogeneously divided into four groups of six rats each. Each group consisted of three males and three females and treated daily for 4 week as follows; group 1 treated with reference substance (Ranferon®-12) at a dose of 5 mg/kg from day D2 and D28; group 2 treated with total aqueous extract of *Moringa oleifera* (400 mg/kg) from day D2 and D28; group 3 treated with total aqueous extract of *Moringa oleifera* (800 mg/kg) from day D2 and D28; group 4 treated with total aqueous extract of *Moringa oleifera* (1600 mg/kg) from day D2 and D28. All administration was done orally using oropharyngeal cannula once per day for 28 days (4 weeks).

Analysis of erythrocyte parameters

The venous blood was collected from the rats through retro orbital sinus of the eye into EDTA using a sterilized Pasteur pipette after anesthetized with ether before induction of anemia (D0), after induction of anemia with PHZ (D2) and D5 D14 D28 of treatment. In addition, venous blood was taken 30 days (D30) after stopping treatment to observe possible persistence, reversibility or late onset of effect. The erythrocyte parameters such as erythrocytes cells, hematocrit (Ht), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and concentration corpuscular hemoglobin (MCHC) were determined at days D0, D2, D5, D14 and D28 using an automatic blood cell 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, California/USA, 2007).

RESULTS

Body weight

Phenylhydrazine did not influence ($p > 0.05$) body weight at D2 in all groups of rats compared to D0. Treatment of rats with aqueous total extract from the leaves of *Moringa oleifera* (TAEMo) at all doses tested and Ranferon®-12 had no significant change ($p > 0.05$) on body weight on day 7 (D7), 14 (D14) and 28 (D28) compared to D2 (Figure 1).

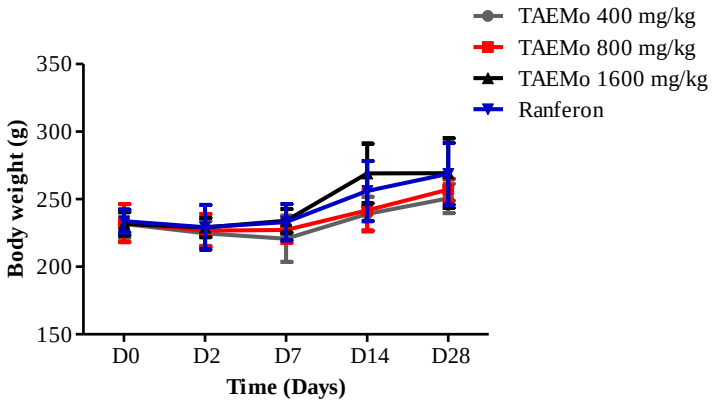

Figure 1: Effect of TAEMo on body weight before and after induction of anemia in rats by phenylhydrazinem Values were expressed as Mean ± SEM; with n=6 in each group. TAEMo: Total aqueous extract of *Moringa oleifera* leaves

Erythrocyte cells

Table 1: Mean values of erythrocyte parameters before and after induction of anemia

Erythrocyte parameters	Day 0	Day 2	Normal values	p values
Erythrocyte cells (10^6/µL)	7.64 ± 0.09	3.6 ± 0.30	5 - 12.5	p < 0.001
Hemoglobin (g/dL)	12.73 ± 0, 19	8.14 ± 0.27	11 - 18	p < 0.001
Hematocrit (%)	3 8.67 ± 0.63	22.24 ± 1.01	36 - 52	p < 0.001
MCV (fL)	50.08 ± 0.9 2	6 1.62 ± 1.28	44.4 - 69	p < 0.001
MCH (pg)	16.58 ± 0.44	23.33 ± 0.64	12 - 24.5	p < 0.001
MCHC (g/dL)	32.65 ± 0, 30	3 8.27 ± 0.64	21.6 - 42	p < 0.001

Values expressed as mean ± SEM; n=18 rats in each group. p < 0.001vs D0. D0: Day before administration of phenylhydrazine; D2: Two (2) days after administration of phenylhydrazine; MCV: Mean Corpuscular Volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular concentration of hemoglobin.

