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**Biosciences** 

## Curative Effect of Total Moringa oleifera Leaf Extract on Reticulocyte **Parameters in Anemic Rats**

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#### Abstract

#### **Original Research Article**

This study aims to evaluate the evolution of reticulocyte parameters in rats made anemic and treated with an aqueous extract of Moringa oleifera leaves. A total of 70 rats aged 8 to 16 weeks were divided into seven groups of ten rats each, composed equally of males and females. Anemia was induced by intraperitoneal injection of 40 mg/kg body weight of phenylhydrazine (PHZ), administered twice a day for two days. Different doses of the aqueous extract (200 mg/kg, 400 mg/kg, 800 mg/kg, and 1600 mg/kg body weight) were administered, compared to a positive control group and a group treated with Ranferon<sup>®</sup>. Treatments were administered from day 3 to day 14, with blood samples taken on days 1, 3, 7, and 14. The reticulocyte parameters evaluated include reticulocyte count, immature reticulocyte fraction (IRF), reticulocyte hemoglobin content (Ret-He), reticulocyte production index (RPI), or corrected reticulocyte count (CRC). The results indicate significant correction of anemia by the aqueous extract of Moringa oleifera leaves as early as the seventh day of the experiment. The mean hemoglobin level of rats treated with the Moringa extract was  $14.21\pm0.07$ g/dL, and the mean reticulocyte rate was  $4.27\pm0.30$  %, unlike the positive control, which had a hemoglobin level of  $10.8\pm0.46$  g/dL and a reticulocyte rate of  $8.76\pm0.39$  %.

Keywords: Moringa oleifera, Anemia, Reticulocytes, Phenylhydrazine, Curative Treatment.

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### **INTRODUCTION**

Anemia is defined as a pathological condition in which hemoglobin concentration is insufficient to meet the body's physiological needs due to a reduced number of red blood cells (Ndiaye et al., 2013). It is a widespread nutritional deficiency affecting approximately a third of global population (Arinda et al., 2022). In sub-Saharan Africa, the prevalence of anemia is alarming, reaching 72.4 % according to some studies. This situation has led the World Health Organization (WHO) to classify this condition among the priority diseases in Africa (Diouf et al., 2015). WHO and the Food and Agriculture Organization (FAO) estimate that globally, 50% of anemia cases are due to iron deficiency (WHO and FAO, 2011). In Côte d'Ivoire, the situation is equally concerning, with high rates among adolescents and schoolchildren. This disorder is more prevalent in developing countries where nutritional causes play an important role in the onset of anemia. For example, a study conducted by Bléyéré et al., (2014) in three communes of Abidjan reported that the prevalence of anemia among pregnant and non-pregnant adolescents aged 15 to 19 years was 77.7% and 42.7%, respectively. Therefore, anemia represents both a nutritional and pathological issue characterized by a reduction in erythrocyte mass or hemoglobin concentration in the blood, leading to a reduced oxygen transport capacity. Many efforts have been made to eradicate this disease, but it remains one of the serious public health problems, ranked among the top ten most serious health issues globally (Zinebi et al., 2017).

Fighting anemia is partly based on good nutritional education, appropriate use of antianemic drugs, and recourse to phytotherapy (WHO, 2005). Medicinal plants are currently used in various regions of the world, particularly in the tropics, for treating different forms of anemia. Most vegetables and plants have been shown to contain hematinic agents such as folic acid, vitamin B6, and iron, which could stimulate the erythropoietic pathway (Adedapo et al., 2002).

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In light of this, alternative solutions such as the use of medicinal plants, particularly *Moringa oleifera*, are being explored for their potential beneficial effects (Obi *et al.*, 2018). Moringa oleifera leaves are a rich source of  $\beta$ -carotene, proteins, vitamin C, calcium, and potassium, and they serve as a good source of natural antioxidants. These compounds, such as ascorbic acid, flavonoids, phenolic compounds, and carotenoids, give Moringa oleifera leaves properties that may improve hematological health and prolong the preservation of fat-containing foods (Rabeh *et al.*, 2021). This study aims to evaluate the antianemic potential of the aqueous extract of Moringa oleifera leaves by determining reticulocyte parameters in rats made anemic by phenylhydrazine.

