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Original Research Article

Moringa oleifera Total Leaf Extract on Anthropometric and Hematological Parameters in Anemic Rats

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Abstract

Study aims to evaluate the evolution of anthropometric and hematological parameters in anemic rats treated with an aqueous extract of *Moringa oleifera* leaves. Seventy rats aged 8 to 16 weeks were divided into seven groups of ten rats each, including five males and five females. Various doses of the aqueous extract (200 mg/kg, 400 mg/kg, 800 mg/kg, and 1600 mg/kg) were administered in comparison with a positive control group and a group treated with Ranferon®. Treatments were administered from day 3 to day 14, with blood samples taken on days 1, 3, 7, and 14. Anemia was induced by injecting 40 mg/kg of phenylhydrazine twice a day for two days. The samples allowed for the determination of red blood cell count, hemoglobin levels, hematocrit levels, mean corpuscular volume, mean corpuscular hemoglobin concentration, and platelet count. The results showed a correction of anemia by the aqueous extract of *Moringa oleifera* leaves from the seventh day of the experiment, unlike the positive control group.

Keywords: Anemia, *Moringa oleifera*, Hematological parameters, phenylhydrazine, Wistar rats.

INTRODUCTION

Anemia is the most widespread nutritional deficiency disorder globally, affecting approximately one-third of the world's population. Iron deficiency accounts for nearly half of all anemia cases worldwide (Arinda *et al*., 2022). In sub-Saharan Africa, the prevalence of anemia is 72.4% (60.2-87.8%). This alarming situation has led the World Health Organization (WHO) to classify this condition among the priority diseases in Africa (Diouf *et al*., 2015). In Côte d'Ivoire, the general trend remains equally concerning. According to a study by Bléyéré *et al*., (2014) in three communes of Abidjan, the prevalence of anemia among pregnant and non-pregnant adolescents aged 15 to 19 years was 77.7% and 42.7%, respectively. Another study by Kokoré *et al*., (2013) showed that the prevalence of anemia among schoolchildren aged 5 to 11 years was 14.2% in boys and 16.1% in girls in Abidjan. Anemia is therefore both a nutritional and pathological problem. Despite numerous efforts to eradicate this disease, it remains a serious public health issue. The WHO ranks it among the ten most serious health problems in the world (Zinebi *et al*., 2017). Consequently, many people, particularly in rural areas of developing countries, turn to alternative medicinal plants like *Moringa oleifera*, which is widely accepted, accessible, cheaper, and supposedly has fewer side effects (Obi *et al*., 2018). Several studies have highlighted the exceptional nutritional qualities of *M. oleifera* leaves used in food due to their richness in proteins, vitamins, and minerals. It has been reported that *M. oleifera* leaves are a rich source of β-carotene, proteins, vitamin C, calcium, and potassium. They act as a good source of natural antioxidants and can improve the shelf life of fat-containing foods thanks to various antioxidant compounds such as ascorbic acid, flavonoids, phenolic compounds, and carotenoids (Rabeh *et al*., 2021).

This study aims to explore the effectiveness of the total extract of *M. oleifera* leaves in treating anemia induced in rats over two weeks. By examining the evolution of anthropometric and hematological parameters, this research aims to provide scientific evidence on the therapeutic benefits of *M. oleifera*, thus contributing to the search for natural and affordable solutions to combat anemia.

MATERIALS AND METHODS

Plant Material

M. oleifera leaves used in this experiment were harvested in the city of Bouaké in Côte d'Ivoire and dried in the Laboratory of Physiology, Pharmacology, and Pharmacopoeia at the University Nangui ABROGOUA under constant air conditioning set at 25°C for 72 hours. After drying, the leaves were powdered using a mortar.

Animal Material

The animal material consisted of Wistar rats (*Rattus norvegicus*) weighing between 116 and 170 g and aged 8 to 16 weeks. These rats were provided by the animal facility of the École Normale Supérieure d'Abidjan (ENS) and that of the University Nangui ABROGOUA (UNA).