Table 2: Changes of erythrocyte parameters 30 days after stopping of various treatments

Erythrocyte parameters	Ranferon	TAEMo 400 mg/kg	TAEMo 800 mg/kg	TAEMo 1600 mg/kg	Normal values
Erythrocytes (10^6/µL)	6.388 ± 0.48	7.35 ± 0.13	7.813 ± 0.44	7.293 ± 0.20	5 - 12.5
Hemoglobin (g/dL)	11.53 ± 3.00	14.20 ± 1.50	13.80 ± 1.00	19.53 ± 2.54	11 - 18
Hematocrit (%)	37.53 ± 1.55	44.03 ± 0.89	46.60 ± 4.23	44.23 ± 1.78	36 - 52
MCV (fL)	59.35 ± 2.83	59.90 ± 1.26	59.43 ± 2.23	60.63 ± 0.99	44.4 - 69
MCH (pg)	19.20 ± 5.80	19.25 ± 1.73	17.57 ± 0.27	26.77 ± 3.38	12 - 24.50
MCHC (g/dL)	31.85 ± 9.37	32.30 ± 3.36	29.67 ± 0.91	44.33 ± 6.04	21.6 - 42

Values expressed as Mean ± SEM, with n=6 in each group. TAEMo: Total aqueous extract of *Moringa oleifera* leaves; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin content; MCHC: Mean corpuscular concentration of hemoglobin.

After injection of phenylhydrazine to rats (D2), there was a significant decrease (p < 0.001) in all groups compared to D0. The values obtained were lower than the reference values (Table 1). But, the reference solution (Ranferon®-12) and total aqueous extract from the leaves of *Moringa oleifera* ((TAEMo) at doses of 400, 800 and 1600 mg/kg increased significantly (p < 0.01, p < 0.001) at D5, D14 and D28 compared to D2 (Figure 2). The results were shown the fast recovery from D5 in all groups of rats. Thirty days after stopping the administration of the reference solution (Ranferon®-12) and extract at doses of 400, 800 and 1600 mg/kg, the values of many of the erythrocytes remained normal (Table 2).

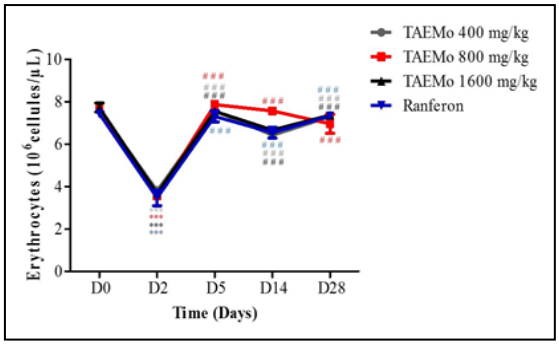

Figure 2: Effect of TAEMo on the number of erythrocytes before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Total aqueous extract of *Moringa oleifera* leaves. Values expressed as mean ± SEM; with n=6 in each group. ***p < 0.001 vs D0 in each group. $^{\#\#\#}$p < 0.001; $^{\#\#}$p < 0.01 vs D2 in each group.

Hemoglobin

The administration of phenylhydrazine at day D2 caused a significant decrease (p < 0.001) hemoglobin in all groups of rats compared to D0. The values obtained were lower than the reference values (Table 1). An increase hemoglobin concentration was observed after treatment with Ranferon®-12 and the total aqueous extract of the leaves of *Moringa oleifera* (TAEMo) at doses of 400, 800 and 1600 mg/kg (p < 0.01 ; p < 0.001) at D5, D14 and D28 compared to D2. The results showed that the rats of all groups were recupered quickly from D5 (Figure 3). Thirty days after stopping the administration of the Ranferon®-12 and the total aqueous extract of *Moringa oleifera* leaves at doses of 400 and 800 mg/kg, the values of hemoglobin concentration remained normal (Table 2). On the other hand, extract at 1600 mg/kg caused a slight increase of this parameter compared to normal reference values.

