

Repeated Oral Administration of Aqueous Leaf Extract of *Moringa oleifera* modulated immunoactivities in Wistar Rats

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Abstract

The immunomodulatory capability of aqueous leaf extract of *Moringa oleifera* was investigated in male Wistar rats. Dry leaf powder of *Moringa oleifera* was extracted with water and lyophilized. Twenty four Wistar rats with body weight of 86.2 ± 4.43 g were grouped equally into four (A-D) and distilled water, 250, 500 and 1000 mg/kg body weight of extract were orally administered once daily for 56 days groups respectively. Serum interleukin-2 concentration increased ($p < 0.05$), while interleukin-6 and tumour necrosis factor- α reduced ($p < 0.05$). Erythrocyte parameters, neutrophils and monocyte concentrations were reduced ($p < 0.05$). Total leukocyte and lymphocyte counts, and liver reduced glutathione were increased ($p < 0.05$), while malondialdehyde and serum uric acid were decreased ($p < 0.05$). The findings of this study supported the folkloric use of the aqueous leaf extract of *Moringa oleifera* as an immunomodulating regime for the prevention and/ or cure of infective and degenerative diseases. However, the extract caused anaemia.

Keywords: Immunomodulatory capability, *Moringa oleifera*, supported, folkloric use, degenerative diseases and anaemia.

1. Introduction

Immune modulation is the manipulation of immune response to suppress unwanted responses resulting from autoimmunity, allergy, and transplant rejection, and to stimulate protective responses against pathogens that largely elude the immune system. 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 (Fagnoni *et al.*, 2000). They have broad effects on the entire immunity system, but affect primarily cell-mediated immunity, while the humoral immunity may be affected indirectly (Goldsby *et al.*, 2000). Immune modulators achieve their effects by boosting specific areas of the immune system, most especially, the innate immunity and the activities of T lymphocytes. They are known to have amplifier and suppressor activities, depending on the immune status of the user (Spelman *et al.*, 2006). 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 etc (Spelman *et al.*, 2006). 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 (Spelman *et al.*, 2006; Oyewo and Akanji, 2011).

Moringa oleifera Lam (syn. *M. pretygosperma* Gaertn) belongs to the family of *Moringaceae*, a fast growing drought-resistant tree native to northern India. It is the most widely distributed species of the monogeneric family, *Moringaceae* (Fuglie, 1999). *Moringa oleifera* is an important food commodity, which has enormous attention as the natural nutrition of the tropics. The leaves, fruits, flowers and the immature pods serve as highly nutritive vegetable (Anwar *et al.*, 2005). However, the roots of the plant was reported to contain spirochin, an alkaloid with fatal nerve paralysing properties and used as condiment in the same way as horseradish (Makkar and Becker, 1997).

The consumption of the leaf of *Moringa oleifera* has been alleged to balance or boost the energetic, soothing ability, prevent ulcer, inflammation, pain, skin problems, detoxify the blood and gastrointestinal tract, promote wound healing and promote immune functions (Siddhuraju and Becker, 2003; Carrasco *et al.*, 2009). In Nigeria, leaf preparations of *Moringa oleifera* is widely used in folklore for the treatment of immune system-related disorders, but so far, no study has scientifically proved how the leaf preparations modulate the activities of the immune system and promote healthy lifestyles. Therefore, the objective of this study was to investigate the

immunomodulatory potentials of the oral administration of aqueous leaf extract of *Moringa oleifera* in male Wistar rats.

2. Materials and methods

2.1 Materials

2.1.1 Leaf material for analysis

Fresh mature leaf of *Moringa oleifera* was collected before sun rise in August from the natural habitat around Masifa area, Ogbomoso, Oyo State. The plant was authenticated in the Department of Crop Science, Ladoko Akintola University of Technology, Ogbomoso, Oyo State. The fresh leaf was rinsed thoroughly in distilled water and dried in the shade for 18 days. The dried leaf was ground to fine powder, using a domestic electric grinder and suspended in distilled water at room temperature. The filtrates were pulled together and lyophilised using a freeze dryer. The yield of the aqueous leaf extract of *Moringa oleifera* was 18.22% (^{w/w}). The lyophilised extract was stored air tight and kept in the dark till when needed.

