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Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in Wistar albino rats

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Abstract

Background—Globally, *Moringa oleifera* is used by different communities to treat various ailments including modulation of the immune system though with limited scientific evidence.

Aim—To study the immunomodulatory activity of *M. oleifera* methanolic leaf extract in Wistar albino rats.

Methods—An experimental laboratory-based study was done following standard methods and procedures. Nine experimental groups (I, II, III, IV, V, VI, VII, VIII, IX) each comprising of six animals were used. Group I received normal saline. Groups II to IX received 200 mg/kg bwt cyclophosphamide at the beginning of the study. Group III received 50 mg/kg bwt of an immunostimulatory drug levamisole. Groups IV to IX were dosed daily for 14 days with extract at

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doses of 250, 500, and 1000 mg/kg bwt, respectively, using an intragastric tube. Complete blood count (CBC), delayed-type hypersensitivity reaction (DTH), neutrophil adhesion test, and hemagglutination antibody titer were determined using standard methods and procedures. Statistical analysis was performed using GraphPad prism 5.0a Software.

Results—There was an increment in WBC, lymphocyte, and neutrophil counts at a dose of 1000 mg/kg bwt similar to the levamisole-positive control group. The neutrophil adhesion was statistically significant ($p = 0.05$) for treatment groups that received 1000 mg/kg bwt (29.94%) and 500 mg/kg bwt at 17.28%. The mean percentage increment in footpad thickness was highest (26.9%) after 8 h of injection of antigen in the footpad of rats dosed 500 mg/kg bwt and this later reduced to 25.6% after 24 h. There was a dose-dependent increment in the mean hemagglutination antibody titer to sheep red blood cells (SRBC) from 10.73 ± 0.57 HA units/ μ L for the 250 mg/kg bwt to 26.22 ± 1.70 HA units/ μ L for the 1000 mg/kg bwt.

Conclusions—Methanolic leaf extract of *M. oleifera* caused a significant immunostimulatory effect on both the cell-mediated and humoral immune systems in the Wistar albino rats.

Keywords

immune system; immunomodulation; *Moringa oleifera* extract; Wistar albino rats

Introduction

Currently, world wide, there is an increase in diseases especially infectious diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation. Malnutrition and infectious diseases have remained a challenge especially in developing nations as they greatly compromise the body's immune system responses in the affected individuals [1]. Inadequate balanced dietary intake, most especially proteins, and diseases are immediate causes of malnutrition and these reinforce one another synergistically [2]. The major causes of immunodeficiency include stress, infectious diseases such as acute respiratory tract infections, diarrheal diseases, yellow fever, hepatitis A and E, tuberculosis, and HIV/AIDS [3]. However, proper nutrition by individuals strengthen the body's immune system and its defense capabilities [2–5]. Globally, it was estimated that about 870 million people were undernourished in the period from 2010 to 2012 and this represented 12.5% of the global population of which about 852 million people live in developing countries, where malnutrition is estimated at 14.9% [6]. However, malnutrition greatly affects the individual's immune system physiology and in most cases, there is need to stimulate it in circumstances of immunosuppression or suppress it in case of overexaggerated stimulation as in case of autoimmune disease conditions.

Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people, they are not easily accessible, and in most cases they are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the use of alternative herbal medicines from medicinal plants such as *Moringa oleifera* that are widely accepted, accessible, cheaper, and assumed to have fewer side effects [7]. In Africa and Asia, about 80% of the population have been reported to depend on traditional medicine for their