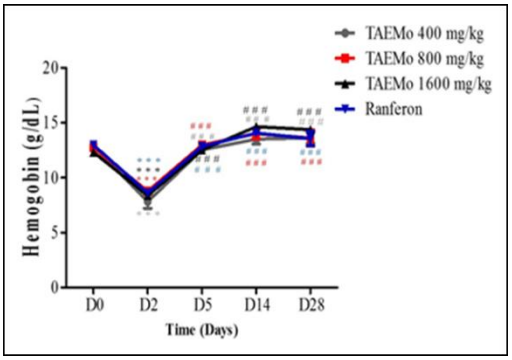

Figure 3: Effect of TAEMo on hemoglobin concentration before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Total aqueous extract *of Moringa oleifera* leaves. Values expressed as mean ± SEM, with n=6 in each group. ***p < 0.001; **p < 0.01; D0 in each group. $^{\#\#\#}$p < 0.001; $^{\#\#}$p < 0.01; $^{\#}$p < 0.05 vs D2 in each group.

Hematocrit

The results revealed that the administration intraperitoneally of phenylhydrazine decreased significantly (p < 0.01, p < 0.001) hematocrit at D2 compared to D0 (Figure 4). The hematocrit values obtained were below the reference values (Table 1). After treatment, a significant increase (p < 0.01, p < 0.001) was observed at days D5, D14 and D28 in rats received the TAEMo at doses of 400, 800 and 1600 mg/kg and Ranferon®-12 when compared to D2 (Figure 4). Thirty days after stopping the administration of the reference solution (Ranferon®-12) and the total aqueous extract of *Moringa oleifera* leaves (TAEMo) at doses of 400, 800 and 1600 mg / kg, the values of hematocrit levels remained normal (Table 2).

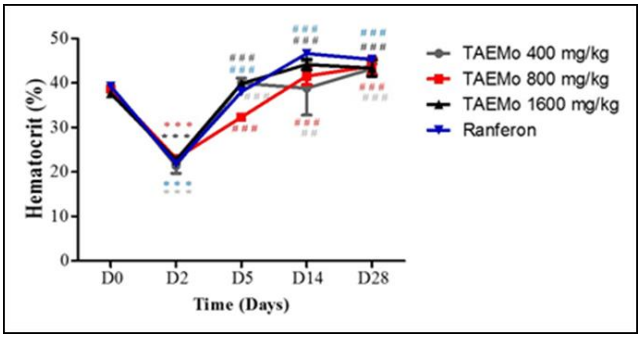

Figure 4: Effect of TAEMo on the hematocrit level before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Total aqueous extract *of Moringa oleifera* leaves. Values expressed as mean ± SEM, with n=6 in each group. ***p < 0.001; **p < 0.01 D2 vs D0 in each group. $^{\#\#\#}$p < 0.001; $^{\#\#}$p < 0.01 D5, D14 and D28 vs D2 in each group.

Mean corpuscular volume (MCV)

The administration of phenylhydrazine at day (D2) increased significantly (p < 0.01, p < 0.001) of MCV in all groups of rats compared to D0 (Figure 5). MCV values obtained are included within the values of reference (Table 1). On the other hand, after treatment, the TAEMo at doses of 800 and 1600 mg/kg induced a significant reduction (p < 0.001) only at day D5 and a non-significant increase (p > 0.05) at days D14 and D28 with compared to D2. Ranferon®-12 decreased significantly (p < 0.001) only at D5 and increased significantly at D14 compared to D2. In contrast, TAEMo at 400 mg/kg not influenced (p > 0.05) MCV during all periods treatment (Figure 5). Thirty days after stopping the administration of the reference solution (Ranferon®-12) and the total aqueous extract of *Moringa oleifera* leaves (TAEMo) at doses of 400, 800 and 1600 mg/kg, the values of VGM remained normal (Table 2).