Method

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

This preparation was made according to the modified method of Guédé-Guina *et al*., (1993). It involved dissolving 150 g of powdered dried *M. oleifera* leaves in a 500 ml Erlenmeyer flask with distilled water. The solution was macerated under magnetic agitation for 24 hours. The macerate underwent double filtration through cotton and Wattman No.1 filter paper to exhaust the chemical constituents contained in the *M. oleifera* leaf powder. The filtrate obtained was subjected to evaporation in an oven at 45°C, resulting in a powder that was weighed and stored in a refrigerator. The different doses of the total aqueous extract tested during the experiment were prepared extemporaneously by dissolving the obtained powder in distilled water.

Anemia Induction

Anemia was induced using phenylhydrazine hydrochloride (PHZ) over two days according to the protocol of Gbenou *et al*., (2006). The induction was done from day 1 to day 2 of the experiment. The PHZ was administered intraperitoneally using a 1 ml syringe. The dose injected into each rat was 40 mg/kg of body weight (bw), and the volume injected was determined based on the animal's mass.

RESULTS AND DISCUSSION

Changes of weight during anemia induction

The administration of PHZ hydrochloride induced a significant decrease in the weight of rats in all experimental groups. Table I shows the body weight values on the first day, which were 120±7.2, 116±7.0, 118.2±9.7, 170±6.6, 145±6.3, and 156±5.9 for the positive control, reference control (Ranféron®), and D 200 mg/kg to D 1600 mg/kg groups, respectively. These values decreased to 96.3 ±7.9 (p=0.039), 98±12.3 (p=0.035), 90±4.5 (p=0.025), 140±8.3 (p=0.018), 121±4.9 (p=0.016), and 138±4.3 (p=0.034), respectively. These decreases are represented by the percentage decreases of 19.75% for the positive control, 15.51% for the reference control, and 23.85%, 17.64%, 16.55%, and 11.53% for the D 200 mg/kg to D 1600 mg/kg groups.

Effect of treatments on body weight of rats at day 7

Table II shows the weight evolution of the rats in different groups during the first week of treatment. During this period, the weight of the rats in the groups treated with EAfMo increased non-significantly, as did the rats in the Ranferon® group, in contrast to the positive control group where the weight decreased in the absence of treatment. These weights increased by 5.10%, 7.77%, 4.28%, 6.76%, and 5.79% for the Ranferon® group and the D 200 mg/kg to D 1600 mg/kg groups, respectively, compared to (-6.36%) for the positive control group.

Effect of EAfMo on Body Weight from Day 7 to Day 14

Table III indicates the evolution of rats' weights from day 7 to day 14 of treatment. The weights of rats in the groups treated with EAfMo and the Ranferon® group showed a non-significant increase, unlike the positive control group where weight decreased significantly. This decrease was significant in the D 1600 mg/kg group.

Table I: Effect of PHZ on Weight from day 1 to day 3

Control and Experimental Groups								
	Periods	Control +	Ranferon®	D 200 mg/kg	D 400 mg/kg	D 800 mg/kg	D 1600 mg/kg	P values
body weight (g)	1	120±7.2	116±7.0	118.2±9.7	170±6.6	145±6.3	156±5.9	<0.001
	3	96,3 ±7,94	98±12,32	90±4.5	140±8.3	121±4.9	138±4.3	<0.001
	P values	0,039	0,035	0.025	0.018	0.016	0.034	
Weight gain	3	-23,7±0.2 (-19.75%)	-18±0.7 (-15.51%)	-28,2±0.7 (-23,85%)	-30±0.7 (-17.64%)	-24±0.2 (-16.55%)	-18±0.3 (-11.53%)	

Table II: Effect of EAfMo on Weight from day 1 to day 3

Control and Experimental Groups								
	Periods	Control +	Ranferon®	D 200 mg/kg	D 400 mg/kg	D 800 mg/kg	D 1600 mg/kg	P values
body weight (g)	3	96.3 ±7.9	110±12.3	111±16.5	164±28.3	139,6±19,4	150±24,3	<0.001
	7	90.17±10.2	103±8.5	97±12.9	146±13.6	129.19±8.7	146±12.4	<0.001

	P values	0.630	0.583	0.621	0.715	0.466	0.556	
Weight gain	7	-6.13±2,26 (-6.36%)	5±0.8 (5.10%)	7±0,57 (7.77%)	6±0.6 (4.28%)	8.19±0.6 (6.76%)	8±0.9 (5.79%)	