2.1.2 Quantitative assay kits and other reagents

The ELISA kits for the determination of rat Interleukin-2 (IL-2), Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF- α) were products of RayBiotech, Inc. USA, while Uric Acid was products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain. Reduced Glutathione assay kit was a product of BioAssay Systems, Hayward, USA. All the chemicals and reagents used in the study were of analytical grade and were purchased from the British Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

2.2 Methods

2.2.1 Phytochemical and mineral analyses

The chemical of constituents in the aqueous leaf extract of *Moringa oleifera* were identified and quantified according to the methods described by Harborne (1973) and Trease and Evans (1983). Standard methods of AOAC (1990) were used for the minerals determination. Determinations were done in triplicates.

2.2.2 Experimental animals and procedure

Twenty four male Wistar rats of average body weight of 86.2 ± 4.43 g were obtained from the Animal Care Facility, Ladoko Akintola University of Technology, Ogbomoso, Oyo State. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria). The rats were randomly grouped into four, comprising of six rats per group and were housed in cages made of wooden frames and metal netting. They were fed *ad libitum* with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 14 days before extract administration was commenced. Calculated amount of lyophilized aqueous leaf extracts of *Moringa oleifera* were constituted in distilled water to give doses of 250, 500 and 1000 mg/kg body weight. The various groups were administered as thus: Group A served as the control rats, received 1.0 ml distilled water, while Group B, C and D received 250, 500 and 1000 mg/kg body weight of the extract respectively. Administration of aqueous leaf extract of *Moringa oleifera* was performed orally once daily between 7:20 am \pm 30 minutes, using metal cannula attached to a 2 ml syringe. Administration lasted for 56 days, after which the rats were fasted for 12 hours and body weights determined before the animals were sacrificed by diethyl ether anaesthesia. This study was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (1985).

2.2.3 Haematological analysis and IL-2, IL-6 and TNF-alpha determination

The haematological parameters were analysed by the automated haemology analyzer (SYSMEX K2X1: SYSMEX CORPORATION, JAPAN). The serum levels of IL-2, IL-6 and TNF- α were determined by *in vitro* enzyme linked immunosorbent assay (ELISA) kit, using colourimetric reaction method as instructed in the kit manual with cat #: ELR-IL2-001, ELR-IL6-001 and ELR-TNF alpha-001 respectively.

2.2.4 Serum uric acid, liver reduced glutathione and malondialdehyde determination

The serum uric acid level was determined by uricase-POD enzymatic colorimetric reaction, according to the method described by Schultz (1984). The levels of reduced glutathione (GSH) were determined using 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) enzymatic colorimetric reaction, according to the method described by Baker *et al.* (1990). The concentration of thiobarbituric acid reactive substances, malondialdehyde (MDA) was determined using the method described by Fraga *et al.* (1981).

2.2.5 Statistical analysis

The results were expressed as mean of 5 replicates \pm standard error of mean (SEM) and were analyzed using statistical package for social sciences (SPSS) 16.0 for Window software. One way analysis of variance (ANOVA) was performed to test the effect of each dose level on the parameter under investigation at 95% level of confidence. The Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($p < 0.05$) and denoted by different alphabets.

3.0 Results

3.1 Phytochemicals and mineral constituents

The phytochemical screening of the aqueous leaf extract of *Moringa oleifera* revealed the presence of alkaloids, saponins, flavonoids, phenols, cyanogenic glycosides, tannins, oxalate, cardenolides, triterpenes and anthraquinones (Table 1). Alkaloids were the highest in terms of concentration, while the least was cardenolides. Mineral constituents of interest that were identified in the aqueous leaf extract of *Moringa oleifera* are presented in Table 1. Minerals implicated in immunomodulation such as selenium, zinc, copper, manganese and magnesium were present, while heavy metal like lead and arsenic were also present.

3.1 Rat behaviour and morphology

Administration of the aqueous leaf extract of *Moringa oleifera* to Wistar rats did not result in any obvious sign of toxicity in terms of the physical appearance and behavioural changes. However, the rats administered 1000 mg/kg body weight of the extract consumed less food and the stool was watery with faded colouration.

