

Research Article

Effects of Graded Levels of *Moringa Oleifera* Leaf-Meal In Albino Rat Diet on Some Hematological Parameters

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Abstract

This study was aimed at investigating the effects of *Moringa oleifera* leaf-meal (MOLM) on some haematological parameters of male albino rats. Sixteen (16) albino rats were randomly selected into four (4) groups; a control group (group A) and three experimental groups (groups B, group C and group D) containing four (4) animals per group. Group A was given normal diet, groups B, C and D were provided with MOLM diets containing 37.5 gm, 56 gm and 75 gm respectively. They were fed with water ad libitum daily for 21 days. At the end of the experimental period, haematological parameters (white blood cell count, packed cell volume, red blood cell count, haemoglobin count and differential white blood cells) were determined. The result showed significant differences ($p < 0.05$) in hemoglobin count, red blood cells and packed cell volume in group B while there was a significant difference ($p < 0.05$) in lymphocyte count of animals in group C and D, although all the groups were within normal range while no significant difference ($p > 0.05$) was shown in the white blood cell count when compared to the control group. In conclusion, this study revealed that MOLM possesses nutritional benefits and this was indicated by its effects on RBC, PCV, haemoglobin and lymphocyte of the animals.

Keywords: *Moringa oleifera* leave powder; Hemoglobin count; Red blood cells; Pcv and lymphocyte count

1. Introduction

Plant materials as medicinal agents were mentioned in historical documents dating back many thousands of years Rasonavivo et al. [1] and from time immemorial, humans have remained dependent on plants for medicine. From a historical reports, it shows the evident that the fascination for plants as medicine is as old as mankind. Currently, medicinal plants were reported to be used against wide arrays of health problems which include cold, cough, stomach ache, cataract, constipation and many other ailments [2]. The plant *Moringa oleifera* as one of these plants

was reported to prevent effectively, structural changes and oxidative damage in lens of rats by enhancing the activities of enzymatic anti-oxidants, reducing the level of lipid peroxidation and inhibiting the formation of free radicals or reactive oxygen species (ROS) [3].

In addition, hematological parameters (packed cell volume, white blood cell counts, differentiation of white blood cells, haemoglobin and platelets) were also reported to be positively affected by using this plant [4]. Moreover, *Moringa oleifera* was found to be of a nutritional value as it contains a number of essential vitamins like vitamins A, B complex (which include B1, B3, B6 and B7), C, D, E and K [5, 6]. However, it has been used for the treatment of high blood pressure, diarrhea, intestinal worms, colon inflammation, as a diuretic agent, skin antiseptic, Lowell [7] and to maintain blood glucose level in diabetic patients [4, 8]. Moreover, *M. oleifera* was used as antimicrobial agent, Caceres et al. [9] to treat ulcers, Pal and Sahib [10] and to enhance the immune systems against microbial and viral infections [8]. So far, most of the work on the effects of *M. oleifera* were conducted on the seeds of this plant. Thus the aim of this study was to investigate the effects of the leaves of this plant on some haematological parameters (white blood cell count, packed cell volume, red blood cell count, haemoglobin count and differential white blood cells) in male albino rats.

2. Materials and Methods

2.1 Chemicals and reagents

KOH and ethanol used were purchased from Bridge Biotech. Ilorin, Kwara State, Nigeria.

2.2 Plant collection and preparation

The plant *Moringa oleifera* fresh leaves were obtained from the compound of a house at Oke-ijetu, Osogbo. The plant was identified and the harvested moringa leaves were air-dried under a shed to obtain *Moringa oleifera* leaf powder (MOLP) which was kept in a plastic rubber until needed. The Blanch diet and premix were purchased from Blessing feed mill at Niyi Ibikunle, Osogbo, Osun state. The *Moringa oleifera* leaf powder was then mixed in different proportions per test group before administration to the animals.

2.3 Experimental animal and design

A total of sixteen (16) male albino rats weighing 155.50-165.50 g were assigned into four groups per treatment. Allocation to groups was based on initial weight of the rats and group mean weight. The rats were acclimatized for a period of three weeks, after which group A (Control) was given normal diet, B, C and D were provided with MOLM diets containing 37.5 gm, 56 gm and 75 gm respectively. They were fed with water *ad libitum* for 21 days.