#### **MATERIALS AND METHODS**

#### **Plant Material**

The *Moringa oleifera* leaves used in this experiment were harvested in Bouaké, Côte d'Ivoire. The leaves were dried at the Laboratory of Physiology, Pharmacology, and Pharmacopoeia at NANGUI ABROGOUA University under constant air conditioning at 25°C for 72 hours. After drying, the leaves were ground into powder using a mortar.

#### **Animal Material**

The animal material consisted of Wistar rats (*Rattus norvegicus*) whose weight varies between 116 and 170 g and aged 8 to 16 weeks, provided by the animal facilities of the École Normale Supérieure of Abidjan and NANGUI ABROGOUA University.

#### Method

# Preparation of the total Aqueous Extract of *Moringa* oleifera leaves

The preparation of the aqueous extract followed the modified method of Guédé-Guina *et al.*, (1993). It involved dissolving 150 g of the powdered dried *Moringa oleifera* leaves in 500 ml of distilled water in an Erlenmeyer flask. The solution was left to macerate under magnetic stirring for 24 hours. The macerate was then filtered twice through cotton and Wattman No. 1 filter paper to extract the chemical components from the leaf powder. The resulting filtrate was subjected to evaporation in an oven at 45°C, yielding a powder that was weighed and stored in a refrigerator. The different doses of the total aqueous extract tested during the experiment were freshly prepared by dissolving the powder in distilled water.

#### Induction of anemia

Anemia was induced using phenylhydrazine (PHZ) hydrochloride over two days following the protocol of Gbenou *et al.*, (2006). This induction took place from day 1 to day 2 of the experiment. The PHZ was administered intraperitoneally using a 1 ml syringe. Each rat received a dose of 40 mg/kg body weight (b.w.), with the volume injected determined based on the animal's weight. The rats were considered anemic when their red blood cell count and hemoglobin content were

reduced by 30%, according to the method of Koffuor *et al.*, (2011).

#### **Statistical Analysis**

The data were analyzed using ANOVA (analysis of variance) and the Bonferroni post-hoc test, with a significance level set at p < 0.05. All results were expressed as mean  $\pm$  standard error of the mean (SEM).

## **RESULTS AND DISCUSSION**

#### Anemia Induction

Figures 1 and 2 illustrate the variation of certain erythrocyte and reticulocyte parameters before and after the administration of phenylhydrazine hydrochloride (PHZ). The PHZ injection resulted in a highly significant reduction (p<0.001) in the number of red blood cells, hemoglobin levels, and hematocrit in all experimental rat groups compared to the healthy control group. By day 3, following the induction of anemia, there was a significant increase in the reticulocyte count (Ret), immature reticulocyte fraction (IRF), and reticulocyte hemoglobin content (Ret-He) in PHZ-treated rats compared to the healthy controls.

The administration of PHZ also led to a significant increase (p<0.001) in the reticulocyte production index (RPI) by day 3, along with an increase in serum iron concentration when compared to the healthy control group.

#### Effect of the Aqueous Extract of Moringa oleifera Leaves on Reticulocyte Parameters from Day 3 to Day 7

#### **Effect on Reticulocyte Count**

The induction of hemolytic anemia by PHZ caused a highly significant increase (p<0.001) in the reticulocyte count in all experimental groups by day 3. After treatment with the aqueous extract of Moringa oleifera and Ranféron®, a significant decrease (p<0.001) in the reticulocyte count was observed by day 7, although this reduction was less pronounced in the positive control group. The group treated with the 200 mg/kg dose showed a more pronounced reduction than the other groups, suggesting a dose-dependent efficacy in correcting anemia. A highly significant difference (p<0.001) was observed between the different experimental groups on both day 3 and day 7 (Table I).

#### Effect on Immature Reticulocyte Fraction (IRF)

Table I indicates the variation in the immature reticulocyte fraction from day 3 to day 7 of treatment. The IRF increased on day 3 and then significantly decreased (p<0.001) by day 7 in all treated groups. This decrease was more marked in the groups receiving doses of 200 mg/kg and 1600 mg/kg, demonstrating a progressive improvement in erythropoiesis and reticulocyte maturation compared to the positive control group, where the reduction in IRF was less significant (Table I).

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#### **Effect on Reticulocyte Hemoglobin Content (Ret-He)**

Table I shows the evolution of Ret-He. The results for reticulocyte hemoglobin content indicated a highly significant increase (p<0.001) in the group treated with the 200 mg/kg dose \$of the extract, indicating effective restoration of erythropoietic function. A slight increase was recorded from day 3 to day 7 in the groups treated with 400 and 800 mg/kg of body weight. However, a non-significant decrease was observed in the group treated with the 1600 mg/kg dose.