3.2 IL-2, IL-6 and TNF- α , serum of uric acid, liver reduced glutathione and malondialdehyde concentrations

The effect of the administration of the aqueous leaf extract of *Moringa oleifera* on interleukin-2 (IL-2), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α), serum of uric acid, liver reduced glutathione (GSH) and liver malondialdehyde (MDA) in Wistar rat is presented in Table 2. The administration of the extract resulted in significant increase ($p < 0.05$) in serum IL-2 concentration at the administered doses with no differences ($p > 0.05$) among doses. Serum IL-6 and TNF- α concentrations decreased significantly ($p < 0.05$) in rats administered the extract in a pattern not dose dependent. Uric acid concentrations were reduced significantly ($p < 0.05$) in rats administered the extract with no significant changes ($p > 0.05$) among doses. The aqueous leaf extract of *Moringa oleifera* resulted in significant increases ($p < 0.05$) in reduced GSH concentrations in the liver at all doses and no effects ($p > 0.05$) with increasing doses, while the liver MDA concentrations were decreased significantly in a similar manner (Table 2).

3.3 Haematological parameters

The trends obtained in the count of blood parameters in rats following the administration of the aqueous leaf extract of *Moringa oleifera* is presented in table 3. The administration of leaf extract of *Moringa oleifera* decreased significantly ($p > 0.05$) the packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) counts of rats at high doses. An almost dose dependent significant increase ($p < 0.05$) was obtained in the erythrocyte sedimentation rates (ESR), while significant reduction ($p < 0.05$) were presented in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in a likewise dose dependent manner. The total white blood cell (WBC) and lymphocytes

(L) count were increased significantly ($p < 0.05$) in rats administered the leaf extract with no differences ($p > 0.05$) among the doses. Significant reductions ($p < 0.05$) were presented in the neutrophil counts at all doses of the extract, while the monocyte count reduced significantly ($p < 0.05$) at high doses of the extract. However, the eosinophil and basophil counts were not affected ($p > 0.05$) in rats administered the extract. The platelet counts was decreased significantly ($p < 0.05$) in an almost dose dependent manner in rats administered the aqueous leaf extract .

4. Discussion

The level of alkaloids in the aqueous leaf extract of *Moringa oleifera* (Table 1), may suggest that the extract has immunomodulatory activity, since some bitter alkaloids (tropane alkaloids) are metabolised in the liver into dimethylxanthine and finally methyl uric acid by cytochrome P₄₅₀ oxygenase systems. Methyl uric acid in the liver stimulates the expression of tumour necrosis factor (in the endothelia cells of the liver by macrophages), which modulates the immune system (Yoshida *et al.*, 1997). Also, saponins, which concentration was high also, are implicated in the modulation of the immune system by serving as adjuvant (saponins-cholesterol-phospholipid complexes) at low concentrations that stimulate cell mediated immune system by inducing the production of interleukins, especially by the antigen-presenting cells in mast cells (Oda *et al.*, 2000; Zahid *et al.*, 2007).

The concentration of phenolic compounds in the extract may help among others, in preventing oxidative stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver (Khanna *et al.*, 2002). Phenolic compounds are implicated to scavenge directly nitric oxide molecule, thereby preventing the oxidation of LDL-C and tissue oxidative damage (Van Acker *et al.*, 1995). Nitric oxide (NO) is constitutively produced in endothelial cells to maintain the dilation of blood vessels and relaxation of smooth muscles (Huk *et al.*, 1998). Peroxynitrite (OONO⁻), the most reactive free radical is formed by further reaction of NO with O₂⁻, which is the major cause of irreversible damage to membranes and degenerative diseases (Van Acker *et al.*, 1995). Flavonoids were reported to decrease also, the immobilization and adhesion of leukocytes to endothelial walls, and degranulation of neutrophils without affecting superoxide production, thereby regulating inflammatory responses in tissue injury and immune responses (Ferrandiz *et al.*, 1996).