2.4 Experimental diets

The basic ingredients used for the experimental (formulated) diets (per 25 kg of feed) used are shown in Table 2. Commercial vitamins and mineral premix were used solely in diet 1 (3 kg per ton of feed), diet 2 consists 37.5 gm of the vitamin-mineral premix and the other half (37.5 gm) replaced by the *Moringa oleifera* leaf meal. Diet 3 consists

19 gm of vitamin-mineral premix in diet and the 56 gm of *Moringa oleifera* leaf meal. Diet 4 contains only *Moringa oleifera* leaf meal (75 gm) as the sole source of vitamins and mineral premix.

2.5 Housing and feeding

In a moderate size wooden cage, four rats to a diet were housed together in a compartment in the cage. The rats were fed *ad libitum* maintained under normal temperature, humidity and light conditions. They were acclimatized for 21 days prior administration of the *Moringa oleifera* leave meal. The experimental diets were offered in separate feeders.

Ingredients	Quantity (Kg)	Calculated Protein %	Calculated Energy (Kcal/E/Kg)
Maize	500	5.00	1717
Wheat offals	200	3.4	374
Groundnut cake	200	3.2	528
Industrial salt	2	-	-
Bone meal	58	-	-
Limestone	40	-	-
	1000	16.60	2619

Protein and energy levels in feed were calculated using standard nutrient levels of feed ingredients (Feed Nutrient standards Pfizer Products Ltd. Lagos, 1989).

Table 1: Basic ingredients of the normal diet- dry matter (kg/ ton of feed).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Maize	12.50	12.50	12.50	12.50
Wheat offals	5.00	5.00	5.00	5.00
Groundnut cake	5.00	5.00	5.00	5.00
Industrial salt	0.50	0.50	0.50	0.50
Bone meal	1.00	1.00	1.00	1.00
Limestone	1.50	1.50	1.50	1.50
Vitamin-mineral premix (gm)	75.00	37.50	19.00	-
<i>Moringa oleifera</i> leaf powder (gm)	-	37.50	56.00	75.00
	25.58	25.58	25.58	25.58

NB: Allowances are made for losses due to milling or mixing which may be in form of dust particles.

Table 2: Basic ingredients of *Moringa oleifera* leave meal per 25 kg of Diet.

2.6 Collection of blood

At the end of the experiment, the rats were fasted overnight and sacrificed. Blood sample was collected via cardiac puncture after being dislocated at the cervical region, into heparinized sampling tubes. Samples were then stored in a refrigerator till when needed.

2.7 Hematological analysis

2.7.1 Erythrocyte Sedimentation Rate (ESR): A wintrobe tube was filled with blood sample and gently placed in an undisturbed place for 60 mins. The downward capillary of the red blood cells in it was measured per hour as ESR.

2.7.2 Packed Cell Volume (PCV): A capillary tube was filled up with blood sample, about 75% of its length and centrifuged with a micro haematocrit centrifuge at 12,000 rpm for 5 minutes.

2.7.3 Red blood cell count: 0.02 ml of blood sample was diluted with 4 ml of diluting fluid in a bijou bottle and mixed together. It was then placed counted in a counting chamber.

2.7.4 White blood cell (WBC): 0.05 ml of the blood was diluted with 0.95 ml of diluting fluid. A little portion was discharged into a counting chamber to count the cells/cubic mm.

2.7.5 Haemoglobin (Hb): 0.02 ml of the blood sample was diluted with 4 ml Drabkin's solution, mixed properly in a tube and allowed to stand for 5 mins for an observable full colour development.

2.7.6 White blood cell differential (WBC differential): Agranulocyte (lymphocytes and monocytes) and granulocytes. Granulocytes (neutrophils, eosinophils and basophils). These were identified and counted after being stained with Giemsa stain. Numbers taken were recorded.