#### Variation in Reticulocyte Production Index (RPI) **During Treatment**

A highly significant decrease (p<0.001) in RPI was observed in all groups by day 7, reflecting a reduction in reticulocyte production in response to the progressive correction of anemia. However, this decrease was less pronounced in rats treated with Ranferon®, suggesting slower recovery compared to the Moringa oleifera extract.

#### Variation in Serum Iron Concentration During Treatment

During the initial treatment period, serum iron concentration decreased in all treated rats as well as in the positive control group. This decrease was highly significant (p<0.001) across all groups (Table I).

Table 1: Reticulocyte parameters during treatment from day 3 to day 7												
Settings Periods		Positive	Ranferon	D1 (200	D2 (400	D3 (800	D4 (1600	P				
		witness	R	mg/kg)	mg/kg)	mg/kg)	mg/kg)	values				
Reticulocytes (%)	J3	$21.05 \pm 5.07$	24.30±1.92	37.65±0.50	23.98±3.99	23.24±8.75	23.91±6.29	< 0.001				
	J7	8.76±0.39	3.83±2.21	3.74±0.13	4.47±0.31	3.85±0.14	5.02±0.64	< 0.001				
		(-58.38%)	(-84.23%)	(-90.06%)	(-81.35%)	(-83.43%)	(-79.00%)					
	P values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001					
IRF (%)	J3	50.75±1.05	62.33±2.03	71.70±0.47	52.20±2.40	50.53±1.42	60.48±3.88	< 0.001				
	J7	34.80±1.09	35.70±1.49	35.43±2.53	31.55±3.25	34.75±5.75	34.30±1.00	0.769				
		(-31.42%)	(-42.72%)	(-50.58%)	(-39.55%)	(-31.22%)	(-43.28%)					
	P values	0.002	< 0.001	< 0.001	0.001	< 0.001	< 0.001					
Ret -He	J3	20.80±0.20	19.07±1.06	18.65±0.05	19.55±0.55	19.17±0.65	20.04±0.25	< 0.001				
	J7	19.70±0.12	16.70±0.14	21.40±0.49	19.60±0.60	19.85±0.35	19.75±0.45	< 0.001				
		(-5.28%)	(-12.42%)	(14.74%)	(0.25%)	(3.54%)	(-1.44%)					
	P values	0.158	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001					
RPI	J3	11.40±0.73	6.70±0.38	18.60±0.28	12.30±0.12	11.50±0.92	11.60±0.49	< 0.001				
	J7	$4.00\pm0.59$	4.40±0.42	4.44±0.39	4.82±0.76	4.13±0.23	5.73±0.39	< 0.001				
		(-66.58%)	(-25.37%)	(-84.08%)	(-73.93%)	(-76.04%)	(-67.18%)					
	P values	< 0.001	0.061	< 0.001	< 0.001	< 0.001	< 0.001					
Iron (µg/L)	J3	2.30±0.14	2.61±0.33	2.32±0.4	3.01±0.11	$2.85 \pm 0.48$	3.17±0.51	0.122				
	J7	1.64±0.12	1.44±0.24	$1.50\pm0.14$	1.31±0.33	1.29±0.30	1.34±0.29	0.073				
		(-28.73%)	(-44.70%)	(-35.37%)	(-56.60%)	(-54.63%)	(-57.66%)					
	P values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001					

Table I: Reticulo	yte	paramete	ers during	tre	atment	from	day	3 to day	7

#### Effect of the Aqueous Extract of Moringa oleifera Leaves on Erythrocyte and Reticulocyte Parameters from Day 7 to Day 14

#### **Effect on Red Blood Cell Count**

When rats were subjected to the various treatments, there was a non-significant decrease (p>0.05) in red blood cell count by day 14 in most groups, except for the 1600 mg/kg group where the decrease was highly significant (p=0.002). However, the rats treated with Ranferon® showed a non-significant increase in their red blood cell count (Figure 3-A). No significant difference (p>0.05) was observed between the different groups from day 7 to day 14.

#### **Effect on Hemoglobin Levels**

Figure 3-B shows a decrease in hemoglobin levels in all rats, both treated and untreated, by day 14. However, this decrease was more significant (p<0.001)in the Ranferon® and 200 mg/kg groups. In the 1600 mg/kg group, this decrease was highly significant (p=0.002). A highly significant difference was recorded when comparing the different groups.

