

# Comparative Effects Between A Total Aqueous Extract and A Diet Enriched with *Moringa oleifera* Leaves in Wistar Rats with Anemia

Narcisse Boua GNANGORAN<sup>2</sup>, Mathieu Nahounou BLEYERE<sup>4\*</sup>, Jean-Baptiste N'Guessan OUSSOU<sup>1</sup>, Aboubacar COULIBALY<sup>3</sup> and Paul Angoué YAPO<sup>3</sup>

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<sup>1</sup>Laboratory of Physiology, Pharmacology and Pharmacopeia; Nangui Abrogoua University, 02 BP 801 Abidjan 02 (Côte d'Ivoire).

<sup>2</sup>Animal Physiology and Pharmacology, Training and Research Unit of Nature Sciences/Laboratory of Physiology, Pharmacology and Pharmacopeia, Nangui Abrogoua University, Côte d'Ivoire

<sup>3</sup>Physiology of Human Nutrition, Training and Research Unit of Nature Sciences/Laboratory of Physiology, Pharmacology and Pharmacopeia, Nangui Abrogoua University, Côte d'Ivoire

<sup>4</sup>Animal Physiology and Physiology of Nutrition, Training and Research Unit of Nature Sciences/Laboratory of Physiology, Pharmacology and Pharmacopeia, Nangui Abrogoua University, Côte d'Ivoire.

## ABSTRACT

The leaves of *Moringa oleifera* have exceptional nutritional qualities and are used in traditional medicine to treat several diseases including anemia. This study is to propose a formulation for the optimal use of *M. oleifera* leaf powder in the treatment of anemia in rats. Phenylhydrazine (40 mg/kg) was administered intraperitoneally for 2 days to induce anemia in rats (*Rattus norvegicus*). The animals were divided into six groups of 6 rats each and treated orally from day D2 to D28. Rats groups 1, 2, 3 and 4 were treated orally, respectively with 400, 800 and 1600 mg/kg of *M. oleifera* aqueous extract of leaves (AEMo) and Ranferon<sup>®</sup>. Groups 5 and 6 were fed ad libitum with diets containing 50% (P50) and 100% (P100) of *M. oleifera* leaves as a substitute for soybean meal, respectively. Red blood cell (RBC) parameters such as RBCs, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were analyzed on days D0, D2, D5, D14 and D28. Results showed a prevalence of 0% in anemic rats treated with P50, AEMo 1600 mg/kg and Ranferon<sup>®</sup> versus 16.67% in those treated with P100, AEMo 400 and 800 mg/kg at D28. In addition, P50, AEMo 1600 mg/kg and Ranferon<sup>®</sup> significantly ( $p < 0.001$ ) increased RBCs, Hb, Ht with total recovery especially RBCs and Ht in anemic rats treated with P50 and Ranferon<sup>®</sup>. In conclusion, the incorporation in small quantities of *M. oleifera* leaf powder as a substitute for soybean meal has a better efficacy on anemia than the total leaf extract of the same plant.

**Keywords:** Aqueous extract, Anemia, Effectiveness, Food formulation, *Moringa oleifera*, *Rattus norvegicus*.

Corresponding author. Email: [chridandre@gmail.com](mailto:chridandre@gmail.com), + 225-45439944 / + 225-78342833

## INTRODUCTION

The World Health Organization (WHO) defines anemia as a decrease in hemoglobin concentration (Hb) below 13 g/dL in men or 12 g/dL in women (Beutler, 2006). In tropical areas, due to the endemicity of malaria, 10 to 20% of the population has less than 10 g/dL of Hb