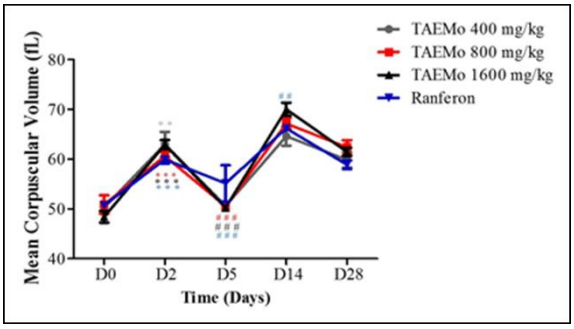

Figure 5: Effect of TAEMo on mean corpuscular volume before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Total Aqueous total extract *of Moringa oleifera* leaves. Values expressed as mean ± SEM, with n=6 in each group. ***p < 0.001; **p < 0.01 D0 in each group. $^{###}$p < 0.001 vs D2 in each group.

Mean corpuscular hemoglobin (MCH)

The administration of phenylhydrazine at day D2 caused a significant (p < 0.001) increase MCH compared to D0 (Figure 6). However, the MCH values was normal (Table 1).

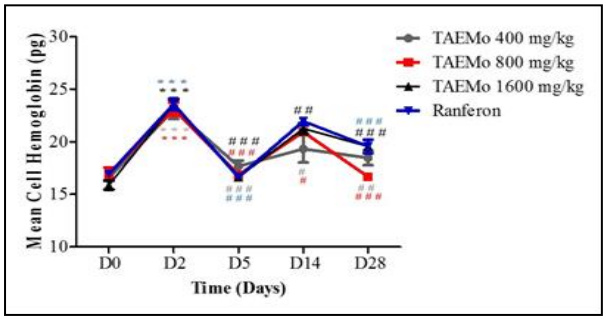

Figure 6: Effect of TAEMo on mean corpuscular hemoglobin before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Total aqueous extract of *Moringa oleifera* leaves. Values expressed as mean ± SEM, with n=6 in each group. ***p < 0.001 vs D0 in each group. $^{###}$p < 0.001; $^{##}$p <0.01; $^{#}$p <0.05 vs D2 in each group.

The treatment of rats with the Ranferon®-12 and TAEMo at doses of 400, 800 and 1600 mg/kg decreased significantly (p < 0.0 1 - 0.001) MCHC at days D5 and D28 when compared to D2. ETAMo caused a significant decrease (p < 0.05 - 0.01) only at D14 compared to D2 (Figure 6). The best recovery of TAEMo and Ranferon®-12 treated anemic rats was D5. Thirty days after stopping the administration of the Ranferon®-12 and the total aqueous extract of *Moringa oleifera* leaves (TAEMo) at doses of 400, 800 and 1600 mg/kg, the values of MCH remained normal (Table 2).

Concentration means corpuscular hemoglobin (MCHC)

The administration of phenylhydrazine at day D2 caused also a significant (p < 0.01, 0.001) increase MCHC compared to D0 (Figure 7). However, the TCMH values obtained are normal (Table 1). After treatment, the results revealed a significant decrease (p < 0.01, 0.001) in groups of rats that received oral Ranferon®-12 and TAEMo at doses of 400, 800, 1600 mg/kg at D5, D14 and D28 compared to D2 (Figure 7). Thirty days after stopping the administration of the Ranferon®-12 and the extract at doses of 400 and 800 mg/kg, MCHC values remained normal. In contrast, TCMH value increased significantly in group of rats treated with TAEMo at 1600 mg/kg compared to the reference values (Table 2).