3. Results

3.1 Hematological parameters

Parameters	Normal Range	Group A Control	Group B	Group C	Group D
PVC (%)	36 - 50	46.33 ± 4.04	47.67 ± 4.73*	44.00 ± 3.61	44.00 ± 3.00
RBC×10 ⁶ /mm ³	6.9 – 11.2	6.79 ± 0.02	8.17 ± 1.21*	7.21 ± 0.51	6.97 ± 0.42
Hb g/dL	12 - 16	15.67 ± 1.53	16.00 ± 1.73	14.67 ± 1.53	15.00 ± 1.00
WBC×10 ³ /mm ³	4 - 10	8.80 ± 5.09	6.33 ± 0.49	4.07 ± 0.70	6.30 ± 2.29
MCHC (g/dL)	32 - 36	33.79 ± 0.40	33.55 ± 0.82*	33.29 ± 0.77	34.09 ± 0.06

DIFFERENTIAL WBCs (%)	-	-	-	-	-
Neutrophils	40 -60	39.67 ± 26.27	28.67 ± 3.21*	20.67 ± 6.42	22.00 ± 8.00
Lymphocytes	65 - 85	59.00 ± 26.06	70.33 ± 1.53	76.67 ± 5.77	76.00 ± 7.55
Monocytes	2 – 8	0.33 ± 0.53	0.33 ± 0.58	2.67 ± 1.15	1.33 ± 1.53*

The results showed the haematological parameters measured, where* indicates significant difference at level p<0.05

Table 3: Effects of Graded levels of *Moringa oleifera* leaf meal on hematological parameters in albino rats.

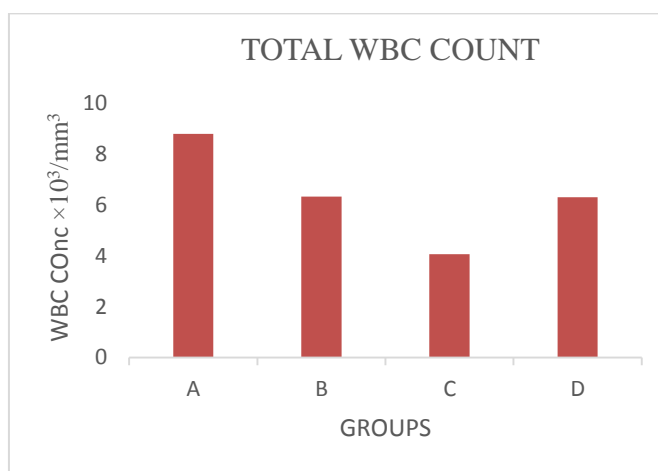


Figure 1: The effect of graded levels of *Moringa oleifera* in the total white blood cell count of the albino rats.

Compared with the control (Group A), animals treated with 37.5 gm of *Moringa oleifera* (Group B) and animals treated with 75 gm of *Moringa oleifera* (Group D) showed decrease in white blood cells and a significant decrease (p>0.05) in WBC of animals treated with 56gm of *Moringa oleifera* (Group C).

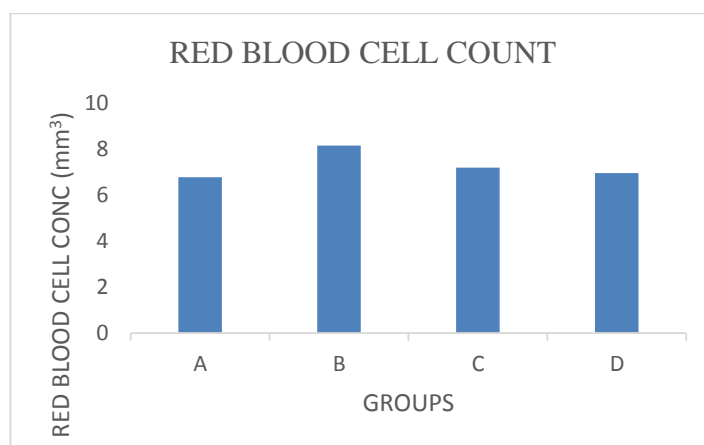


Figure 2: The effect of graded levels of *Moringa oleifera* in the Red blood cell of albino rat. Animals treated with 37.5 gm of *Moringa oleifera* (Group B) showed significant increase (p<0.05), while there was an observed slight increase in RBC of animals treated with 56 gm of *Moringa oleifera* (Group C) and animals treated with 75 gm of *Moringa oleifera* (Group D) when compared with the control (Group A).