(Diallo et al., 2008). Anemia remains a major public health problem in many developing countries, particularly in Africa. It leads to decreased work capacity, impaired cognitive performance, decreased immunity to infections, complications of pregnancy,

reduced psychomotor skills and poor learning capacity (Oladiji et al., 2007). There are several types of anemia, including hemorrhagic anemia, aplastic anemia, megoblastic anemia and hemolytic anemia (Ross and Wilson, 2006; Guyton and Hall, 2007). Several studies have shown that hemolytic anemia is associated with oxidative stress in RBC. Hemolytic damage is accompanied by the generation of reactive oxygen species (ROS), depletion of glutathione, oxidation of Hb and the formation of Heinz bodies in RBC. Hemolytic agents have been reported to cause membrane lipid peroxidation and denaturation of cytoskeletal protein (Jollow et al., 2001). Traditional medicine has been practiced for many years in developing countries, particularly in Africa. According to WHO (2002), its use is widespread and is becoming increasingly important in terms of health and economics. Moreover, more than 80% of the population uses it for primary health needs. Thus, many plants are used locally for the treatment of anemia and one of these plants is *M. oleifera* Lam (Moringaceae). It is a plant long used by African communities to treat malnutrition (Fahey, 2005). The leaves are the most widely used part of this plant. Phytochemical studies have revealed that fresh leaves of *M. oleifera* contain more vitamins C and A, calcium, potassium, iron and protein than other food products such as oranges, carrots, milk, bananas, yoghurt and spinach (Falowo et al., 2018). Flavonoid pigments such as alkaloids, kaempferol, rhamnetine, isoquercitrin and kaempferitrin are also present in leaves (Siddhuraju and Becker, 2003). They also contain certain phytoconstituents that are believed to have anti-oxidant, anti-microbial, anti-diabetic, anti-inflammatory, anti-asthmatic, anti-ulcer, anti-tumor, anti-cancer, hypocholesterolemia and hypotension activities (Farooq et al., 2012). This plant is traditionally used for anemia, anxiety, asthma, cholera, conjunctivitis, cough, diarrhoea, eye and ear infections and fever (Anwar et al., 2007). Due to its high nutritional value and its content of many biologically active compounds, the powder from the leaves of *M. oleifera* has been enriched in feed and food as a growth promoter and/or for medicinal purposes (Babiker et al., 2017). The purpose of this study is to compare the effect of an aqueous extract and a diet of *M. oleifera* leaves in order to propose an effective mode of use between two preparations in the treatment of anemia.

## MATERIALS AND METHODS

### Plant Material

The plant material consisted of *M. oleifera* leaves. They were harvested in the city of Abidjan (Côte d'Ivoire) in 2017. The plant was identified by us and authenticated by a botanist from Nangui Abrogoua University, Abidjan,

Côte d'Ivoire.

### Animal Material

The animal material consisted of albino rats Wistar, *R. norvegicus*, with a mean weight of  $177.66 \pm 25$  g. They were reared at the Laboratory of Animal Physiology, Pharmacology and Pharmacopoeia, Unit of Training and Research in Natural Sciences of Nangui Abrogoua University according to good laboratory practices.

The rats have been acclimated to the breeding conditions of the animal facility of the Laboratory of Physiology, Pharmacology and Pharmacopoeia at the Training and Research Unit in Natural Sciences of the Nangui Abrogoua University. They were fed daily with IVOGRAIN® food and had access to water at will in their bottles.

### Total Aqueous Extract Preparation of *M. oleifera* Leaves

The total aqueous extract of *M. oleifera* leaves (AEMO) was obtained according to the maceration methods developed at the Laboratory of Physiology, Physiology, Pharmacology and Pharmacopoeia of Training and Research Unit of Nature Sciences of Nangui Abrogoua University. The fresh leaves of *M. oleifera* were carefully cleaned and then dried in the laboratory for two weeks at a temperature of  $25 \pm 2^\circ\text{C}$ . After drying, the leaves were reduced to a fine powder using an electric grinder (Culatti, France). Thus, 150 g of *M. oleifera* leaf powder was macerated in 2500 mL of distilled water for 24 h on a magnetic stirrer. The macerate obtained was double-filtered on cotton wool and Whatman filter paper No 1. The filtrates were evaporated, dried in an oven at  $45^\circ\text{C}$  for 72 h and 30.16 g of aqueous extract were obtained.