The levels of minerals such as selenium, zinc, iron, manganese and magnesium in the leaf extract (Table 1) may contribute to its immunomodulatory action, since these minerals have been implicated in immune modulation (Prasad, 2000; Ravaglia *et al.*, 2000). This submission is strengthened by the report of Girodon *et al.* (1999) that deficiencies of selenium, copper and zinc induced attenuation of immune functions including phagocytic activity, natural killer cell activity, macrophages, antigen-specific antibody production, and the proliferative response of T cells. In addition, the presence of copper, zinc, selenium and iron may enhance the activities of antioxidant enzymes, since these elements serve as cofactors for such enzymes and ultimately modulate the immune system (De la Fuente *et al.*, 2005).

The aqueous leaf extract of *Moringa oleifera* could enhance erythropoietin activity due to the level of iron present (Table 1). However, high iron level in blood has been reported in enhancing oxidative stress by generating reactive oxygen species via the Fenton reaction (Han *et al.*, 2005). The levels of heavy metals in the aqueous leaf extract may suggest a note of caution in the use of the crude extract. Heavy metals have been reported in induce inflammatory responses in biological cells. For example, chronic exposure to lead and arsenic has been implicated in kidney failure (Champe *et al.*, 2005). Furthermore, the presence of lead in the blood could precipitate acquired porphyria, hypochromic-microcytic anaemia and hyperuricemia as lead inhibits the incorporation of iron into the protoporphyrin ring during haem biosynthesis (Jeremy *et al.*, 2001; Champe *et al.*, 2005).

The trend obtained in the serum IL-2, IL-2 and TNF- α concentrations in rats administered the aqueous leaf extract of *Moringa oleifera* (Table 2) suggest that the aqueous leaf extract has immune modulation activities. IL-2 is normally produced by the body to regulate immune response to environmental substances (molecules or microbes) that gain access to the body (Smith, 1988). These substances (antigens) are recognized as foreign by antigen receptors that are expressed on the surface of lymphocytes. Antigen binding to the T cell receptor stimulates the secretion of IL-2 and the expression of IL-2 receptors. The IL-2/IL-2R interaction then stimulates the growth, differentiation and survival of antigen-selected cytotoxic T cells via the activation of the expression of specific genes (Beadling and Smith, 2002). After exiting from the thymus, T-reg function to prevent other T cells from recognizing and reacting against "self antigens", which could result in "autoimmunity". That is, IL-2 is required to discriminate between self and non-self, another one of the unique characteristics of the immune

system (Thornton et al., 2004). Increasing doses of the extract had no remarkable effect on the serum IL-2 concentrations, thus, suggesting that the inherent immunomodulatory activities of the aqueous extract of *Moringa oleifera* is not dose dependent.

IL-6 acts as both a pro-inflammatory and anti-inflammatory cytokine. It protects against tissue damage and increases the synthesis of fibrinogen as part of healing processes (Murtaugh et al., 1996). IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha (Heinrich et al., 2003). It has major effects on haematopoiesis, stimulation of acute phase reaction and tyrosine kinase, which enhance diapedesis and cell-cell communication of innate immune cells (Abbas et al., 1997). However, the over-expression of IL-6 has been reported in some disease conditions such as atherosclerosis due to increased fibrinogen production, sepsis, liver diseases, degenerative disease (cancer), oedema, massive weight loss and inflammatory disorders (Dubinski and Zdrojewicz, 2007). Therefore, the reduction in the serum IL-6 concentrations at 250 and 500 mg/kg body weight of the extract suggested that *Moringa oleifera* leaf regulated the release of IL-6 in muscles and tissues, probably indicating no tissue damages and inflammatory processes. Increasing dose of the extract did not enhance the suggested the immunomodulatory activity of *Moringa oleifera* leaf.

The trend obtained in serum TNF- α concentration (Table 2) suggest that there was reduced possibilities of systemic inflammation and/ or stimulation of the acute phase reaction (supported the immunomodulatory potential of the extract), which could be due to tissue damage. TNF- α is involved in systemic inflammation and stimulates the acute phase reaction (Locksley et al., 2001). The primary role of TNF- α is in the regulation of the growth of normal cells and immune cells by inducing apoptotic cell death, inflammation and inhibition of tumour igenesis and viral replication. TNF- α has receptors on the surface of all organs in the body and aids in maintaining homeostasis by the regulation the body's circadian rhythms. The over-expression of TNF- α , however, has been implicated in increased risk of mortality, heart disease, septic shock, dehydration, anorexia, net catabolism, weight loss, anaemia, hepatosplenomegaly, autoimmune disorders and increased risk of cancer (Dubinski and Zdrojewicz, 2007; Oyewo and Akanji, 2011).

