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Effect of Acute Administration of Aqueous Leaf Extract of *Moringa oleifera* on Immunoglobulin levels in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the study and performed the statistical analysis. Author OO wrote the protocol and wrote the first draft of the manuscript. Author DVD managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This present study aims to determine the effect of acute administration of aqueous extract of *Moringa oleifera* leaf on immunoglobulins in wistar rats.
Methods: Acute toxicity study of aqueous *Moringa oleifera* leaf extract was done using 24 mice divided into six (6) groups of four (4) were used. The graded doses of the extract (0.2, 0.5, 1.0, 1.5 and 2.0 i.p) corresponding to group 2, 3, 4, 5, and 6 served as test groups; group 1 received distilled water as control. Forty (40) male rats were randomly divided into 5 groups of 8 rats each. Group 1 served as control and received distilled water, while groups 2, 3, 4 and 5 served as test groups and received 20 mg/kg bw, 40 mg/kg bw, 60 mg/kg bw and 80 mg/kg bw of the extract respectively. The administration was for 14 days. At the end of administration, blood samples were collected by cardiac puncture and IgA, IgG and IgM levels were estimated by the immunoturbudrimetric method. Data were subjected to statistical analysis. A p value less than 0.05 was considered significant.
Results: The acute toxicity study showed LD_{50} of 1 g/kg. Result obtained show a significant

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($p<0.05$) reduction in the serum concentration of immunoglobulin G (IgG); and a significant ($p<0.05$) increase in the serum levels of immunoglobulins A and M (IgA and IgM) at a dose of 40 mg/kg of the extract. However, a significant ($p<0.05$) increase was observed for immunoglobulin A at 60 mg/kg when compared to the control. There were no significant changes in the immunoglobulins at the other concentrations.

Conclusion: The present study demonstrates possible beneficial therapeutic effect on the amelioration of immunological diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriasis etc in human clinical trials, especially at low concentration for immunoglobulins A and M. This finding supports the anecdotal use of leaf extracts of *Moringa oleifera* as an immune boosting agent.

Keywords: Moringa oleifera; immunoglobulins; wistar rats; immunomodulation.

1. INTRODUCTION

An immune modulator is any substance that affects directly or indirectly the immune response to external agents or therapeutics and prevents or reduces the development of degenerative diseases [1]. They have broad effects on the entire immunity system, both cell mediated immunity and humoral immunity [2]. Immune modulators achieve their effects by boosting specific areas of the immune system, most especially, the innate immunity and the activities of B lymphocytes through the action of plasma cell which secrete immunoglobulins. IgA protects the mucous membrane from attack by bacteria and viruses and infections by activating the alternative pathway of the compliment and prevent binding with of virus to epithelial cells of the respiratory, gastrointestinal and urogenital tracts. IgG crosses the placenta to give passive immunity to fetus and provides majority of antibody-based immunity against invading pathogens, while IgM eliminates pathogens in the early stages of B-cell mediated (humoral) immunity before there is sufficient IgG in the body and is also responsible for complement fixation [3].

Immunoglobulins are known to have amplifier and suppressor activities, depending on the immune status of the user [4]. However, there were no true immune modulating pharmaceutical drugs, due to their low efficacy and vast adverse effects like central obesity, hyperglycemia, osteoporosis, indiscriminate killing of all dividing cells, increasing the risk of opportunistic infections, sacrificing the "self" - "not self" regulatory mechanisms of lymphocytes [4]. Reductions in the levels immunoglobulins, especially IgA is the most common among all primary immunodeficiencies. Conditions associated with immunodeficiencies include systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriasis, etc. 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 [4].

M. oleifera, belongs to family Moringaceae, is also known as 'Horse radish' or 'Drumstick'. *Moringa oleifera* tree also known as drumstick tree is a rapid growing deciduous shrub or small tree of about 13 m tall and 35 cm in diameter with an umbrella-shaped open cap [5]. It has an impressive range of medicinal uses with high nutritional value throughout the world. *Moringa oleifera* is an important food commodity, which has enormous attention as the natural nutrition of the tropics. Native to Western and sub-Himalayan, India, Pakistan, Asia, and Africa [6,7].