### Formulation of Enriched Food with *M. oleifera* Leaves

The feed formulation was carried out at the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Training and Research Unit of Nangui Abrogoua University, Abidjan, Côte d'Ivoire. The diets were P<sub>50</sub> and P<sub>100</sub> in which *M. oleifera* leaf powder was incorporated, respectively at 50 and 100% in substitution of soybean meal amount contained in food intended for rat rearing.

Thus, *M. oleifera* leaf powder incorporated in 100 g of the control diet represented 7 g (P<sub>50</sub>) and 14 g (P<sub>100</sub>). The centesimal composition of the diets is recorded in Table 1.

### Experimental Study

Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (PHZ) at 40 mg/kg

**Table 1.** Composition of diets.

Food Ingredients	Composition in (%) of Diet		
	Control	P50	P100
Dry bread baking powder (%)	44.5	44.5	44.5
Crushed yellow corn spray (%)	25	25	25
Dry fish spray (%)	16	16	16
Soybean meal (%)	14	7	0
<i>M. oleifera</i> leaf powder (%)	0	7	14
Salt (%)	0.5	0.5	0.5
Total	100	100	100

P50: Control food with half substitution of soybean by *M. oleifera* leaf powder; P100: Control food with all substitution of soybean by *M. oleifera* leaf powder.

body weight daily for 2 days (D0 and D1) (Naughton et al., 1995). In this study, rats that had a Hb < concentration of 13 g/dL were considered anemic (Osafanme et al., 2019). Oral administration of total aqueous extract of *M. oleifera* (AEMo) and consumption of the diet was done daily from D2 to D28. Thus, 36 anemic rats were divided into 6 groups of 6 rats each. One group of anemic rats was orally administered the reference molecule (Ranferon®). Three groups of anemic rats were given the total aqueous extract of *M. oleifera* leaves at doses of 400 mg/kg (Group 1), 800 mg/kg (Group 2), and 1600 mg/kg (Group 3) body weight, respectively. Two groups of anemic rats were fed P50 (Group 4) and P100 (Group 5) diets, respectively.

#### Collection of Blood Samples and Determination of Hematological Parameters

Blood samples were taken from rats on days 0 (D0), 2 (D2), 5 (D5), 14 (D14) and 28 (D28) (Coulibaly et al., 2020a and b). On days D0 and D2, anemic rats were selected and on days D5, D14 and D28, the effects of both dietary formulations were evaluated. At each sampling, the rats were anesthetized with ether and blood samples were taken from the retro-orbital sinus using a sterile Pasteur pipette. Blood collected in tubes containing an anticoagulant, ethyl diamine tetraacetic acid (EDTA) was immediately used for hematological analysis. The blood count was performed using an automatic hematological analyzer (Sysmex XT-2000 i, Japan). This analysis determined erythrocyte parameters such as RBCs count, Ht, Hb, MCV and MCHC.

#### Statistical Analysis

The statistical analysis of the data was performed using Graph Pad Prism 5.01 software (San Diego, California, USA). The results are given as the mean followed by

the standard error on the mean ( $M \pm SEM$ ). In addition, some results have been expressed as percentages. Whenever a significant difference ( $p < 0.05$ ) was revealed, ANOVA 1 was supplemented by the Tukey-Kramer post-hoc test.

## RESULTS

### Changes in Anemia Prevalence During Treatment Periods

Intraperitoneal administration of PHZ at 40 mg/kg bw in rats at D2, induced anemia in all rats of each group with a hb concentration of less than 13 g/dL. The prevalence of anemia in each group of rats was 100%. At 5 days (D5) after oral treatment in anemic rats, aqueous extract of *M. oleifera* leaves at 400, 800 and 1600 mg/kg bw and Ranferon® gave anemia prevalence of 67.67, 33.33, 67.67 and 67.67%, respectively. Similarly, results from anemic rats fed P50 and P100 diets revealed identical anemia prevalence of 67.67%. At D14 and D28, anemia prevalence was 16.67% in anemic rats treated with AEMo at 400 and 800 mg/kg and anemic rats fed P100 diet. In contrast, the prevalence was 0% in anemic rats treated with Ranferon®, AEMo at 1600 mg/kg bw and P50 (Table 2).