#### Effect on Hematocrit Levels

Figure 3-C shows a decrease in hematocrit levels in all treated and untreated rat groups from day 7 to day 14 of treatment. This decrease was highly significant (p=0.003) in the positive control and Ranferon® groups. In the 200 mg/kg group, the decrease was highly significant (p=0.004) and very significant in the 1600 mg/kg group. A highly significant difference (p<0.001) was observed between the different groups.

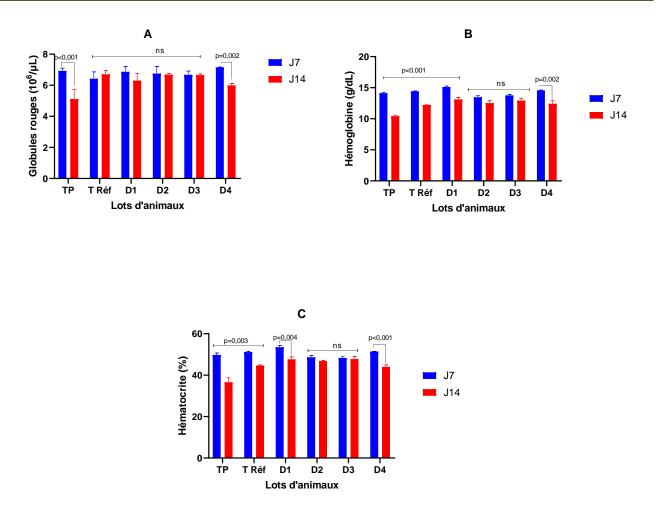


Figure 3: Variations of some erythrocyte parameters during treatment of anemia from day 7 to day 14 TP: positive control; T Ref: reference control (ranferon-12); D1: dose 1 (200 mg/kg); D2: dose 2 (400 mg/kg); D3: dose 3 (800 mg/kg); D4: dose 4 (1600 mg/kg)

#### Effect of the Aqueous Extract of Moringa oleifera Leaves on Reticulocyte Parameters from Day 7 to Day 14

#### **Effect on Reticulocyte Count**

The reticulocyte count increased in all groups treated with *Moringa oleifera* and in the positive control (TP) group. This increase was non-significant (p=0.672) at the 1600 mg/kg dose and highly significant for the doses of 200 mg/kg (p=0.003) and 400 mg/kg (p<0.001) of body weight. In contrast, the reference control group showed a significant decrease (p=0.015) in its reticulocyte count, with a significant difference (p=0.004) observed when comparing the different groups (Figure 4-H).

#### Effect on the Immature Reticulocyte Fraction (IRF)

An increase in the immature reticulocyte fraction (IRF) was observed in the Ranferon® group and in the 400 mg/kg, 800 mg/kg, and 1600 mg/kg treatment groups. In contrast, the IRF significantly decreased in the positive control and 200 mg/kg groups (Figure 4-I).

#### Effect on Reticulocyte Hemoglobin Content (Ret-He)

Figure 4M shows the variation in reticulocyte hemoglobin content on the 14th day of treatment. By this day, no significant variation was observed after one week of treatment in the 400 mg/kg, 800 mg/kg, and 1600 mg/kg groups, although this parameter slightly decreased in these groups, similar to the positive control. However, a significant decrease was observed in the 200 mg/kg group (p=0.025) and in the reference control group (p=0.042) on the 14th day.

#### Variation in Reticulocyte Production Index (RPI) During Treatment

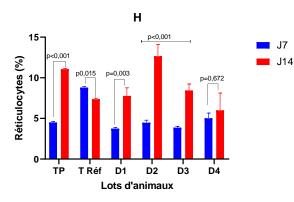
On the 14th day of treatment, the RPI significantly increased (p<0.001) in all groups treated with *Moringa oleifera*, unlike the positive control group, where the increase was not significant, as well as in the 1600 mg/kg group (p=0.838) (Figure 4-P).