It is widely cultivated throughout Tropical 'countries and Sub-Himalayan of India. Its leaves, flowers, and fruits are used as vegetables. As all the parts of plant are very nutritious so it has also been called as 'Multipurpose Tree' or 'The Miracle Tree of Life' [8].

Moringa oleifera oil and micronutrients contain antitumor, antiepileptic, antidiuretic, anti-inflajmmatory and venomous bite characteristics. *Moringa oleifera* (MoE) contains specific plant pigments which demonstrate powerful antioxidative ability such as vitamins C, E, A, caffeoylquinic acids, carotenoids - lutein, alpha-carotene and beta carotene, kaempferol, quercetin, rutin [9,10]. These activities of *Moringa oleifera* may be due to one or more constituents such as gallic tannins, catechol tannins, steroids and triterponoids, saponins, anthraquinones, alkaloids, and reducing sugars as identified by [11].

Modulation of immune responses, by various plants materials, for alleviation of diseases has

been an interesting approach since ancient time [12,13]. Herbal drugs possess immunomodulatory property and generally act by stimulating both specific and non-specific immunity [5].

In Nigeria, leaf preparations of *Moringa oleifera* is widely used in folklore for the treatment of immune system related disorders. Literatures have also shown its scientific immunomodulatory activities. Hence this study is aimed at determining the effect of the oral administration of aqueous extract of *Moringa oleifera* leaf on serum *immunoglobulins* in albino wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extracts Preparation

The *Moringa Oleifera* leaves were harvested from Mopol barrack officers' quarter, Saakpewa in Tai Local Government Area in Rivers State. The plant was botanically identified and authenticated by a botanist at the University of Port Harcourt Herbarium, Department of Plant Science and Biotechnology.

2.2 Extraction Process

The leaves of *Moringa Oleifera* were thoroughly rinsed in tap water to remove any residual dirts, dried in an air-oven at 40℃ for 14 days and then milled into fine powder. 100 g of the grinded plant material (100 g) was subsequently extracted with 1000 mL of 80% deionized water using Soxhlet apparatus. The resulting crude aqueous extract was filtered by passage through a Whatmann No. 3 filter paper followed by concentration in vacuole at 40℃ using a rotary evaporator and subsequently freeze dried. The yield of the freeze-dried sample representing the aqueous extract was calculated to be 45%.

2.3 Acute Toxicity Test (LD_{50})

The acute toxicity of aqueous *Moringa oleifera* leaf extract was determined using [14] (sawadogo et al. 2005) method. 24 albino mice divided into six (6) groups of four (4) were used. The graded doses of the extract (0.2, 0.5, 1.0, 1.5 and 2.0) corresponding to group 2, 3, 4, 5, and 6 served as test groups and were separately administered by intraperitoneal administration. The control group (group A) received distillated water (3 ml) only. All the mice were then allowed free access to food and water and their gross behavioural, neurologic, autonomic and toxic effects were observed and number of animals that died in 24 h were recorded. The LD_{50} was calculated as the geometrical means of the maximum dose producing 100% (a) and minimum dose producing 0% mortality (b) $LD_{50} = \sqrt{ab}$.

2.4 Experimental Design

Forty (40) male albino wistar rats weighing between 160 g to 200 g were procured and housed in Animal house of the Department of Human Physiology, University of Port Harcourt, Nigeria under 12-hours light/dark cycle. The rats were allowed free access to tap water and rat feed (Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria). The animals were allowed to acclimatize for 14 days before extract administration was commenced. "Principles of laboratory animals care" (NIH publication No. 85, revised 1985, were followed as well as specific national laws where applicable [15].

The animals were randomly divided into five groups comprising of eight rats per group and designated groups 1 to 5. The rats were subsequently treated as follows: Group I: Served as control and received 1.0ml of distilled water (DW); Groups 2, 3, 4 and 5 received 20. 40, 60 and 80 mg/kg bw of the extract respectively. The doses of the extract chosen were based on the result of acute toxicity (LD_{50}) study conducted in this report, which was obtained as 1 g/kg for aqueous leaf extract *Moringa oleifera*. The extract was administered orally once daily between 8 am and 9 am using a cannula attached to a 2 ml syringe.

The extract was administered for 14 days. The rats were then fasted for 12 hours and body weights determined before sacrifice using 25% urethane (ethyl carbamate) at the dose of 0.6 ml/100 g bw ip.