### Effect of Total Aqueous Extract and Diet on RBC Parameters in Rats

Red blood cell counts at D0 ranged from  $7.43 \pm 0.16$  to  $7.53 \pm 0.14 \times 10^6/\mu\text{L}$  in groups of rats. After intraperitoneal administration of phenylhydrazine (D2), it decreased significantly ( $p < 0.001$ ) in different groups of rats and ranged between 3.135 and  $3.678 \times 10^6/\mu\text{L}$ . Treatment of anemic rats with aqueous extract of 1600 mg/kg bw, P50 diet and Ranferon® resulted in a significant increase ( $p < 0.001$ ) and total recovery at D5 of red blood cell count, 105.87, 140.14 and 111.05%,

**Table 2.** Prevalence percentage of anemia in rats during treatment periods.

Different Extracts and Foods	D2	D5	D14	D28
Ranferon®	100	66.67	0	0
AEMo 400mg/kg	100	66.67	16.67	16.67
AEMo 800mg/kg	100	33.33	16.67	16.67
AEMo 1600mg/kg	100	66.67	0	0
P50	100	66.67	0	0
P100	100	66.67	16.67	16.67

AEMo: Total aqueous extract of *M. oleifera* leaves; P50: Control food with half substitution of soybean by *M. oleifera* leaf powder; P100: Control food with all substitution of soybean by *M. oleifera* leaf powder.

**Table 3.** Effects of treatment on red blood cells in rats.

Groups	Red Blood Cells (10 <sup>6</sup> cell/ $\mu$ L)				
	D0	D2	D5	D14	D28
AEMo 1600 mg/kg	7.73 $\pm$ 0.22	3.68 $\pm$ 0.13 <sup>a***</sup> -55.92%	7.57 $\pm$ 0.16 <sup>b***</sup> 105.87%	6.67 $\pm$ 0.190 <sup>b***</sup> 81.43%	7.37 $\pm$ 0.09 <sup>b***</sup> 100.38%
P50	7.53 $\pm$ 0.14	3.14 $\pm$ 0.216 <sup>a***</sup> -58.38%	7.53 $\pm$ 0.15 <sup>b***</sup> 140.47%	7.57 $\pm$ 0.13 <sup>b***</sup> 141.47%	7.07 $\pm$ 0.15 <sup>b***</sup> 125.52%
Ranferon®	7.43 $\pm$ 0.16	3.47 $\pm$ 0.37 <sup>a***</sup> -53.36%	7.31 $\pm$ 0.270 <sup>b***</sup> 111.05%	6.55 $\pm$ 0.26 <sup>b***</sup> 69.70%	7.34 $\pm$ 0.17 <sup>b***</sup> 111.83 %

Values expressed as Mean  $\pm$  SEM with n=6 rats in each group; \*\*\*P < 0.001; a: comparison with D0; b: comparison with D2; AEMo: Total aqueous extract of the leaves of *M. oleifera*; P50: Control feed with 50% substitution of soybean meal by *M. oleifera* leaf powder.

**Table 4.** Effects of treatment on Hb concentration in rats.

Groups	Hemoglobin (g/dL)				
	D0	D2	D5	D14	D28
AEMo 1600 mg/kg	12.33 $\pm$ 0.36	8.38 $\pm$ 0.34 <sup>a***</sup> -32.01%	12.52 $\pm$ 0.24 <sup>b***</sup> 49.35%	14.65 $\pm$ 0.20 <sup>b***</sup> 74.76%	13.98 $\pm$ 0.32 <sup>b***</sup> 66.76%
P50	12.58 $\pm$ 0.22	7.72 $\pm$ 0.35 <sup>a***</sup> -38.66%	12.75 $\pm$ 0.15 <sup>b***</sup> 65.22%	14.77 $\pm$ 0.84 <sup>b***</sup> 91.39%	14.53 $\pm$ 0.18 <sup>b***</sup> 88.28%
Ranferon®	12.98 $\pm$ 0.23	8.60 $\pm$ 0.30 <sup>a***</sup> -33.74%	12.84 $\pm$ 0.12 <sup>b***</sup> 49.30%	14.06 $\pm$ 0.318 <sup>b***</sup> 3.49%	14.15 $\pm$ 0.64 <sup>b***</sup> 64.53%