#### Variation in Serum Iron Concentration

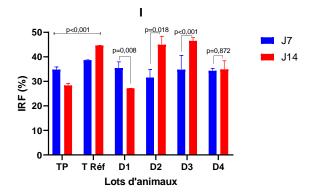
By the 14th day of treatment, no significant variation was observed in the serum iron concentration in any of the rat groups (Figure 4-O). This concentration

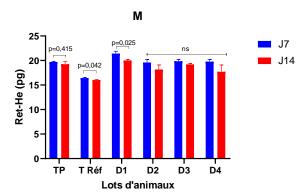
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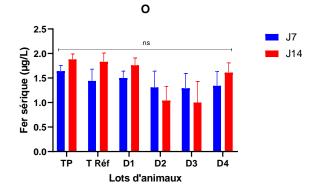
increased in the 200 mg/kg and 1600 mg/kg groups, as well as in the reference control group. However, it



slightly decreased in the 400 mg/kg and 800 mg/kg groups.







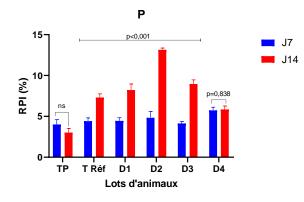


Figure 4: Variation of reticulocyte parameters and serum iron from day 7 to day 14 TP: positive control; T Ref: reference control (ranferon-12); D1: dose 1 (200 mg/kg); D2: dose 2 (400 mg/kg); D3: dose 3 (800 mg/kg); D4: dose 4 (1600 mg/kg)

#### DISCUSSION

Phenylhydrazine (PHZ) is a non-immunogenic chemical substance that induces hemolytic anemia by selectively destroying mature red blood cells through oxidative stress (Adebayo *et al.*, 2017). Reticulocytes are red blood cell precursors with an average lifespan of 1 to 2 days, making them a crucial diagnostic tool for assessing, categorizing, and monitoring the effectiveness of anemia treatment (Bashir Abdrhman, 2024). Our results show a highly significant increase in reticulocyte count, IRF, Ret-He, and RPI after the induction of anemia, which is consistent with those of Bobee *et al.*, (2018), who demonstrated that hemolytic diseases are characterized by an elevated IRF, indicating an increase in immature reticulocytes.

Recent studies, such as those by Saxena and Saxena (2023), also confirm the importance of regulating reticulocytes in the treatment of anemia, related to the effects of *Moringa oleifera* on hematopoiesis. The reticulocyte count is a good indicator of bone marrow activity, as it represents recent production and reflects the erythropoietic status of the subject, indicating whether production is normal or not. Our various treatments helped restore red blood cell count, reticulocyte rate, hemoglobin levels, and hematocrit in all anemic rats, unlike the positive control.

Given that PHZ induces anemia by oxidative denaturation of hemoglobin initiated by free radicals, the antioxidant potential of *Moringa oleifera* leaves could be responsible for restoring red blood cell count, hemoglobin levels, and hematocrit. Our results are consistent with those of Lee *et al.*, (2014), who studied the antianemic activity of Raktavardhak Kadha, a plant composition rich in antioxidant elements and iron. These authors established that their composition had regenerative properties due to its antioxidant power.

Palanisamy et al., (2022) also demonstrated that the components of Moringa oleifera play a crucial role in cellular regeneration, particularly in anemia models. This recovery is likely due to the antianemic potential of Moringa oleifera leaves, which are rich in micronutrients necessary for effective hematopoiesis. These results are confirmed by the Reticulocyte Production Index (RPI), which is an index used to evaluate erythropoiesis efficiency and bone marrow productivity in anemia cases. The elevated RPI reflects an increased erythropoietic response following PHZ-induced anemia. These results are further supported by the Ret-He, which measures iron supply to erythropoiesis in real-time and assesses the quality of newly produced cells. These effects are in line with those reported by Oyetunde et al., (2023), who highlighted the protective role of Moringa oleifera against hemolytic anemia by stimulating antioxidant and erythropoietic pathways.

#### CONCLUSION

This study highlighted the antianemic potential of the aqueous extract of Moringa oleifera leaves through the restoration of reticulocyte parameters and serum iron concentration. These parameters provide information on cellular characteristics such as size, hemoglobin concentration, or RNA content, Indeed, conducting blood counts, reticulocyte parameters, reticulocyte indices, and serum iron assays can track the real-time effectiveness of an antianemic treatment. The establishment of these parameters showed the recovery of red blood cell, hemoglobin, and hematocrit levels, as well as reticulocyte count, reticulocyte hemoglobin concentration, immature reticulocyte fractions. reticulocyte production index, and serum iron concentration in all anemic subjects compared to the positive control that received no treatment. In conclusion, the aqueous extract of Moringa oleifera leaves effectively treated experimentally induced anemia in rats.

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