Table III: Effect of EAfMo on Weight from day 7 to day 14

Control and experimental batches								
	Periods	Control +	Ranferon®	D 200 mg/kg	D 400 mg/kg	D 800 mg/kg	D 1600 mg/kg	P values
body weight (g)	7	90.17±10.2	103±8.5	97±12.9	146±13.6	129.19±8.7	146±12.4	<0.001
	14	83±7.3	119±12.1	120±10.8	181±23.9	153±12.7	169±21.5	<0.001
	P values	0.580	0.306	0.204	0.057	0.153	0.376	
Weight gain	14	-7.17±0.9 (-7.95%)	16±0.67 (15.53%)	23±0.9 (23.71%)	35±0.25 (29.97%)	23.81±0.97 (18.43%)	23±0.91 (15.75%)	

Effect of Aqueous Extract of *Moringa oleifera* Leaves on Hematological Parameters from day 3 to day 14

The administration of the aqueous extract and Ranferon® restored the red blood cell count by the seventh day of treatment in all anemic rats and in the positive control group that received no treatment after PHZ injection (Table IV).

This increase was highly significant (p<0001) in rats treated with Ranferon® at doses of 200, 800, and 1600 mg/kg bw. The counts were respectively $4.62x10^6$ ±024/µL (200 mg/kg group); $5.02x10^6$±0.12/µL (400 mg/kg); $4.78x10^6$±0.2/µL (800 mg/kg); $5.05x10^6$ ±0.2/µL (1600 mg/kg); $3.74x10^6$ ±0.59/µL (Ranferon®) and $3.03x10^6$ ±0.19/µL for the positive control group. These counts increased to 6.86±0.35 (48.48%) for the 200 mg/kg group, 6.75±0.45 (34.46%) for the 400 mg/kg group, 6,99±055 (46.23%) for the 800 mg/kg group, and 7,15±022 (43.08%) for the 1600 mg/kg group. For the Ranferon® group, the count was 6,50±0,35 (38.24%). For the positive control group, it was 5,30±040 (74.58%).

The decrease in red blood cell count induced by PHZ was significantly corrected by EAfMo at the different doses administered (p<0001).

Effect of the aqueous extract of *Moringa oleifera* leaves on hematological parameters from day 3 to day 14

Effect of treatments on red blood cell count

The administration of the aqueous extract and Ranferon® restored the red blood cell count on the seventh day of treatment in all anemic rats, including the positive control group that did not receive any treatment after PHZ injection (Table IV). This increase was highly significant (p<0.001) in rats treated with Ranferon® and at doses of 200, 800, and 1600 mg/kg of body weight. These counts were respectively 4.62×10^6 ±0.24/µL (200 mg/kg); 5.02×10^6 ±0.12/µL (400 mg/kg); 4.78×10^6 ±0.2/µL (800 mg/kg); 5.05×10^6 ±0.20/µL (1600 mg/kg); 3.74×10^6 ±0.59/µL (Ranferon®) and 3.03×10^6 ±0.19/µL for the positive control. These counts increased to 6.86 ±0.35 (48.48%) for the 200 mg/kg group, 6.75 ±0.45 (34.46%) for the 400 mg/kg group, 6.67 ±0.24 (39.53%) for the 800 mg/kg group, 7.15 ±0.02 (41.58%) for the 1600 mg/kg group, 6.42 ±0.44 (71.65%) for the Ranferon® group, and 3.92 ±0.18 (17.69%) for the positive control. There was no significant difference (p>0.05) between the different groups of rats on days 3 and 7.

Despite the administration of treatment, there was a non-significant decrease (p>0.05) in this parameter on day 14 in all rats except for those treated with Ranferon®, where the count increased non-significantly (Table VI). This decrease was highly significant (p<0.001) in the 1600 mg/kg group. There was no significant difference (p>0.05) between the different groups on day 14.

Effect of treatments on hemoglobin levels

The various treatments with the aqueous extract of *M. oleifera* leaves and Ranferon® induced a highly significant increase (p<0.001) in all experimental groups and in the positive control group, where a significant increase (p=0.046) was observed. The percentage increase was 66.85% for the 200 mg/kg group, 43.08% for the 400 mg/kg group, 53.45% for the 800 mg/kg group, 61.12% for the 1600 mg/kg group, 79.01% for the Ranferon® group, and 11.34% for the positive control. However, a highly significant difference was observed on day 7 of treatment between the different experimental groups (Table IV).