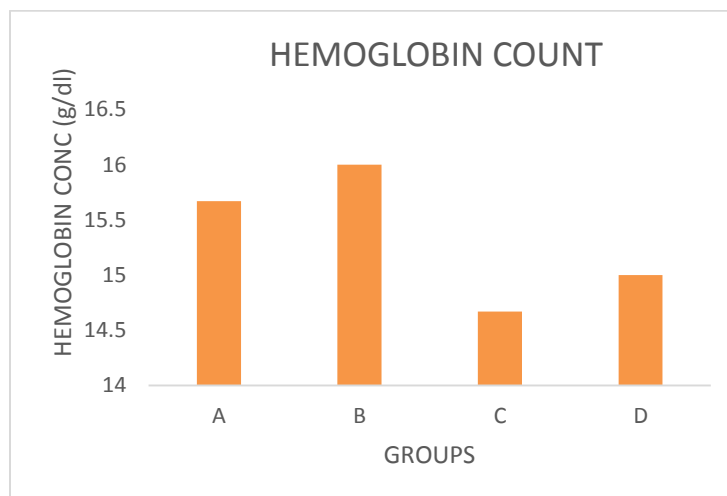


Figure 3: The effect of graded levels of *Moringa oleifera* in the hemoglobin count of albino rat. Animals treated with 37.5 gm of *Moringa oleifera* (Group B) showed a significant increase ($p < 0.05$) in hemoglobin count, while there was a significant decrease ($p > 0.05$) in animals treated with 56 gm of *Moringa oleifera* (Group C) and those treated with 75 gm of *Moringa oleifera* (Group D) respectively.

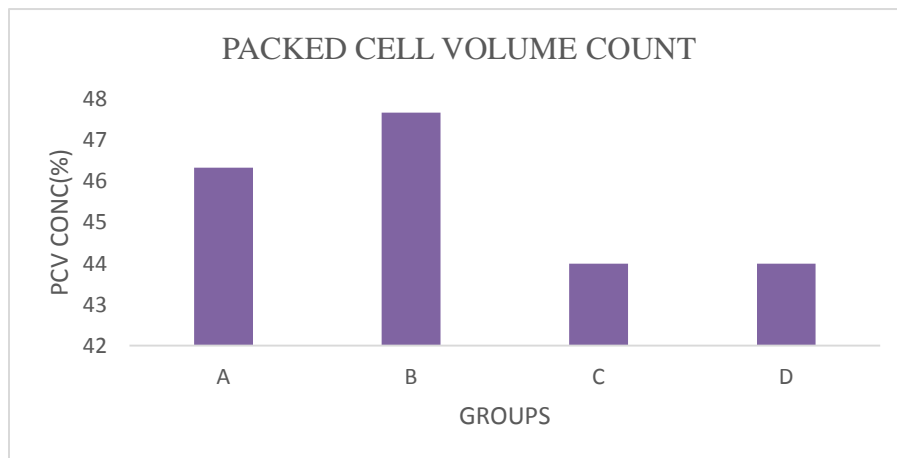


Figure 4: The effect of graded levels of *Moringa oleifera* in the packed cell volume count of Albino rat. Animals treated with 37.5 gm of *Moringa oleifera* (Group B) showed a significant increase ($p < 0.05$) in packed cell volume count, while there was a significant decrease ($p > 0.05$) in PCV count of animals treated with 56 gm of *Moringa oleifera* (Group C) and animals treated with 75 gm of *Moringa oleifera* (Group D) when compared to the control (Group A).