primary health care needs including immunomodulation [7]. A number of medicinal herbs have long been used and reported to boost the immune system or to modulate it and they are used putatively to treat and prevent various disease conditions worldwide [8]. Among the herbs used are *Morus alba* Linn, *Triphala megaExtract (megaExt)* is a traditional Ayurvedic herbal formulation consisting of a mixture of different equal parts or extracts of three medicinal plant fruits including *Terminalia chebula*, *Terminalia bellerica*, and *Emblica officinalis* [9, 10], *Fiscus racemosa*, *M. oleifera*, and many others. *M. oleifera* is a common herb and has been documented to have various phytochemicals, macronutrients, and micronutrients that contribute to its vast medicinal value including the management of diseases such as asthma, bronchitis, mastitis, skin conditions, worm infestations, and HIV/AIDS symptoms among others [11]. Its ability to treat most of these conditions has been attributed to the nutritional and immunomodulatory properties it possesses including its antioxidant and anticancer activity among others [12]. The herb is commonly available and widely used in local communities and by traditional herbalists in Uganda as an immune booster in the management of various disease conditions [11]. Previous study on use of *M. oleifera* and activated charcoal on intoxicated Wistar albino rats with lead acetate demonstrated improvements in biochemical, hematological parameters as well as protective effects on various organs such as the liver and kidneys and tissues like blood [13]. Also, various studies have reported the immunomodulatory activity of *M. oleifera* leaf extracts in cyclophosphamide immunosuppressed Wistar albino rats [14, 15]; however, no studies have been done in normal rats as depicted by its use in the various local communities in Uganda. Also, previous studies on the immunomodulatory activity of *M. oleifera* were done in different geographical regions of the world and this has been reported to affect the nutritional and the phytochemical composition of the plant [16] and hence its medicinal value effectiveness may differ geographically. The study therefore, determined the immunomodulatory activity of methanolic leaf extracts of the Ugandan *M. oleifera* in nonimmunosuppressed Wistar albino rats.

Materials and methods

Study design and setting

The study was a preclinical laboratory-based experimental study. It was conducted at Makerere University College of Veterinary Medicine, Animal resource and Biosecurity (CoVAB) Pharmacology Laboratory, Uganda.

Sample collection and identification

The *M. oleifera* leaves were harvested in the Wakiso district during the dry season (June, 2013) due to its abundance in the district and its proximity. The plant was identified by a taxonomist and a voucher specimen number (41302) was deposited at the Makerere University herbarium.

Processing and extraction

The leaves were collected and air-dried in the shade until constant weight was achieved. They were then pounded into a fine coarse powder, using a mortar and pestle. The extraction procedures were carried out at Makerere University CoVAB, nutrition laboratory following

the already established extraction procedure of plant materials [17, 18]. Three hundred grams of the powder was weighed using an electronic weighing scale (Mettler PJ3000, Mettler-Toledo GmbH, Ockerweg, Germany) and was then soaked in 1.6 L of absolute methanol (Sigma-Aldrich Chemie GmbH, Munich, Germany) in an amber-colored bottle for 3 days with occasional shaking. The mixture was filtered on the 3rd day using a gauze cloth and the fine filtrate was obtained using Whatman No: 1 filter paper in a Buchner funnel. The filtrate was concentrated using a Büchi Rotavapor R-200 (Büchi Labortechnik, Flawil, Switzerland) into slurry which was further dried into a semi-solid extract in an oven set at 50 °C. The dry extract was stored at 4 °C until the immunomodulatory experimental bioassays were carried out. The percentage yield of the extract was determined. Concentrated stock solution of the *M. oleifera* leaf extract was prepared by dissolving 2 g of extract in 40 mL of distilled water to make a working concentration of 50 mg/mL. Control solutions were normal saline and levamisole that was given at a dose of 50 mg/kg bwt as the positive control.

Drugs

Cyclophosphamide (Baxter Healthcare Corporation, Deerfield, IL, USA, LOT; 1D680L; Expiry: 04, 2014) was used as a negative control. Levamisole Hydrochloride BP 40 mg syrup (Regal Pharmaceuticals Limited, Nairobi, Kenya, batch no; 130680; Expiry: 06, 2016) was used as a positive control. All chemicals and reagents used were of analytical grade and were checked to ensure that they were not expired before the experimentation.