On the 14th day of treatment, Table VI indicates a decrease in all treated and untreated rats. However, this decrease was more significant (p<0.001) in the Ranferon® and 200 mg/kg groups. Also, this decrease was very significant (p=0.002) in the 1600 mg/kg group. A highly significant difference was noted when comparing the different groups.

Effect of treatments on hematocrit levels

On the 7th day of treatment, the hematocrit level increased significantly (p<0.001) in all groups. However, this increase was less significant (p<0.01) in the positive control group. Comparing the positive control group with the different experimental groups showed a highly significant difference (p<0.001) on days 3 and 7. Table VI indicates a decrease in hematocrit levels in all treated and untreated rats on the 14th day of

treatment. This decrease was highly significant ($p<0.001$) for the positive control, Ranferon®, and 1600 mg/kg groups, and very significant ($p=0.003$) in the 200 mg/kg group. The decrease was non-significant in the 400 mg/kg and 800 mg/kg groups.

Effect of treatments on mean corpuscular volume (MCV)

The administration of PHZ led to an increase in MCV in all groups on day 3. Our treatments did not inhibit this increase after one week of treatment. However, there was a highly significant increase ($p<0.001$) in the positive control group and at doses of 400 and 1600 mg/kg. This increase was less significant ($p<0.01$) in the Ranferon®, 200 mg/kg, and 800 mg/kg groups. Nonetheless, a highly significant difference ($p<0.001$) was observed between the different groups on days 3 and 7.

The treatments with our extract and Ranferon® induced a decrease in MCV on day 14 as indicated in Table VII. This decrease was highly significant ($p<0.001$) in the Ranferon® group, unlike the groups treated with the aqueous extract of *M. oleifera* leaves, where the decrease was non-significant ($p>0.05$). However, a highly significant difference ($p<0.001$) was observed when comparing the different experimental groups.

Effect of treatments on mean corpuscular hemoglobin (MCH)

Table V shows the variation of MCH from day 3 to day 7 during treatment. A slight non-significant increase ($p>0.05$) was observed on the seventh day of treatment in all groups treated with the aqueous extract and Ranferon®. Conversely, this parameter decreased non-significantly ($p>0.05$) in the positive control group. No significant difference ($p>0.05$) was noted when comparing the means of the different groups on days 3 and 7.

On the 14th day of treatment, as indicated in Table VII, MCH decreased in all treated groups, unlike in the positive control group where the level slightly increased non-significantly. This decrease was highly significant ($p<0.001$) in the Ranferon® group.

Effect of treatments on mean corpuscular hemoglobin concentration (MCHC)

Table V shows the variation of MCHC during the first week of treatment. Except for the Ranferon® and 800 mg/kg groups where a non-significant decrease ($p>0.05$) was observed, a slight non-significant increase was observed in all other groups. No significant variation ($p=0.599$) was noted when comparing the mean values of the different groups on day 7, unlike on day 3 where a highly significant difference was observed.

This rate decreased in all treated groups, unlike in the positive control group on day 14 where no significant difference was noted. MCHC decreased non-significantly ($p>0.05$) in the Ranferon® group and all other treated groups except for the 800 mg/kg group where the decrease was highly significant ($p<0.001$).

Effect of treatments on blood platelets

On the 7th day of treatment, a non-significant decrease ($p>0.05$) was recorded in all groups as indicated in Table V. After the administration of PHZ on day 3, no significant difference ($p=0.128$) was noted, nor on day 7 ($p=0.172$) between the different groups.

Table VII indicates the evolution of blood platelets on the 14th day of treatment. This table shows a highly significant decrease ($p<0.001$) in the 400 mg/kg and 800 mg/kg groups, as well as in the positive control group. Conversely, in the Ranferon®, 200 mg/kg, and 1600 mg/kg groups, a non-significant increase ($p>0.05$) in platelets was noted, and no significant difference was observed between the different groups.

