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Original Research Article

Haematological Effects of Oral Administration of Aqueous Leaf Extract of *Moringa Oleifera* in Wistar Rats: Further Evidence of Immunomodulatory Potential

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ABSTRACT

Haematological effects of oral administration of aqueous leaf extract of Moringa Oleifera in male wistar rats was investigated in this study. Twenty five (25) wistar rats were used, which were divided into 5 groups of 5 rats each. Group I served as control and received water and normal feed. Group II served as positive control and received 40mg/kg levamisole (a potent immune booster). Groups III-V served as test groups and were administered with 125mg/kg, 250mg/kg and 500mg/kg of the leaf extract once a day for 28 days. Blood samples were collected through cardiac puncture for analyses of hematological parameters. The result showed significant ($p<0.05$) dose dependant increase in Total WBC count, Neutrophil count, Lymphocyte count, Monocytes count, Total lymphocyte count (TLC) and Platelets count. However, non significant increase in Eosinophil and Basophile were seen. The results therefore scientifically confirm the Immunomodulatory claim of M. Oleifera leaf extract at high dose. However, further studies that can explore its effects on other immunological parameters, such as cytokines which were not assayed in this study are recommended.

Key words: Moringa Oleifera, immunomodulation, haematology, wistar rats

INTRODUCTION

Moringa oleifera Lam (syn. *M. pterygosperma* Gaertn) is a member of *Moringaceae* family, which is a fast growing drought-resistant tree that is native to northern India. It is the most widely spread species of the family, "*Moringaceae"* Fuglie, (1999). *M. oleifera* is a little beat tall tree, with a height of 10–12 m (32-40 ft) and the trunk can reach a diameter of 45 cm (1.5 ft). The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up a feathery foliage of trip innate leaves (Parrott and John, 1993).

It has been shown that plants in general have contributed crucial task in enhancing the human quality life and also have maintained healthy life in human and animals for many decades and have used well as important components for medicines, beverages, seasonings, dyes and cosmetics. Herbal medicine which is on the basis that plants are made up of likely chemical substances that has the ability to improve health life and eliminate illness .In current times, focus on medicinal plants studies has became popular all over the

scientific world and a large number of proof has gathered to show enormous prospective of medicinal plants uses in diverse traditional practical systems. One of such plant is *Moringa oleifera Lam*, which attracts attention of the researchers worldwide owing to its medicinal and pharmacological potentials; from anti-inflammatory to anticancer abilities. It has been shown that its pharmacological potentials is due to various Phytochemical constituents resident in various parts of the plants. Several researchers have in their studies reported that the leaves contain alkaloids, flavonoids, proanthocyanidins, anthocyanins, and cinnamates (Pal *et al.*, 1995, Caceres *et al.,* 1991, Faizi *et al*., 1995).

Traditional medicine practitioners claim that some herbal preparations detoxify toxins in the body, cleanse the body of such toxins, and ultimately modulate the immune system (Spellman *et al*., 2006).

The consumption of *Moringa oleifera* leaf has also been alleged to balance or boost the energetic, soothing ability, prevent ulcer, inflammation, skin problems, pain, detoxify the blood and gastrointestinal tract, promote wound healing and ultimately enhance immune functions (Carrasco *et al*., 2009).

In Nigeria, anecdotal results indicate that leaf preparations of *Moringa oleifera* is commonly used to enhance immune function, but so far, no clear study has scientifically investigate how the leaf preparations modulate the actions of the immune system (Carrasco *et al*., 2009).

This study therefore, seeks to specifically determine the haematological effects of oral administration of aqueous leaf extract of *Moringa Oleifera* in wistar rats, with the following

Objectives:

To determine the effects of levamisole and aqueous leaf extract of *Moringa oleifera* on body weights of the rats.

To determine the effects of levamisole and aqueous leaf extract of *Moringa oleifera* on Total white blood cell count (TWBCs), Red blood cell and platelets.