Laboratory animals used in the study

Fifty-four disease-free Wistar albino rats aged between 6 and 8 weeks were randomized into nine experimental groups ($n = 54$; $n_1 = 6$) with three males and three females separated in each group. They were maintained under standard laboratory conditions and temperature (25 ± 1 °C) and light/dark cycle (12 h light:12 h dark cycle). They received rat pellets and clean water ad libitum. Male rats were kept in separate cages from their female counterparts, before and during the time of the study to avoid conception during the study time. The animals were acclimatized for 2 weeks before the experimental study was carried out. The protocol was approved by the Makerere University college of Health Sciences Research and Ethics Committee and the Uganda National Council for Science and Technology in compliance with the international biosafety guidelines (WHO laboratory biosafety manual, 2004) and the international guidelines for the care and use of laboratory animals in Biomedical Research (National Academies Press, 2011).

Antigen preparation

Fresh blood was collected from healthy sheep at Makerere University CoVAB and mixed with sterile Alsever's solution (1:1). The blood was then centrifuged at $1609.92 \times g$ for 5 min to enable red blood cells to settle at the bottom of the test tube. The supernatant was discarded, leaving sheep red blood cells (SRBC) pellets that were washed three times with pyrogen-free phosphate buffered saline (pH 7.2). They were then kept under refrigeration for use in the immunization and challenge study.

Group treatments and dosing of animals

Rats in the normal control group (Group I) received normal saline throughout the 2 weeks of study period. Rats in the test groups (Group II–IX) were treated with an immunosuppressant (cyclophosphamide) at doses of 200 mg/kg bwt on day 0 of the study by subcutaneous injection (Table 1).

Determination of complete blood count (CBC)

Two milliliters of fresh blood was drawn by intraventricular puncture for each of the animals in Group IV, V, and VI on the 14th day into ethylenediaminetetraacetic acid (EDTA)-containing vacutainers. It was then analyzed at the Mulago National Referral Hospital hematology laboratory using an automated Beckman coulter A-T Pierce hematology analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) for the complete and differential blood cell counts.

Determination of neutrophil adhesion

On day 14 after administration of the extract, blood samples from rats in Group IV, V, and VI were obtained by ventricular puncture and analyzed for total leucocyte counts (TLC) and differential leukocyte counts (DLC). After the initial counts, the blood samples were incubated with 80 mg/mL of nylon fibers for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil were given as the neutrophil index (NI) of blood sample [19]. Percent neutrophil adhesion was calculated as follows:

$$\text{Neutrophil adhesion} = (\text{NIu} - \text{Nit}) \times 100 / \text{NIu}$$

where, NIu, neutrophil index of untreated blood sample; Nit, neutrophil index of treated blood sample

$$\text{Neutrophil index (NI)} = \text{TLC} \times \% \text{ neutrophil}$$

Determination of delayed-type hypersensitivity responses

On day 7 of the study, all the Group III, IV, and V rats were primed by subcutaneously injecting 0.1 mL of suspension containing 1×10^8 SRBC into the right hind footpad. The contralateral paw also received an equal volume of 0.1% phosphate buffered saline (PBS). The administration of *M. oleifera* methanolic leaf extracts was continued until the 14th day. On the 14th day, the animals were challenged by subcutaneously injecting 0.1 mL of 1×10^8 SRBCs into the left hind footpad of the rats. The extent of delayed-type hypersensitivity (DTH) response in the rats was determined by measuring the footpad thickness after 4, 8, and 24 h of challenge using vernier calipers. The difference in the thickness of the right hind paw and the left hind paw was then used as a measure of DTH reaction and was expressed as a mean percent increment in thickness/edema.

$$\frac{[\text{Left footpad challenged with antigen} - \text{Right foot pad control}]}{\text{Left footpad challenged with antigen}} \times 100$$

Determination of humoral antibody response to SRBC (hemagglutination antibody titer test)