Table IV: Evolution of Hematological Parameters During Treatment from Day 3 to Day 7

Parameters	Periods	Positive Control	Ranferon	200 mg/kg Group	400 mg/kg Group	800 mg/kg Group	1600 mg/kg Group	P values
Red blood cells **(10^6/µL)**	J3	3.03±0.1	3.74±0.59	4.62±0.24	5.02±0.12	4.78±0.2	5.05±0.2	0.117
	J7	3.92±0.18 (29.37%)	6.42±0.44 (71.65%)	6.86±0.35 (48.48%)	6.75±0.45 (34.46%)	6.67±0.24 (39.53%)	7.15±0.02 (41.58%)	0.701
	P values	0.481	0.001	<0.001	0.413	<0.001	<0.001	
Hemoglobin (g/dL)	J3	9.7±0.15	8.1±0.44	9.05±0.35	9.40±0.2	8.96±0.46	9.03±0.32	0.051
	J7	10.8±0.46 (11.34%)	14.5±1.91 (79.01%)	15.1±0.05 (66.85%)	13.45±0.05 (43.08%)	13.75±0.15 (53.45%)	14.55±0.05 (61.12%)	<0.001
	P values	0.046	<0.001	<0.001	<0.001	<0.001	<0.001	
Hematocrit (%)	J3	33.6±1.82	24.97±1.46	33.35±2.75	34.7±0.8	33.4±2.28	32.87±1.65	0.004
	J7	30.77±0.92 (21.23%)	51.7±1.7 (107.04%)	53.47±0.87 (60.32%)	48.6±0.9 (40.05%)	48.3±0.6 (44.61%)	51.4±0.1 (56.37%)	0.004
	P values	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	

Table V: Evolution of Erythrocyte Indices During Treatment

Parameters	Periods	Positive Control	Ranferon	200 mg/kg Group	400 mg/kg Group	800 mg/kg Group	1600 mg/kg Group	P values
MCV (fL)	J3	66.68±1.69	68.1±3.47	72.3±2.2	69.05±1.95	70.03±5.5	65.33±4.97	0.083
	J7	71.93±2.9 (7.87%)	79.63±2.72 (16.93%)	78.13±2.72 (8.06%)	72.2±3.5 (4.56%)	72.5±1.7 (3.52%)	72.3±0.4 (10.66%)	<0.001
	P values	<0.001	0.039	0.018	0.001	0.042	<0.001	
MHC (pg)	J3	21.27±0.75	21.97±2.51	19.6±0.3	18.7±0.5	18.77±1.14	17.93±0.88	<0.001
	J7	20.43±0.57 (-3.94%)	22.5±1.82 (2.41%)	22.13±1.01 (12.9%)	20±1.3 (6.95%)	20.65±0.55 (10.01%)	20.9±1.4 (16.56%)	0.132
	P values	0.394	0.215	0.072	0.373	0.168	0.103	
MCHC (g/dL)	J3	29.13±0.98	32.53±0.71	27.15±1.15	27.05±0.05	26.9±0.52	27.57±0.73	0.012
	J7	28.37±0.52 (-2.6%)	28.37±0.12 (-12.78%)	28.27±0.35 (4.12%)	27.7±0.4 (2.4%)	28.45±0.65 (5.76%)	28.35±0.15 (2.82%)	0.599
	P values	0.509	0.071	0.373	0.138	0.092	0.320	
PLT (10^3/µL)	J3	855±142.9	1070.33±169.3	777±108	812±31	1128±84.35	714.7±143.4	0.128
	J7	593.3±104.4 (-30.6%)	680.33±28.99 (-36.43%)	671.0±96.67 (-13.64%)	741±66 (-8.74%)	726.5±59.5 (-35.59%)	548.5±26.5 (-23.25%)	0.329
	P values	0.215	0.405	0.151	0.157	0.072	0.098	

Legend: MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration
PLT: Platelet

Table VI: Evolution of Hematological Parameters During Treatment from day 7 to day 14