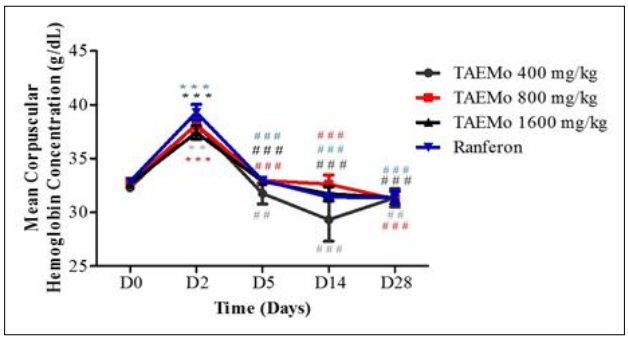

Figure 7: Effect of TAEMo on mean corpuscular hemoglobin concentration before and after induction of anemia in rats by phenylhydrazine.

TAEMo: Aqueous total extract of *Moringa oleifera* leaves. Values expressed as mean ± SEM, with n=6 in each group. ***p < 0.001; **p < 0.01 vs D0 in each group. $^{###}$p <0.001; $^{##}$p < 0.01 vs D2 in each group.

DISCUSSION

In this study, the results showed that phenylhydrazine (PHZ) did not decrease significantly body weight of rats when compared with D0. The loss of body weight observed in anemic rats is the one of the symptoms anemia. It would be due to a lack of appetite. The results of (Shravan, K.D. *et al.*, 2016) revealed a decrease in body weight of rats after induced by PHZ. According to these authors, this observation could be explained by a reduction in the activities of disaccharidases, enzymes that catalyze the last step of carbohydrate digestion. The treatment of anemic rats with an aqueous extract of *Moringa oleifera* leaves (TAEMo) and Ranferon®-12 did not increased significantly body weight of the rats during the different treatment periods. Thus, TAEMo would activate the appetite in rats by phytoconstituents present in this plant. These results were similar to of (Shravan, K.D. *et al.*, 2016; & Luka, C.D. *et al.*, 2014). They obtained in their respective studies, a non significant increase of the anemic rats treated with an aqueous extract of *Spinacia oleracea* leaves and an ethanolic extract of *Punica*

Granatum seeds for four weeks compared to their initial values.

Concerning to erythrocyte parameters, mean values of erythrocyte number, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean hemoglobin corpuscular concentration in rats before to phenylhydrazine administration were normal. After induction anemia by phenylhydrazine (PHZ) for two days, there was a significant reduction in erythrocyte cells, hemoglobin and hematocrit compared to D0. In addition, the obtained values are lower than the reference values mentioned by (Descat, F. 2002). On the other hand, a significant increase of MCV, MCH and MCHC was observed after administration of PHZ with values between the limits of the reference values. The phenylhydrazine has therefore induced a normocytic normochromic hemolytic anemia. These results corroborate those some authors (Nwaehujor, C. O. 2015 ; & Droucoula, G. C. *et al.*, 2017). They showed a significant reduction ($p < 0.001$) erythrocytes, hemoglobin, hematocrit and significant elevation ($p < 0.001$) of MCV, MCHC and MCH. However, our results are contrary to those of (Droucoula, G C et al. 2017) who observed a significant decrease in MCV after PHZ administration for two days. In these studies, the values of MCH, MCHC and MCV remained normal. According to (Flanagan, J. P., & Lessler, M. A. 1970), PHZ decreased erythrocytes, hemoglobin, hematocrit levels and increased MCV, MCHC, MCH, extramedullary hematopoiesis in the liver and spleen. In addition (Murakami, A. *et al.*, 1998), showed that MCV, MCH and MCHC are increased in pathological conditions such as liver cirrhosis and hemolytic anemia.