To determine the effects of levamisole and aqueous leaf extract of *Moringa oleifera* on Differentials White blood cells and Total lymphocyte count.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh leaves of *Moringa oleifera* were collected in June, 2015 from a local garden in Rumuji town Emohua Local Government Area, Rivers State, Nigeria. The plant material was identified by Dr. C. Okeke of the department of plant Science and Biotechnology, University of Port Harcourt, Nigeria, with the reference number UPH/V/1214. Voucher specimen was also deposited for future reference.

Preparation of Aqueous Leaf Extract

The fresh leaves were sorted to remove any contaminant, dead matters and sand particles and were carefully washed in tap water. They were allowed to air dry for 28 days. The dry leaves were then grounded to a fine powder with the aid of domestic electric mill. 750g of the dried leaves were obtained and soaked in distilled water at room temperature. The solution was filtered into a conical flask with Watman filter paper number one. The filtrate was pulled together and lypholized using a freeze dryer. The yield of the aqueous extract was 18.22% (W/W). The lypholized extract was stored in tight container at -12°C until ready for use.

Acute Toxicity Test: Determination of LD50

The acute toxicity of *Moringa Oleifera* extract was estimated using 42 mice weighing between 50-60g. The mice were divided into 7 groups consisting of 6 mice per group. Each group was given different dosages of the extract. The number of death in each group within 24 hours was recorded. The doses were administered intraperitoneally using 1 ml disposable syringe. The LD50 was calculated using the formular of Kerber (Matselyukh etal., 2005)

Experimental Animals

25 male wistar rats weighing between 120-200g were used in this study. They were kept in animal house of Department of Physiology, University of Port Harcourt in spacious and well ventilated cages at room temperature (27±1°C) under natural dark and light cycle. The animals were allowed two weeks to acclimatize, during which they were allowed free access to rat feed.

All animals were treated according to Natural Institute of Health (NIH) guidelines for care and use of experimental laboratory animals (1985).

Experimental Design

The rats were randomly divided into five (5) groups (group's I-V) of 5 rats in each group. The rats were allowed for 14 days to fully acclimatize to the laboratory environment at the end of which they were weighed and treated as follows:

Group I this group served as standard control and received tap water only for 28 days

Group II served as positive control and received 40mg/kg levamisole from 24^{th}-28^{th} day

Groups III, IV and V served as treatment groups

Group III received 125 mg/kg body weight of extract orally once daily for 28 days

Group IV received 250mg/kg body weight of extract orally once daily for 28 days

Group V received 500mg/kg body weight of extract orally once daily for 28 days

All the animals were sacrificed on the 29^{th} day and blood collected by direct cardiac puncture into EDTA bottles. All blood samples were analysed within 2 hours of collection at room temperature.

Haematological parameters were analyzed using an automated haematological analyzer Beacon

Statistical Analysis

The results obtained were presented as mean ±SEM in tables 1-3. Statistical significance was determined using analysis of variance (ANOVA). $P<0.05$ was considered statistically significance.

RESULTS

The values for weight are as presented in table 1. Similarly, the values for Total white blood cell counts, Red blood cell and Platelets are presented in table 2. Furthermore, the values for Differential white blood cell counts (neutrophils, lymphocytes, monophils, eosinophils basophiles) and total lymphocyte count (TLC) are presented in table 3.

Table 1 shows a significant ($p<0.05$) increase in animals' body weight when group 2 (40mg/kgb.w levamisole: 165.00 ± 5.00) and group 5 (500mg/kgb.w MO:196.67 ± 3.33) were compared to the control group (156.00 ± 5.01). However, the results show a non-significant ($p>0.05$) increase in the rats' body weight when group 3 (125mg/kg b.w MO: 157.50 ± 2.50) and group 4 (250mg/kg b.w: 163.75 ± 2.39) were compared to the control group.