IL-6 and TNF- α are implicated as better predictors of inflammatory related problems in people with no conventional risk factors (Morrow and Ridker, 2000). Thus, controlling the release of these cytokines is very important if there is the need to enhance immunity and reduce the possibility of developing degenerative diseases (Oyewo and Akanji, 2011). The overall trends in the serum cytokine concentrations (IL-2, IL-6 and TNF- α) following the administration of the aqueous leaf extract of *Moringa oleifera* indicate that increasing doses had no balanced regulatory effects on the immune system, but rather upward regulation that could result in over stimulation of the immune system.

The administration of the aqueous leaf extract of *Moringa oleifera* suggested a good means of the recovery of reduced glutathione (GSH), which is in line with the trend obtained in the liver GSH level in rats administered the extract (Table 2). However, increasing the dose of extract did not have any effect on the recovery of reduced glutathione. GSH is the one of the most proactive endogenous antioxidants in the body, because it is involved in many detoxification processes (Becker, 1993). The levels of saponins and polyphenolic compounds in the aqueous extract (Table 1), inferably suggest antioxidant properties and thus, the recovery of reduced GSH that could prevent the development of degenerative diseases caused by oxidative stress. The trend obtained in liver GSH following administration of the leaf extract is consistent with the reports of Anderson et al. (1997) and Xiang et al. (2001) that decrease in liver GSH concentration resulted in the increase in tissue inflammation and over-expression of IL-6.

The result obtained in the serum uric acid concentration following the administration of the aqueous leaf extract of *Moringa oleifera* (Table 2) was probably due to the concentration of reduced glutathione in the liver as the concentration of uric acid in the blood was reported to be tightly regulated by the level of reduced glutathione in the liver. Increase in GSH concentration in liver facilitates the excretion of uric acid in the blood, thereby reducing the risk of the formation of kidney stones and gout (Becker, 1993). However, there is increase in the demand for uric acid as an antioxidant (greater than 50% of total antioxidant pool in the body) when the concentration of GSH in the liver is low, in conditions such as oxidative stress, liver diseases and chronic inflammatory diseases (Becker, 1993; Xiang et al., 2001). The result of the serum uric acid concentration supported that of the serum IL-6 and TNF- α concentrations that suggested a reduced risk of systemic inflammatory responses in rats administered the aqueous extract. Chronic systemic inflammatory responses would result in an increase in the serum uric acid concentration, which has been implicated in triggering localized inflammatory responses mediated by increasing the serum IL-6 and TNF- α concentrations (Cesari et al., 2003).

The administration of the aqueous leaf extract of *Moringa oleifera* reduced the formation of lipid peroxidation product, malondialdehyde (MDA) concentration in the liver (Table 2), thereby, supporting the antioxidative property of the aqueous leaf extract. However, the antioxidative property of the leaf extract was not dose dependent. The result obtained in the MDA concentrations in the liver is consistent with the trends reported in the liver GSH concentrations and serum uric acid concentrations, all indicating no correlation in the endogenous antioxidant capability with increase in dose of the leaf extract. The overall result of the oxidative stress indices supported the immunomodulatory activity of the aqueous leaf extract, since oxidative stress is a key factor that compromises the immune system through the induction of inflammatory responses by free radicals.

The administration of aqueous leaf extract of *Moringa oleifera* might have induced anaemia as indicated by the packed cell volume, red blood cell and haemeoglobin counts at 500 and 1000 mg/kg body weight (Table 3). The increase in erythrocyte sedimentation rate at all doses further strengthened the anaemic capability of the extract at those doses. The anaemia might result from the haemolysis of erythrocytes, due to the levels of saponins and heavy metals (lead) in the aqueous leaf extract *Moringa oleifera* (Table 1). Saponins have been implicated in the permeabilization of plasma membranes of biological cells due to the bipolar structure of saponins, in which the lipophilic components integrate easily and complex with the lipid fraction of plasma membranes, while the hydrophilic glycosidic portion forms complexes with transmembrane proteins, thereby causing irreversible disorder and disruption of the plasma membrane (Choi *et al.*, 2001). Permeabilization of erythrocyte plasma membranes would result in the distortion of the outer lipid bilayer, which reduces the osmotic pressure, thereby causing the swelling and lysis of the erythrocyte (haemolysis) (Trease and Evans, 1989; Choi *et al.*, 2001).