Blood samples were obtained through cardiac puncture for analyses of immunoglobulin A, G and M using the immunoturbidimetric Method. Determination of immunoglobulins concentration was through photometric measurement of immunocomplex.

2.5 Statistical Analysis

The results were expressed as mean of 5 replicates ± standard error of mean (SEM) and

were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. One way analysis of variance (ANOVA) was performed to test the effect of each dose on the parameter under investigation at 95% level of confidence. Values were considered statistically significant at ($p<0.05$).

3. RESULTS

The animals having received aqueous extract exhibited marked behavioural changes especially at the dose of 1.0 gram, 1.5 gram and 2.0 grams of the extract administered. Dose of 0.2 and 0.5 grams showed a stabilized behaviour. The ones that died just became weak and less active followed by gradual death. The LD_{50} of aqueous leaf extract *Moringa oleifera* was calculated as 1.5 g/kg.

Table 2 shows the mean serum levels of immunoglobulins following the administration of graded doses of *Moringa oleifera* in wistar rats. There was a significant ($p<0.05$) increase in the serum level of immunoglobulin A (IgA) following the administration of 40 mg/kg and 60 mg/kg of the extract when compared with the control. A non – significant reduction was recorded for the dose of 80 mg/kg when compared. However, there was no particular change in serum level of immunoglobulin A (IgA) when compared with the control.

A significant ($p<0.05$) reduction was recorded in the serum level of immunoglobulin G (IgG) for the 40 mg/kg administered dose when compared with the control. There was a slight decrease in the serum level of immunoglobulin G (IgG), for the dose of 60 mg/kg, though not statistically significant. However, a non-significant increase was observed for the dose of 80mg/kg administered when compared. The serum level of immunoglobulin G (IgG) was also observed to be unchanged for the dose of 20 mg/kg when compared.

A significant increase in the serum level of immunoglobulin M (IgM) was seen following the administration of 40 mg/kg of the extract when compared with the control. However, a non – significant increases in the serum level of immunoglobulin M (IgM) was recorded for the doses of 60 mg/kg and 80 mg/kg when compared with the control.

Table 1. Results of acute toxicity study of aqueous leaf extract of *Moringa oleifera* in rats

Groups	Medium	Quality in grams	Observation	Number of death (Mortality)	Lethal conce	Lethal dose	Safe dose
1	Distilled water		Normal stable	None	1 g, 1.5 g, 2 g and or greater	1.5 g	0.5 g/kg 0.2 g/kg Between and or less
2	Aqueous.	0.2	Normal stable	None			
3	Aqueous	0.5	Normal stable	None			
4	Aqueous	1.0	Restless, confused, anorexic and death	Death recorded though not significant			
5	Aqueous	1.5	Withdrawal behaviour, sluggish death	Death recorded though not significant			
6	Aqueous	2.0	Neurological deficit, slow death	High mortality rate			

Table 2. Mean serum levels of immunoglobulins following the administration of graded doses of *Moringa oleifera* in wistar rats

Groups	Treatment	Parameters		
		IgA (g/L)	IgG (g/L)	IgM (g/L)
Group 1	Control	0.25±0.16	3.25±0.16	0.75±0.16
Group 2	20 mg/kg	0.25±0.16	3.25±0.16	0.75±0.16
Group 3	40 mg/kg	1.50±0.27*	2.75±0.16*	1.38±0.32*
Group 4	60 mg/kg	2.00±0.78*	3.00±0.00	0.88±0.23
Group 5	80 mg/kg	0.57±0.20	3.71±0.29	0.86±0.14

*Values are expressed as mean ± SEM; n=5; *: Significant at p value less than 0.05*

4. DISCUSSION

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. B cell lymphocytes are responsible for antibody-mediated immunity (humoral immunity). They produce immunoglobulins (antibodies), which are proteins that bind with and neutralize specific antigens.

The effect of the aqueous extract of *Moringa oleifera* leaves on the immunoglobulins in albino wistar rats was investigated in this study. The assessment of immune parameters such as immunoglobulins is a biomarker for evaluating immune function and autoimmune conditions.