Values expressed as Mean  $\pm$  SEM with n=6 rats in each group; \*\*\*P < 0.001; a: comparison with D0; b: comparison with D2; AEMo: Total aqueous extract of *M. oleifera* leaves; P<sub>50</sub>: Control food with 50% substitution of soybean meal by *M. oleifera* leaf powder.

respectively, compared to D2. This increase was 81.43, 141.47, and 69.70% at D14; 100.38, 125.52, and 111.83% at D28 in anemic rats treated with AEMo 1600 mg/kg bw, P50 and Ranferon®, respectively (Table 3). With respect to hb, PHZ administration at D2 resulted in a significant decrease ( $p < 0.001$ ) of 32.01, 33.74, and 38.66% in groups of AEMo 1600 mg/kg bw, P50 and Ranferon® rats, respectively ( $p < 0.001$ ). After the treatment of anemic rats, hb increased significantly ( $p < 0.001$ ) at D5, D14, D28 with a peak at D14. The increase was 74.76, 91.39 and 63.49% in groups of rats treated with AEMo 1600 mg/kg bw, P50 and Ranferon®, respectively (Table 4). For Ht, PHZ administration significantly ( $p < 0.001$ ) decreased Ht levels in all three groups of rats. Treatment of anemic rats with AEMo at 1600 mg/kg bw, P50 and Ranferon® indicated a significant increase ( $p < 0.001$ ) in Ht at all times. However, this increase was greatest at D14 with 94.45,

113.17, 120.47% recovery in anemic rats treated with AEMo at 1600 mg/kg bw, Ranferon® and P50, respectively (Table 5). With respect to MCV and MCHC, phenylhydrazine induced a significant increase at D2 in the groups of AEMo at 1600mg/kg bw, P50 and Ranferon® rats compared to D0. After treatment of anemic rats, low recovery of MCV and MCHC was observed at D5, D14 and D28 (Tables 6 and 7). Considering the overall results, the diet of P50 was more improved erythrocyte parameters at best compared to 1600 mg/kg bw of total aqueous extract.

## DISCUSSION

This study showed that intraperitoneal administration of phenylhydrazine at 40 mg/kg bw resulted in a decrease in Hb below 10 g/dL. According to Hariom et al. (2017);

**Table 5.** Effects of treatment on Ht in rats.

Groups	Hematocrit Percentage				
	D0	D2	D5	D14	D28
AEMo 1600 mg/kg	37.63±0.93	22.70±0.94 <sup>a***</sup> -39.67%	39.86±0.36 <sup>b***</sup> 75.59%	44.14±1.20 <sup>b***</sup> 94.45%	43.30±1.85 <sup>b***</sup> 90.75%
P50	38.73±0.53	20.42±1.33 <sup>a***</sup> -47.27%	38.73±0.57 <sup>b***</sup> 89.66	45.02±2.31 <sup>b***</sup> 120.47%	42.22±0.63 <sup>b***</sup> 106.76%
Ranferon®	39.28±0.67	21.86±0.72 <sup>a***</sup> -44.35%	38.03±0.69 <sup>b***</sup> 73.95%	46.60±0.97 <sup>b***</sup> 113.17%	45.28±0.59 <sup>b***</sup> 107.14%

Values expressed as Mean ± SEM with n=6 rats in each group; \*\*\*P < 0.001; a: comparison with D0; b: comparison with D2; AEMo: Total aqueous extract of *M. oleifera* leaves; P50: Control feed with 50% substitution of soybean meal by *M. oleifera* leaf powder.