The results of the erythrocyte function indices (Table 3) supported the anaemia indicated at high doses of the extract. The result of the mean cell volume (MCV) signifies that the sizes of RBCs were reduced, indicating microcytic anaemia due to probably due to iron deficiency and not due to chronic inflammatory response, since the trends obtained in the serum IL-6 and TNF- α did not suggest systemic inflammatory condition. Furthermore, the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) supported the possibility of the extract predisposing the consumer to iron deficiency anaemia and/ microcytic hypochromic anaemia. The trend in the erythrocyte function indices indicated that the aqueous leaf extract predisposed to anaemia dose dependently, which is due to increasing concentrations of erythrocyte toxicants in the extract, such as saponins, lead etc (Choi *et al.*, 2001) and lead (Champe *et al.*, 2005).

The aqueous leaf extract of *Moringa oleifera* boosted the total white blood cell and lymphocytes counts in rats (Table 3), suggesting immunomodulation capability, as white blood cells are involved in fighting infection and clearing off injured or dead cells and tissues in body (Jeremy *et al.*, 2001). The trend in serum IL-2 concentration (Table 2) supported the result of the total white blood cell and lymphocyte counts. In addition, the reduction in the neutrophils concentrations following the administration of *Moringa oleifera* leaf extract (Table 3) did not suggest inflammatory tissue damage like splenomegaly, hepatic inflammation, but a decrease in the production of neutrophils due to the obtained levels of IL-6 and TNF- α (Table 2). IL-6 and TNF- α regulate the activities of innate immune cells, especially neutrophils and macrophages (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 (Stvr̃tinova *et al.*, 1995). Thus, the drop in the neutrophils count in rats administered the aqueous leaf extract of *Moringa oleifera* is not suggesting localized tissue inflammation that could have increased the demand for neutrophils production and margination with the endothelial cells of the inflamed tissue.

The decreases in the platelets count (Table 3) supported the anaemic capability of the extract as suggested by the results of the erythrocyte parameters (Table 3). Anaemia has been reported in cases of reduced number of platelets (Topley, 1998). In addition, the trends obtained in the serum IL-6 and TNF- α concentrations supported the result of the blood platelets in rats administered the aqueous leaf extract of *Moringa oleifera*.

Conclusion

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. However, high doses of the extract are not recommended, because the immunodulatory activities of the extract were not dose dependent. In addition, high doses of the extract resulted in anaemia. Therefore, this study established scientifically the use of the aqueous leaf extract of *Moringa oleifera* as an immune modulator. The toxicity of the aqueous leaf extract in male Wistar rat is currently being investigated.

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Table 1: Some phytochemicals and minerals in aqueous leaf extract of *Moringa oleifera*

Phyto-constituent	% Composition	Leaf Minerals	analyses (mg/kg)
Akaloids	11.115 ± 0.06	Magnesium	1.211 ± 0.00
Tannins	0.324 ± 0.01	Manganese	0.612 ± 0.03
Oxalate	3.010 ± 0.03	Copper	3.551 ± 0.04
Flavonoids	1.004 ± 0.03	Iron	4.717 ± 0.07
Saponins	6.172 ± 0.06	Zinc	3.417 ± 0.03
Cardenolides	0.071 ± 0.02	Selenium	1.056 ± 0.00
Phenols	2.275 ± 0.03	Sodium	4.495 ± 0.06
Anthraquinone	0.558 ± 0.00	Potassium	2.812 ± 0.07
Triterpenes	1.002 ± 0.01	Calcium	1.001 ± 0.02
		Lead	2.171 ± 0.03
		Arsenic	0.943 ± 0.02

Values are means ± SEM; n=3.