Parameters	Periods	Positive Control	Ranferon	200 mg/kg Group	400 mg/kg Group	800 mg/kg Group	1600 mg/kg Group	P values
Red blood cells (10^6/µL)	J7	3.92±0.18	6.42±0.44	6.86±0.35	6.75±0.45	6.67±0.24	7.15±0.02	0.701
	J14	4.11±0.13 (4.84%)	6.7±0.24 (4.36%)	6.29±0.48 (-8.30%)	6.68±0.07 (-1.03%)	6.65±0.08 (-0.29%)	5.99±0.12 (-16.22%)	0.576
	P values	0.127	0.589	0.360	0.881	0.939	<0.001	
Hemoglobin (g/dL)	J7	10.8±0.46	14.5±1.91	15.1±0.05	13.45±0.05	13.75±0.15	14.55±0.05	<0.001
	J14	10.4±0.18 (-3.7%)	12.2±0.04 (-15.86%)	13.1±0.3 (-13.24%)	12.5±0.4 (-7.06%)	12.9±0.4 (-6.18%)	12.4±0.5 (-14.77%)	<0.001
	P values	0.176	<0.001	<0.001	0.072	0.075	0.002	
Hematocrit (%)	J7	40.77±0.92	51.7±1.70	53.47±0.87	48.6±0.9	48.3±0.6	51.4±0.1	0.004
	J14	36.57±0.16 (-10.30%)	44.6±0.38 (-13.73%)	47.65±1.25 (-10.88%)	46.75±0.35 (-3.80%)	47.8±1.2 (-1.03%)	43.95±0.9 (-14.49%)	<0.001
	P values	<0.001	<0.001	0.003	0.084	0.717	<0.001	

Table VII: Evolution of Erythrocyte Indices During Treatment from day 7 to day 14

Parameters	Periods	Positive Control	Ranferon	200 mg/kg Group	400 mg/kg Group	800 mg/kg Group	1600 mg/kg Group	P values
MCV (fL)	J7	71.93±0.9	79.63±0.72	78.13±0.72	72.2±0.5	72.5±1.7	72.3±0.4	<0.001
	J14	70.33±0.65 (-2.22%)	65.8±0.35 (-17.36%)	76±0.9 (-2.72%)	70±1.3 (-3.04%)	71.8±0.9 (-0.96%)	73.4±3.1 (1.52%)	<0.001
	P values	0.743	<0.001	0.664	0.569	0.723	0.732	
MHC (pg)	J7	20.43±0.57	22.5±1.82	22.13±1.01	20±1.3	20.65±0.55	20.9±1.4	0.132
	J14	20.5±0.78 (0.34%)	18.3±0.08 (-18.66%)	20.9±1.1 (-5.55%)	18.7±0.8 (-6.5%)	19.35±0.35 (-6.29%)	20.7±1.3 (-0.95%)	0.159
	P values	0.944	<0.001	0.431	0.414	0.074	0.886	
MCHC (g/dL)	J7	28.37±0.52	28.37±0.12	28.27±0.35	27.7±0.4	28.45±0.05	28.35±0.15	0.599
	J14	29.3±1.37 (3.27%)	27.8±0.11 (-2%)	27.5±0.1 (-2.72%)	26.75±0.65 (-3.42%)	26.95±0.15 (-5.27%)	28.2±0.5 (-0.52%)	0.112
	P values	0.542	0.034	0.060	0.242	<0.001	0.780	
PLT (10^3/µL)	J7	593.3±104.4	680.33±28.99	671±96.67	741.0±66	726.5±59.5	548.5±26.5	0.329
	J14	298.7±158.4 (-49.65%)	715±29.6 (5.09%)	730±237.0 (8.79%)	421.5±143.5 (-43.11%)	424±167 (-41.63%)	633.5±149.5 (15.49%)	0.338
	P values	<0.001	0.358	0.822	0.001	<0.001	0.588	

Legend: MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration
PLT: Platelet

DISCUSSION

The results of this study revealed that the aqueous extract of *Moringa oleifera* leaves (EAfMo) possesses significant therapeutic properties for the correction of PHZ-induced anemia in rats. The administration of the extract led to a notable improvement in anthropometric and hematological parameters, suggesting its potential as an effective anti-anemic agent.