Table 2 shows the effects of oral administration of levamisole and aqueous leaf extract of moringa oleifera on total white blood cell (WBC) count, red blood cell (RBC) and platelets. The results reveal a significant ($p<0.05$) increase in total white blood cell count when group 2 (40 mg/kg b.w levamisole: 24.22 ± 0.97) and group 5 (500 mg/kg b.w MO: 24.55 ± 1.84) were compared to the control (20.28 ± 1.19); but a non significant ($p>0.05$) increase when group 3 (125 mg/kg b.w MO: 21.98 ± 2.82) and group 4 (250 mg/kg b.w MO:23.38 ± 2.82) were compared to the control group (20.28 ± 1.19). In the same vein, platelets count increased significantly ($p<0.05$) when group 2 (40 mg/kg b.w levamisole: 294.40 ± 41.05), group 4 (250 mgkg b.w MO: 297.50 ±5.64) and group 5 (500 mg/kg b.w MO 309.50 ± 8.21) were compared to the control (285.00 ± 47.59); but a non significant ($p>0.05$) increase when group 3 (125 mh/kg b.w MO: 280.00 ± 25.70) were compared to group 1. However, there was no significant differences in red blood cell count, when all the test groups (groups 2, 3 and 5) were compared to the control group (9.38 ± 0.18),because the increase observed in all

the test groups when compared to the control were all not significant.

Table 3 shows the effects of oral administration of levamisole and aqueous leaf extract of moringa oleifera on Differentials white blood cells (i.e. neutrophils, lymphocytes, monocytes, eosinophils, basophiles) and total lymphocyte count (TLC). The results reveal a significant ($p<0.05$) increase in neutrophils when group 2 (40mg/kgb.w levamisole: 79.40 ± 5.97) and group 5 (500mg/kg b.w MO: 74.00 ± 11.08) were compared to the control group (70.60 ± 5.29); but a non significant ($p>0.005$) increase when group 3 (125 mg/kg b.w MO: 74.00 ± 11.08) was compared to the control. At 250mg/kg b.w, neutrophils decreased non significantly relative to the control group. There was significant ($p<0.05$) increase in lymphocytes when only group 5 (500 mg/kg bw MO: 21.25 ± 1.49) was compared to the control group (18.00 ± 3.00); but no significant ($p>0.05$) increase in lymphocytes were observed when groups 2, 3 and 4 with these respective values; 19.80 ± 3.15, 20.75 ± 1.55 and 20.00 ± 3.08 were compared to the control group. Also, monocytes increased significantly ($p<0.05$) when group 2 (40 mg/kg b.w levamisole :7.00 ±0.71) and group 5 (500 mg/kg b.w MO: 7.00 ± 0.91) were compared to the control (4.50 ± 0.93); but also decreased significantly ($p<0.05$) when group 4 (250 mg/kg b.w MO: 4.50 ± 0.96) was compared to the levamisole group (an immune booster). However, non significant ($p>0.05$) increase was observed when group 3 (125 mg/kg b.w MO: 5.00 ± 0.41) was compared to the control. Furthermore, TLC showed a significant ($p<0.05$) increase when group 2 (40 mg/kg b.w levamisole: 1915.30 ± 45.09) and group 5 (500 mg/kg b.w MO) were compared to the control group (1434.88 ± 43.08); but non significant ($p>0.05$) increase when group 3 (125mg/kg b.w MO:1572:60 ± 33.33) and group 4 (250 mg/kg b.w MO: 1654.02 ± 26.22) were compared to the control group.

On the other hand, eosinophils and basophils do not show any significant increase at all concentrations (40mg/kg b.w levamisole, 125 mg/kg b.w MO, 250 mg/kg b.w MO and 500 mg/kg b.w MO) administered to the rats when compared to the control. Although both eosinophils and basophiles increased at all doses administered to the experimental rats, but these increases was not significant ($p>0.05$) at all levels.