Previous reports and phytochemical screening of extracts has shown the presence of flavonoids, alkaloids, proteins, glycosides, antioxidative such as vitamins C, E, A, caffeoylquinic acids, carotenoids - lutein, alpha-carotene and beta carotene, kaempferol, quercetin, rutin [11,12,13]. The activities of *Moringa oleifera* may be due to one or more constituents such as gallic tannins, catechol tannins, steroids and triterponoids, saponins, anthraquinones, alkaloids, and reducing sugars as identified by [9].

4.1 Acute Toxicity Studies

Results of acute toxicity study tests with aqueous and ethanol extracts of *M. oleifera* leaf showed a safe range from 0.2 g to 0.5 g. The LD_{50} for the aqueous extract was 1.5 g/kg body weight. The results are in agreement with those of [16], which reported the plant leaves being relatively safe for both nutritional and medicine uses. However this study identified the lethal dose that can assist those who wish to standardize the *M. oleifera* leaf as herbal medicine.

The trend obtained in serum level of immunoglobulins A and M (IgA and IgM) in the rats administered with the aqueous leaf s extract of *Moringa oleifera* (Table 2) suggest that the aqueous leaf extract markedly enhanced the production of (IgA and IgM), has immune modulatory activities. A significant reduction in serum level of immunoglobulin G at the concentration of 40mg/kg of the extract was observed. This trend was most prominent at the concentration of 40 mg/kg of the extract. However, the higher dose of the extract showed a significant dose dependent high serum levels of Immunoglobulin A. Immunoglobulins are normally produced by B cell to regulate immune system especially humoral immunity. Their production is in response to environmental substances (molecules or microbes) that gain access into the body [17]. These substances (antigens) are recognized as foreign by antigen receptors that are expressed on the surface of T lymphocytes particular cluster of differentiation cells (CD4). Antigen binding to the T cell receptor stimulates the secretion of IL-2 and the expression of IL-2 receptors which stimulates humoral immunity.

Saponin are either triterpenoid or steroidal glycosides proven as important phytoconstituent with different pharmacological activities such as antiallergic, antiplogostic, cytotoxic antitumour, antiviral, immunomodulating, antihepatotoxic, molluscicidal and antifungal activity. Allergen-specific IgA inhibits the absorption of allergens. The association between systemic lupus erythematosus (SLE) and IgA was first described in 1962 by West et al. [18], and have shown that increased serum levels of immunoglobulins A and M (IgA and IgM) correlates to a better autoimmune disease outcome, particularly SLE. It has also been documented that autoimmune diseases, and not only systemic lupus erythrematosus, are more common in individuals with IgA deficiency [19]. Serum IgA may possess an anti – inflammatory property.

This extracts may in turn suppress the expression of allergic symptoms. Previous studies review the antibacterial activity and significant anti-inflammatory activity of leaf extract of *Moringa oleifera* [20]. This is consistent with the findings of this study, which may be implicated by the presence of phytochemicals like saponin. Role of saponins in the immunomodulating effect of the plant, lymphocyte stimulation tests were performed by [21].

Our findings suggest immunomodulatory potential of the extract of *Moringa oleifera.*

Extracts from *M. oleifera* leaves have been shown to modulate humoral immunity in rats and mice [21,22]. They have exhibited strong anti-inflammatory properties in rodent models of chemically induced inflammation of the paw [23,24]. These properties have been more extensively studied with fruit and seed extracts [25].

Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses, IgG and IgM are the major immunoglobulin which are involved in the complement activation, opsonization, neutralization of toxins, etc [25].

The extract has the potential to boost the capacity of the host to fight the invading parasites. The social implication of exploring the medical potential of the extract will be easy availability and cheap cost. This will go a long way in alleviating the usual problems of healthcare delivery, particularly, in relation to finances, logistic of distribution, and at the same time, atone the cultural yearnings of the people.

5. CONCLUSION

This present study reports the lethal dose of aqueous leaf *Moringa oleifera.*

Moringa oleifera leaf extract may possess a possible beneficial therapeutic effect on the amelioration of immunological diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriasis etc in human clinical trials.

The overall trend obtained in the parameters employed for the assessment of immunomodulatory potentials of the aqueous leaf extract of *Moringa oleifera* indicated that the extract is a good candidate as an immune modulating regime.

Our study could be of value to justify the traditional use of the extract as an immune booster, since previous literatures in this regard especially from this part of Nigeria have been relatively scanty.

However, the mechanism of action could be unfolded only after detailed investigations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) [15] were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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