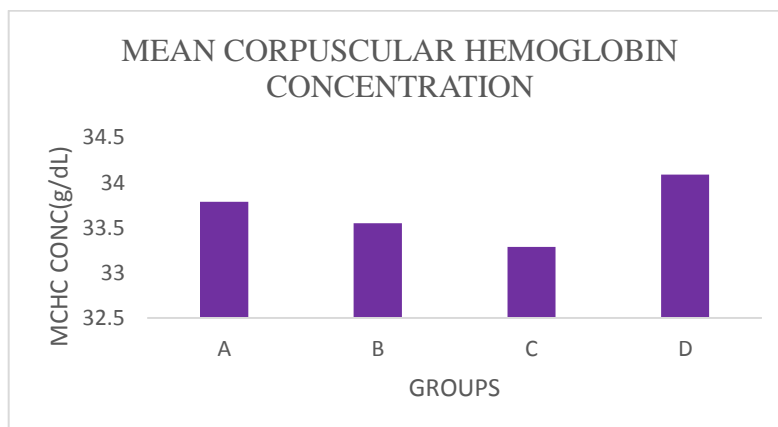


Figure 5: The effect of graded levels of *Moringa oleifera* in the mean corpuscular haemoglobin concentration (MCHC) of albino rats. Animals treated with 75 gm of *Moringa oleifera* (Group D) showed a significant increase ($p < 0.05$), while there was a significant decrease in MCHC of animals treated with 37.5 gm of *Moringa oleifera* (Group B) and animals treated with 56 gm of *Moringa oleifera* (Group C) when compared with the control (Group A).

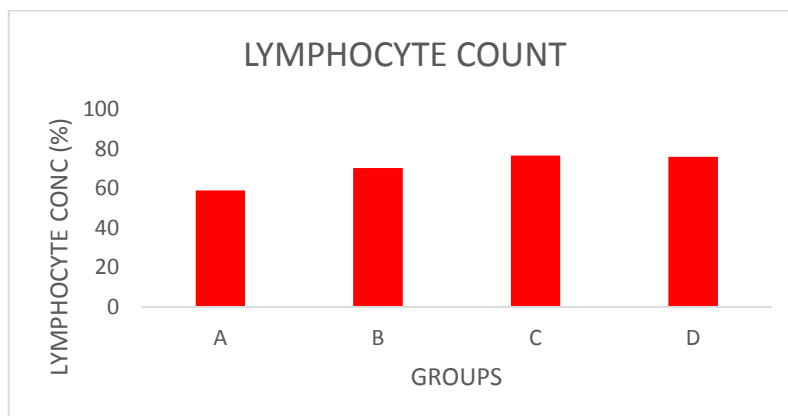


Figure 6: The effect of graded levels of *Moringa oleifera* in the lymphocyte count of albino rats. Animals treated with 37.5 gm of *Moringa oleifera* (Group B) and those treated with 56 gm of *Moringa oleifera* (Group C) and those treated with 75 gm of *Moringa oleifera* (Group D) showed significant increase ($p < 0.05$) in lymphocyte count when compared to the Control group. The increase in the lymphocyte count was with increment in the amount of the *Moringa oleifera* respectively.

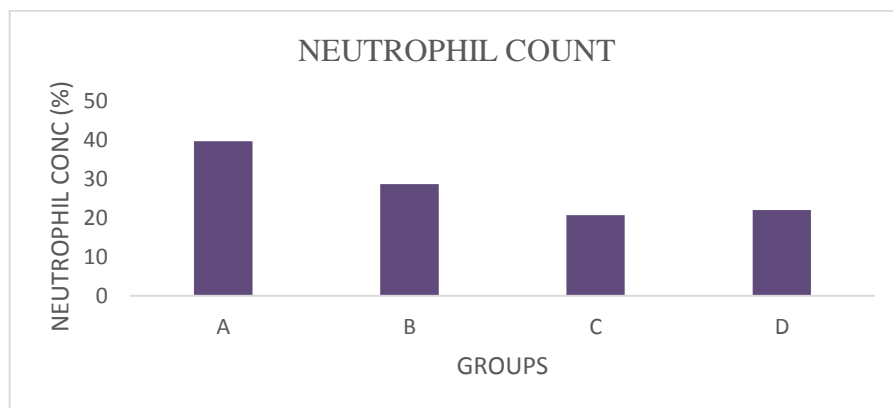


Figure 7: The effect of graded levels of *Moringa oleifera* on the Neutrophil count of Albino rats. Animals treated with *Moringa oleifera* showed a decrease in neutrophils, where animals treated with 56gm of *Moringa oleifera* (Group C) and animals treated with 75 gm of *Moringa oleifera* (Group D) showed significant decrease ($p>0.05$) when compared with the control (Group A).