Table 1: Weight changes following administration of levamisole and aqueous leaf extract of *Moringa Oleifera*

Groups	Weight before administration (g ± SEM)	Weight after administration (g ± SEM)
Group 1 (control)	120.0 ± 3.16	156.00 ± 5.01
Group 2 (Levamisole (40 mg/kg b.w)	121.0 ± 3.32	165.00 ± 5.00*
Group 3 (125mg/kg b.w *Moringa Oleifera* extract)	122.50 ± 2.50	157.50 ± 2.5
Group 4 (250mg/kg b.w *Moringa Oleifera* extract)	115.0 ± 2.89	163.75 ± 2.39
Group 5 (500mg/kg b.w *Moringa Oleifera* extract)	123.33 ± 3.33	196.67 ± 3.33***

All values are presented in mean ± SEM; * means values are statistically significant when compared to the control at $p\leq 0.05$. ** means values are statistically significant compared to Levamisole at $p\leq 0.05$.

Table 2: Changes in total White blood cell, Red blood cell and Platelets following administration of levamisole and aqueous leaf extract of *Moringa Oleifera*

Groups	WBC ($x 10^9$/L± sem)	RBC ($x 10^{12}$/L ± sem)	PLATELETS ($x 10^9$/L± sem)
Group 1 (control)	20.28 ± 1.19	9.38 ± 0.18	285.00 ± 47.59
Group 2 (Levamisole :40 mg/kg b.w)	24.22 ± 0.97*	9.52 ± 0.19	294.40 ± 41.05*
Group 3 (125mg/kg b.w *Moringa Oleifera* extract)	21.98 ± 1.33	9.45 ± 0.18	280.00 ± 25.70
Group 4 (250mg/kg b.w *Moringa Oleifera* extract)	23.38 ± 2.82	10.13 ± 0.17	297.50 ± 5.64*
Group 5 (500mg/kg bw *Moringa Oleifera* extract)	24.55 ± 1.84*	10.55 ± 0.26	309.50 ± 8.21*

All values are presented in mean ± SEM; * means values are statistically significant when compared to the control at p≤ 0.05. ** means values are statistically significant compared to Levamisole at p≤ 0.05

Table 3: Percentage changes in Differential White blood cell counts following administration of levamisole and aqueous leaf extract of *Moringa Oleifera*

Groups	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	Total Lymphocyte Count (TLC)
Group 1 (control)	70.60 ± 5.29	18.00 ± 3.30	4.50 ± 0.93	3.00 ± 0.95	0.80 ± 0.37	1434.88 ± 43.08
Group 2 (40mg/kg b.w Levamisole)	79.40 ± 5.97*	19.80 ± 3.15	7.00 ± 0.71*	4.00 ± 1.05	1.40 ± 0.40	1915.30± 45.09*
Group 3 (125mg/kg b.w *Moringa oleifera* extract)	74.00 ± 7.01	20.75 ± 1.55	5.00 ± 0.41	3.75 ± 0.48	1.15 ± 0.25	1572.60 ± 33.33
Group 4 (250mg/kg b.w *Moringa oleifera* extract)	68.50 ± 8.85	20.00 ± 3.08	4.50 ± 0.96**	3.50 ± 0.87	1.00 ± 0.41	1654.02 ± 26.32
Group 5 (500mg/kg b.w *Moringa oleifera* extract)	74.00 ± 11.08*	21.25 ± 1.49?*	7.00 ± 0.91*	4.25 ± 0.48	1.50 ± 0.29	1935.77 ± 21.33*

All values are presented in mean ± SEM; * means values are statistically significant when compared to the control at p≤ 0.05. ** means values are statistically significant compared to Levamisole at p≤ 0.05.

DISCUSSION

Changes in the body weight after extract administration as observed in this study have been used as valuable and growth index of *M. Oleifera* leaf as stated by (Fuglie, 1999), and thus, have been justified in this study that the reportedly dose dependant increase in the animal's body weight of Levamisole (165.0±5.00), 125mg/kg (157.50±2.50), 250mg/kg (163.75±2.39), 500mg/kg (196.67±3.33) and 500mg/kg (190.0±5.77) in this study were considered to be related to both growth and valuable index, since the increase were dose dependant and significant (p<0.05) (see table 1). This indicates that the leaf extract has growth potentials and agrees with the study conducted by Nwanjo, 2005, which confirms the growth potentials of some medicinal plants.