The induction of anemia in rats led to a disruption in their respiratory rate and a decrease in responsiveness compared to the healthy control group. The symptoms of anemia were visibly apparent, with pale eyes, tails, and ears. The condition of the positive control group deteriorated further in the absence of anti-anemic treatment. However, the administration of EAfMo progressively corrected the body weight loss observed post-induction of anemia. Notably, rats treated with the highest doses of EAfMo exhibited a rapid recovery in body weight, indicating an overall health improvement and a positive response to the treatment. This effect can be attributed to the nutritional properties of *M. oleifera*, which compensates for the nutritional deficits caused by anemia. These findings are consistent with those reported by Tété-Bénissan *et al*., (2013), who found that regular consumption of *M. oleifera* leaves increased weight and height in malnourished children, irrespective of HIV infection status. Similarly, studies in ruminants (Mendieta-Araica *et al*., 2011) have shown that dietary supplementation with *M. oleifera* positively affects weight, growth, and milk production in animals. Additionally, *M. oleifera* is rich in proteins and essential nutrients, which contribute to the overall health improvement observed in anemic rats, as highlighted by Magagi *et al*., (2022).

Intraperitoneal administration of PHZ resulted in a significant reduction in red blood cell count, hemoglobin levels, and hematocrit, all indicative of anemia. PHZ is known to induce hemolytic anemia, characterized by elevated mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), consistent with findings by Singh *et al*., (2014). PHZ is a non-immunogenic chemical that causes hemolytic anemia by selectively destroying mature red blood cells through oxidative stress (Adebayo *et al.*, 2017). Gheith (2018) also demonstrated that intraperitoneal injections of PHZ at a dose of 40 mg/kg over two consecutive days effectively induced an anemic state in rats.

Hematological analyses revealed a significant increase in red blood cell count, hemoglobin levels, and hematocrit in rats treated with various doses of EAfMo. These improvements were comparable to, or even exceeded, those observed in the control group treated with Ranféron®. This suggests that *Moringa oleifera* may stimulate red blood cell production and enhance hemoglobin concentration, likely due to its richness in essential nutrients such as iron, vitamins, and amino acids. These results corroborate the findings of Ahouansou (2009), who highlighted the role of *M. oleifera* in correcting hematological anomalies during nutritional recovery in malnourished subjects.

The Ranféron®-treated control group exhibited similar results to those treated with high doses of EAfMo. However, *Moringa oleifera* extract presents the added advantages of being a natural and potentially cost-effective solution. Moreover, the absence of notable side effects in EAfMo-treated rats underscores its potential as a viable therapeutic alternative.

The anti-anemic effects of *Moringa oleifera* may be attributed to multiple mechanisms. Beyond its iron content, the bioactive compounds in the leaves, such as flavonoids, phenols, and amino acids, likely play a crucial role in stimulating erythropoiesis. Atakpama *et al*., (2014) reported that *Moringa* leaves are rich in iron, which promotes red blood cell formation and increases hemoglobin levels, thereby aiding in the correction of PHZ-induced anemia. Additionally, the antioxidant properties of *Moringa oleifera* may help protect erythrocytes from oxidative stress, promoting their survival and function.

The treatments administered in this study successfully restored red blood cell count, hemoglobin levels, and hematocrit in all anemic rats, in contrast to the positive control group. Since PHZ induces anemia through the oxidative denaturation of hemoglobin initiated by free radicals, the antioxidant potential of *Moringa oleifera* leaves is likely responsible for this recovery. These findings are in agreement with those of Lee *et al*., (2014), who studied the anti-anemic activity of Raktavardhak Kadha, a plant composition rich in antioxidants and iron, and established its capacity for regeneration due to its antioxidant power. The anti-anemic potential of *Moringa oleifera* leaves, rich in micronutrients essential for effective hematopoiesis, is further supported by the observed increases in hemoglobin concentration, which reflects real-time iron supply to erythropoiesis and the quality of newly produced cells.

CONCLUSION

This study highlighted the therapeutic effects of *Moringa oleifera* leaves in rats made anemic by phenylhydrazine (PHZ) injection. Initially, a significant weight loss was observed in the anemic rats. However, under the influence of the aqueous extract of *M. oleifera* leaves, a notable improvement in their weight was observed. Additionally, the nutritional properties and stimulating effects of *M. oleifera* on hematopoiesis significantly restored the hematological parameters disrupted by PHZ induction. These results suggest that *M. oleifera* leaves could constitute a promising therpeutic option for combating anemia and weight loss,

especially in developing countries where access to conventional treatments is often limited.

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