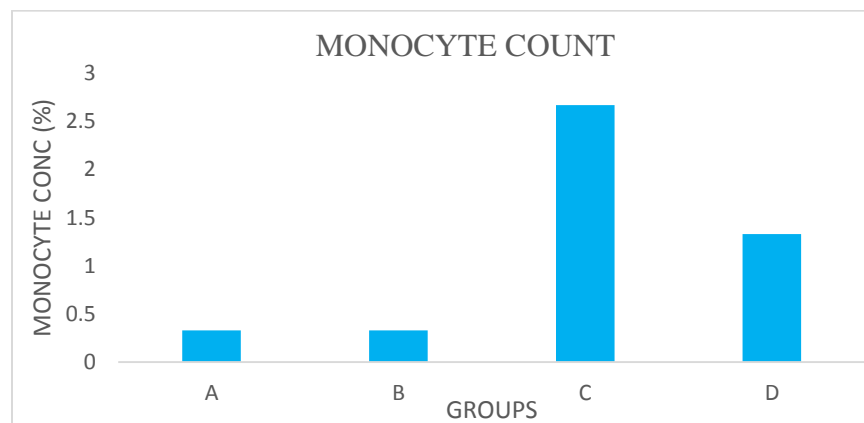


Figure 8: The effect of graded levels of *Moringa oleifera* in the monocyte count of albino rats. Animals treated with 56 gm of *Moringa oleifera* (Group C) and animal treated with 75 gm of *Moringa oleifera* (Group D) showed significant increase ($p<0.05$) in monocyte concentration when compared with the control (Group A). Animals treated with 37.5 gm of *Moringa oleifera* (Group B) normalised with that of the control (Group A).

4. Discussion

Results showed that *M. oleifera* caused a decrease in red blood cell (RBC) in all the groups to which it was administered. The result showed increase in hemoglobin (Hb) count in animals fed with 37.5 gm of *M. oleifera* only. The increase in Hb count could be that MOLM enhances blood production when consumed in small portions than large portions. This observation is in agreement with earlier work reported by Adedapo et al. [11] who reported an

increase in Hb count among rats given 400 mg/kg body weight of *M. oleifera* leave than the quantities greater than 400 mg/kg b.w. Also, Otitoju et al in his study, in which the lowest 1mg/kg body weight quantity of *M. oleifera* brought about the highest Hb count. Similarly, the result showed increase in PCV in animals fed with 37.5 gm of *M. oleifera*. This increase suggests that *Moringa Oleifera* leaves could promote blood formation in anaemic patients. This result suggests the local use of this plant as blood booster in anaemic patients.

However, the result showed a decrease in white blood cells in all the concentrations used, this may infer nutritional deficiency of vitamins B₉ and B₁₂, this observation is supported by the work reported by Fahey [12] in which *M. oleifera* nutritional properties does not include the presence of vitamin B₉ and B₁₂. However mean values of all the groups which fed on graded levels of *M. oleifera* leave meal were within the range of $4 \times 10^3/\text{mm}^3$ to $1 \times 10^4/\text{mm}^3$.

Results showed that *M. oleifera* at 37.5 gm, 56.0 gm and 75.0 gm caused increase in the level of lymphocyte count in all the test groups. This observation showed the function of differential white blood cell (Lymphocyte) to fight against invading microorganisms. This study suggests that the consumption of *M. oleifera* leaves at small quantity seems better. Lymphocytes are produced naturally by the body system in response to any foreign substances in the body. In order to avoid induction of lymphocytes, minimum quantities which are sufficient to perform the nutritional and/or therapeutic function by which it is used for must be taken into consideration during consumption. It is recommended that further studies are conducted to be sure of the best or optimum levels at which the most health and metabolic benefits can be derived.

5. Conclusion

The results of this study thus support some investigations conducted on *M. oleifera*, that it possess medicinal effects on some nutritional diseases like anaemia [13]. This was indicated in this study by the effect of the MO on RBC, PCV, haemoglobin and lymphocyte of the albino rats.

6. Suggestion

It is suggested that more research be carried out to investigate the synergistic effect of *Moringa oleifera* on haematological parameters especially using graded level to ascertain the exert dose healthy for consumption to promote desired performance characteristics.

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