Some hematological parameters can be used to investigate immonomodulation. In this study Total WBC Count, Differential WBC and Platelets were evaluated.

There was significant (p<0.05) dose related increase in WBC count, Platelets level and percentage neutrophils, lymphocytes, Monocytes and total lymphocyte count (TLC) with the administration of the aqueous leaf extract of *M. Oleifera*, where as there is no significant change in Eosinophils and Basophils.

The significant increase effects of the *M. Oleifera* leaf extract on heamatological parameters of the experimental rats in Total WBC, Platelets, Neutrophils, Lymphocytes, Monocytes and TLC when compared to control, has been studied to stimulate humoral and cell mediated immunity (Jeremy *et al*., 2001) , thus, this suggest that the plant could be a good positive immunomodulator. But there no significant changes were observed in Eosinophils and Basophiles.

The aqueous leaf extract of *Moringa oleifera* boosted the total white blood cell counts in rats as displayed in (Table 2), indicating immunomodulating capacity of the leaf extract since WBCs play important role in tackling infection and clearing off

injured or dead cells and tissues in body (Jeremy *et al*., 2001). The increase in neutrophil and lymphocyte levels (Table 3) supported the result of the total white blood cell count.

The neutrophils are mainly involved in acute immunity, while lymphocytes are mainly in charge of chronic immunity (Liszewski *et al.,* 1996).

Also, the increase in neutrophils concentrations following administration of *Moringa oleifera* leaf extract (Table 3), which was dose dependant when compared to the controls suggest inflammatory tissue damage like splenomegaly, hepatic inflammation etc (Abbas *et al*., 1997).

In mammals, half the neutrophils in circulation are detectable in the blood, while the rest adhere to vessel walls as the marginating pool (*Stvrtinova* et al*., 1995*). Thus, the increase in the neutrophils count in rats administered the aqueous leaf extract of *Moringa oleifera* may suggest localized tissue inflammation that might have increased the demand for neutrophils production by the endothelial cells of the inflamed tissue.

The significant increase in lymphocytes suggests presence of lymphocytosis in the treated rats. This may be as a result of immune response of the rats to the extract, which led to the mobilization of immune competent cells. Also, increase in lymphocyte might be indicative that the plants leaves enhanced the animal's ability to wade off infection and this may account for the plants' antimicrobial activity (Abbas *et al*., 1997).

The reportedly increase in the platelets count (Table 2) suggests inflammatory tissue damage. This is because the primary function of platelets is to detect damaged blood vessel endothelium, which accumulates at the site of injury and then cause blood clotting to close the wound.

Also, they are part of adaptive immune and innate system, and plays a role in the initiation of inflammation by interacting with leukocytes, and are further involved in atherosclerosis and tumor growth. Thus this reason may be the root cause of the increased level of platelets (Topley, 1998).

This increase in the platelets count also suggests that there was no anaemic capability of the extract as suggested by the results of the RBCs count (Table 2). This Anaemia has been reported in cases of reduced number of platelets (Topley, 1998).

Moringa leaves contain significant amounts of Vitamins (A, B and C), Calcium, Ions, potassium, proteins, traces of carotenoids, saponins, alkaloids and phenolic constituents (Siddhuraju and Becker, 2003)

These constituents may be responsible for increase in the level of red blood cell and also its immunomodulatory activity. The exact mechanisms of action of MOE involved in stimulation of both the cellular and the humoral immunity is not yet clear. This may be due to an enhanced production of growth factors.

Results of the present study demonstrated that aqueous *Moringa Oleifera* leaf extract may alleviate the myelosuppression and subsequent leucopenia induced by any immune suppressant in rats.

The overall trend obtained in the parameters used for the evaluation of immunomodulatory effects of the aqueous leaf extract of *Moringa oleifera* has a clear indication that the extract is a good candidate as an immune modulator, since there is an overall increase in TWBC, Platelets, differential WBCs and TLC in this present study.

Therefore, this study established scientifically the use of the aqueous leaf extract of *Moringa oleifera* as an immune modulator in myelosuppression and subsequent leucopenia.

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