Treating the rats anemic by Ranferon®-12 and total aqueous extract from the leaves of *Moringa oleifera* administered orally restored all erythrocyte parameters modified by the phenylhydrazine from D5. TAEMo at doses of 400, 800 and 1600 mg/kg body weight induced, on the one hand, a significant increase in erythrocyte, hemoglobin and hematocrit levels ; and on the other hand, a significant reduction of MCV, MCHC and MCH at all treatment periods (D5, D14 and D28). Our results were similar to (Tchogou, A. P. *et al.*, 2016) who reported a recovery of erythrocyte parameters after one week of treatment with an extract aqueous of *Trema guineensis* leaves at doses of 100 mg/kg and 200 mg/kg. In addition (Nwaehujor, C. O. 2015) revealed normalization of erythrocyte parameters after treatment with an ethanolic extract of *Moringa oleifera* leaves at doses of 300 and 600 mg/kg for 21 days in rats induced by hemolytic anemia by PHZ. TAEMo at 1600 mg/kg has an anti-anemia activity substantially similar to that of Ranferon®-12.

The resorption of anemia by the reference substance (Ranferon®-12) is attributed to its composition in folic acid, iron, vitamin B12, ascorbic acid and zinc. Erythropoietin is the regulator of erythrocyte production in the bone marrow. In fact, this hormone increases the number of sensitive erythroblasts in the bone marrow that are converted into reticulocytes and later into mature erythrocytes (Sánchez-Elsner, T. *et al.*, 2004). The improvement in hematopoiesis revealed by the significant increase in the number of erythrocytes, hemoglobin and hematocrit after the use of *Moringa oleifera* is due to a contribution of proteins, iron, vitamins B (thiamine, riboflavin and niacin), E (α-tocopherol) and nicotinamide contained in the leaf powder and highlighted by several authors (Girija, V. *et al.*, 1982 ; & Anwar, F. *et al.*, 2007). 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, G. *et al.*, 1981). Standardization of these parameters observed at doses of total aqueous extract of *Moringa oleifera* in rats induced hemolytic anemia would be attributed to phytochemical constituents, vitamins and minerals (Anwar, F. *et al.*, 2006 ; & Uc, R., & Nair, V. M. G. 2013) revealed a high concentration of these nutrients in the leaves of this plant. These are known for their ability to stimulate erythropoietin (Nwaehujor, C. O. 2015).

Thirty days after stopping the administration of the reference substance (Ranferon®-12) and total aqueous extract of *Moringa oleifera* leaves at doses of 400, 800 and 1600 mg/kg, the values of the erythrocyte cells, hemoglobin, MCV and MCH remained normal. TAEMo, except at doses of 400 and 800 mg/kg and Ranferon®-12, values of the hemoglobin and MCHC have increased substantially in the group of treated rats with TAEMo at 1600 mg/kg compared to the values of reference. However, aqueous extract at 1600 mg/kg did not significantly affect the concentration of hemoglobin and MCHC. These results will indicate that anemia does not reappear 30 days after discontinuation of treatment with TAEMo at doses of 400, 800 and 1600 mg/kg.

CONCLUSION

This study reveals the effect of total aqueous extract of *Moringa oleifera* leaves in Wistar rats induced anemic by phenylhydrazine. The results show that TAEMo allows rapid recovery of erythrocyte, hemoglobin and erythrocyte indices (MCV, hematocrit, MCH and MCHC) deteriorated after administration of phenylhydrazine in rats. The extract at 1600 mg/kg, body weight shows a restoring effect of these parameters almost similar to that of Ranferon®-12. Thirty days after stopping treatment, the reversibility study shows that not all studied erythrocyte parameters were significantly affected. The anti-anemia activity of extract is attribued to phytoconstituents, minerals and vitamins contained in the leaves of this plant. This study demonstrates the antianemic power of total aqueous extract of *Moringa oleifera* leaves and the use of this plant by patients to treat anemia. Further, studies would

be needed to assess the influence of this plant on the blood quality of rats.

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