Table 2: Effect of leaf extract on serum IL-2, IL-6, TNF- α , serum uric acid, liver glutathione and malondialdehyde concentrations in Wistar rats

	Doses (BW)			
	Control	250	500	1000
IL-2 (PL)	28.22 \pm 2.17 ^a	39.67 \pm 3.43 ^b	44.15 \pm 3.11 ^b	43.63 \pm 2.07 ^b
IL-6 (PL)	320.41 \pm 11.54 ^a	241.56 \pm 9.95 ^b	202.47 \pm 6.88 ^c	229.33 \pm 8.84 ^b
TNF- α (PL)	441.32 \pm 12.45 ^a	246.67 \pm 8.85 ^b	252.82 \pm 13.09 ^b	230.55 \pm 11.67 ^b
Uric acid (ML)	4.96 \pm 0.28 ^a	3.55 \pm 0.20 ^b	3.31 \pm 0.35 ^b	3.37 \pm 0.52 ^b
Liver GSH (ML)	1.48 \pm 0.22 ^a	2.36 \pm 0.09 ^a	2.22 \pm 0.16 ^b	2.44 \pm 0.28 ^b
Liver MDA (UP)	4.87 \pm 0.41 ^a	3.41 \pm 0.37 ^b	3.22 \pm 0.44 ^b	3.13 \pm 0.51 ^b

Values are means \pm SEM; n=5. Values bearing different alphabets are significantly different (p<0.05).

Key: BW (mg/kg body weight), PL (pg/ml), ML (mg/ml), UP (nmole /mg of protein).

Table 3: Haematological parameters in Wistar rats administered with the leaf extract

hematological parameters	Doses (BW)			
	Control	250	500	1000
PCV (%)	40.1 \pm 2.10 ^a	39.4 \pm 1.02 ^a	36.1 \pm 2.08 ^b	35.2 \pm 1.61 ^b
RBC (10 ¹²)/L	6.02 \pm 0.49 ^a	5.99 \pm 0.31 ^a	5.22 \pm 0.25 ^b	5.09 \pm 0.46 ^b
Hb (g/dl)	12.34 \pm 0.71 ^a	12.11 \pm 0.45 ^a	11.02 \pm 0.55 ^b	11.10 \pm 0.35 ^b
ESR (mm ³ /hr)	3.5 \pm 0.35 ^a	3.9 \pm 0.60 ^b	4.8 \pm 0.55 ^c	5.3 \pm 0.30 ^c
MCV (fL)10 ¹⁵	59.35 \pm 3.75 ^a	50.45 \pm 4.05 ^b	42.15 \pm 2.65 ^c	39.05 \pm 3.35 ^c
MCH (10 ⁻¹²)	0.20 \pm 0.02 ^a	0.15 \pm 0.02 ^b	0.11 \pm 0.01 ^c	0.09 \pm 0.01 ^d
MCHC (g/L)	3.55 \pm 0.15 ^a	2.95 \pm 0.25 ^b	2.30 \pm 0.10 ^c	2.25 \pm 0.25 ^c
WBC 10 ⁹ /L	5.00 \pm 0.42 ^a	5.85 \pm 0.35 ^b	5.75 \pm 0.65 ^b	6.05 \pm 0.75 ^b
Lymphocyte (%)	40.85 \pm 3.91 ^a	45.25 \pm 3.75 ^b	47.25 \pm 4.05 ^b	48.75 \pm 2.55 ^b
Neutrophil (%)	47.08 \pm 3.11 ^a	40.51 \pm 2.51 ^b	40.5 \pm 2.95 ^b	39.45 \pm 3.02 ^b
Monocyte (%)	9.03 \pm 0.43 ^a	9.34 \pm 0.85 ^a	8.25 \pm 0.29 ^b	7.97 \pm 0.61 ^b
Eosinophil (%)	1.00 \pm 0.25 ^a	1.00 \pm 0.25 ^a	1.00 \pm 0.25 ^a	1.00 \pm 0.25 ^a
Basophil (%)	1.00 \pm 0.00 ^a	Not detected	Not detected	1.00 \pm 0.00 ^a
Platelets(μ l)10 ³	400.0 \pm 9.75 ^a	375.6 \pm 4.05 ^b	325.5 \pm 6.44 ^c	310.3 \pm 7.25 ^c

Values are means \pm SEM; n=5. Values bearing different alphabets are significantly different (p<0.05).

BW (mg/kg body weight).