**Table 6.** Effects of treatment on mean corpuscular volume in rats.

Groups	Mean Corpuscular Volume (fL)				
	D0	D2	D5	D14	D28
AEMo 1600 mg/kg	48.42±1.17	62.920±0.93 <sup>a***</sup> +29.95%	50.26±0.56 <sup>b***</sup> 20.12%	70.03±1.32 <sup>b**</sup> 11.33%	61.47±0.81 <sup>bNS</sup> 2.28%
P50	51.17±0.97	65.35±2.10 <sup>a***</sup> +21.70%	51.15±0.57 <sup>b***</sup> 21.73%	64.55±1.84 <sup>bNS</sup> 1.22%	59.78±0.65 <sup>bNS</sup> 8.52%
Ranferon®	50.730 ±0.51	59.94±0.78 <sup>a***</sup> 18.15%	55.15±3.64 <sup>b***</sup> 16.15%	66.17±0.08 <sup>b***</sup> 16.83%	58.88±0.88 <sup>NS</sup> 2.56%

Values expressed as Mean ± SEM with n=6 rats in each group; \*\*\*p < 0.001; \*\*p < 0.01; NS: Not significant (p > 0.05). a: comparison to D0; b: comparison to D2; AEMo: Total aqueous extract of *M. oleifera* leaves; P50: Control food with 50% substitution of soybean meal by *M. oleifera* leaf powder.

**Table 7.** Effects of treatment on mean corpuscular Hb concentration in rats.

Groups	MCHC (g/dL)				
	D0	D2	D5	D14	D28
AEMo 1600 mg/kg	32.67±0.32	37.42±0.62 <sup>a***</sup> +14.54%	32.88±0.19 <sup>b***</sup> 12.13%	31.72±0.66 <sup>b***</sup> 15.23%	31.37±0.59 <sup>b***</sup> 16.16%
P50	33.02±0.38	36.98±0.88 <sup>a***</sup> +11.98%	33.07±0.17 <sup>b***</sup> 10.57%	32.72±0.48 <sup>b***</sup> 11.52%	32.98±0.36 <sup>b***</sup> 10.70%
Ranferon®	32.88±0.26	39.30±0.73 <sup>a***</sup> +19.52%	32.98±0.20 <sup>b***</sup> 16.08%	31.43±0.31 <sup>b***</sup> 20.02%	31.37±0.59 <sup>b***</sup> 20.17%

Values expressed as Mean ± SEM with n=6 rats in each group; \*\*\*p < 0.001; a: comparison with D0; b: comparison with D2; AEMo: Total aqueous extract leaves of *M. oleifera*; P50: Control food with 50% substitution of soybean meal by *M. oleifera* leaf powder.

Osafanme et al. (2019), rats that exhibited mean Hb below 13 g/dL in the respective studies were considered anemic rats. In this study, all PHZ-treated rats exhibited Hb values below 13 g/dL, representing a 100% prevalence in each group. Treatment of anemic rats with two useful forms of *M. oleifera* leaf, showed variations in the prevalence of anemia during the experimental periods. The results revealed that groups of rats treated with AEMo at 1600 mg/kg bw, Ranferon® and fed a P50 diet gave a prevalence of 0% versus 16.67% in anemic rats treated with AEMo at 400 and 800 mg/kg bw, respectively and anemic rats fed a P100 diet at D14 and D28 (Coulibaly et al., 2020a and b). Among the doses of aqueous extract and percentage of leaves incorporated in the diet, AEMo at 1600 mg/kg bw and P50 showed an improved recovery (100%) comparable to that of Ranferon® (Reference Anti-Anemic substance) (Coulibaly et al., 2020a and b). The results would indicate that the high-dose aqueous extract (1600 mg/kg) and P50 diet would contain satisfactory nutrients and bioactive compounds in the resorption of anemia. RBC and RBC indices are usually used for the diagnosis of anemia. Thus, the study focused on the effect of the aqueous extract at 1600

mg/kg and P50 diet of *M. oleifera* leaves on the RBCs count, Hb concentration, Ht level, MCV and MCHC. PHZ at 40 mg/kg body weight induced hemolytic anemia characterized by a significant decrease in red blood cells (55.89%), Hb (34.80%) and Ht (43.76%) compared to D0.

The results are almost similar to those obtained by Shende et al. (2017) who observed a significant decrease in RBC (48.03%), Hb (37.03%) and Ht (45.23%) after administration of PHZ at 40 mg/kg for 2 successive days. Moreover, the results showed a significant increase in MCV and MCHC at D2 in rats given phenylhydrazine. Unami et al. (1996) and Sènou et al. (2016) obtained similar results as ours. The effect of PHZ could be due to its toxicity caused by the aryl and hydroxyl radicals it generates. It could also be due to a low affinity of oxygen for hemoglobin molecules because the tendency of hemoglobin to bind to oxygen improves blood flow to tissues (Ganong, 2005). Oral use of P50 and AEMo at 1600 mg/kg bw in rats previously injected with PHZ resulted in a significant increase in RBC, Hb and Ht count, a significant decrease in MCHC and fluctuation in MCV during different treatment periods. The results showed that the

rats in P50 group fully recovered in RBC count (141.47% at D14 and 125.52% at D28) and Ht (120.47% at D14 and 106.76% at D28). Thus, P50 diet has better correction of anemia compared to AEMo at 1600 mg/kg bw. In addition, the antianemic effect of P50 is relatively superior to that of Ranferon®, the reference antianemic substance. This observation would be justified by the use of *M. oleifera* leaves in rats and the composition of the diet. Thus, the incorporation of 50% of *M. oleifera* leaf powder in the control diet as a substitute for soybean meal of 7% of *M. oleifera* leaf powder of the control diet in rats would be beneficial in the treatment of anemia. The obtained results are relatively better than those of Madukwe et al. (2013). These authors recorded an increase of 93.16, 54.23 (Hb) and 63.96, 62.27% (RBC), respectively in anemic rats treated with 5 and 10% supplementation of *M. oleifera* leaf powder in commercial food. This difference could be justified by the composition of the control diet or the amount of *M. oleifera* leaf powder supplemented in the base diet.

In this study, the lower values observed in the group of rats treated with AEMo at 1600 mg/kg bw would be due to inadequate intake of the micronutrients and bioactive compounds required to treat anemia compared to the P50 diet. This observation may also be related to the presence of certain chemical compounds in AEMo at 1600 mg/kg bw that would negate the optimal effect on hematopoiesis. According to El Tazi and Tibin (2014), the decrease in Hb concentration is due to the potential toxicity of high levels of flavonoids and tannins in plant leaves. Previous studies have reported that the leaves of this plant taken as food are very rich in nutrients such as vitamins, minerals, proteins, carbohydrates and lipids (Lockett et al., 2000; Thurber and Fahey, 2009). Thus, the improvement of hematopoiesis revealed by the significant increase in the number of erythrocytes, Hb and Ht in rats after the use of *M. oleifera*, would be due to a high intake of protein, minerals mainly iron, vitamins B (thiamin, riboflavin and niacin), E (α-tocopherol) and nicotinamide contained in the leaf powder and highlighted by several authors (Anwar et al., 2007; Thurber and Fahey, 2009). Indeed, amino acids (proteins), vitamins B, E and iron are involved in Hb synthesis and the formation and maturation of red blood cells (Mathé et al., 1981; Madukwe et al., 2013). In addition, the incorporation of 50% *M. oleifera* leaf powder as a substitute for soybean meal of 7% *M. oleifera* leaf powder in the basal diet would act synergistically on hematopoiesis with the nutrients and phytochemicals present in the basal diet.

## CONCLUSION

Incorporation of *M. oleifera* leaf powder as a substitute for soybean meal powder in the staple food induces a

significant increase in RBC, Hb concentration and Ht level compared to the total leaf extract of the same plant in rats with phenylhydrazine-induced hemolytic anemia. Half-substituted soybean meal, P50 leads to a complete recovery of RBC and Ht level from D14. Thus, the dry leaves of *M. oleifera* rich in essential nutrients could be used in small quantities in food supplementation to effectively treat nutritional diseases, particularly anemia.

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