Rats in test Group VII, VIII, and IX were immunized by injecting 0.5 mL of SRBCs intraperitoneally (i.p.) on the 7th day of the experiment. Administration of *M. oleifera* methanolic leaf extract continued for another 7 days until day 14 and blood samples were collected by cardiac puncture. Blood was centrifuged at $1609.92 \times g$ to get serum. Antibody titers were then determined by the hemagglutination technique as described by Gaur et al. (2009). Serial two-fold dilutions of serum were made with normal saline in microtiter plates of 96-well capacity and SRBC (25 μL of 1% SRBC prepared in normal saline) added to each of these dilutions. The hemagglutination plates were then incubated at 37°C for 1 h and then examined for hemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the hemagglutination antibody titer (HA units/ μL).

Results

Percentage yield

The percentage yield of the methanolic leaf extract of *M. oleifera* that was used in the study was calculated as follows:

$$\begin{aligned} \text{Percentage yield} &= \frac{\text{weight of final extract}}{\text{soaked sample material}} \times 100 \\ \text{Percentage yield} &= \frac{18.09 \times 100}{300} = 6.03\% \end{aligned}$$

Effect of varying doses of extract on CBC

Animals in Group II who were left untreated with *M. oleifera* leaf extract during the study period showed a net reduction in the hematological parameters assessed. The animals in Group III who received levamisole during the study period showed significant increment in WBC as compared to the control Group I. The mean WBC counts in rats dosed 50 mg/kg bwt of levamisole and 1000 mg/kg bwt of *M. oleifera* leaf extract were significantly higher than those of the animals in the other groups ($p < 0.05$) with the highest counts observed in Group III (22.53×10^3 cells/ μL). The animals in the group who received 250 mg/kg bwt showed a net reduction in mean WBC counts in relation to all the other group animals. The group that received levamisole 50 mg/kg bwt showed significant elevation in the total RBC counts in relation to the other groups. The group VI which received 250 mg/kg bwt of *M. oleifera* leaf extract and Group III which received 50 mg/kg bwt of levamisole significantly had elevated hemoglobin concentration and hematocrit ($p < 0.05$) compared to the other groups. With regard to the differential counts, statistically significant results ($p < 0.05$) were observed for neutrophil and lymphocyte counts in the treatment group that received levamisole and 1000 mg/kg bwt of *M. oleifera* extract in comparison to all the other groups (Table 2).

Effects of extract on the neutrophil adhesion

The mean % neutrophil adhesion from the rats who were dozed with 50 mg/kg bwt of levamisole, 1000 mg/kg bwt, and 500 mg/kg bwt of *M. oleifera* leaf extract were higher than that of the rats who received 250 mg/kg bwt of extract and cyclophosphamide only. The highest mean % neutrophil adhesion was recorded in the group that was not subjected to immunosuppressant cyclophosphamide at the beginning of the study (55.24%) followed by the group that received the immunostimulant levamisole (30.78%). From the treatment groups, statistically significant results ($p < 0.05$) were observed in the groups that received 1000 mg/kg bwt (29.94%) and 500 mg/kg bwt at 17.28% (Table 3).

Effect of extract on DTH

The mean percentage increment in footpad thickness was highest (26.9%) after 8 h of injection of SRBC antigen in the right footpad for the rats dosed 500 mg/kg bwt of the extract and this later reduced to (25.6%) at 24 h. Generally, there was a reduction in the percentage increment of footpad thickness after 8 h post-antigen injection; this, however, was not true for the group that received 1000 mg/kg bwt of extract that showed a significantly steady increment in footpad thickness up to 24 h post-antigen injection. The group that received levamisole showed a steady increment in footpad thickness with the highest percentage increment for this group observed after 24 h at 24.1% (Figure 1). There was a statistically significant elevation in percentage footpad thickness increment ($p < 0.05$) in the group that received 1000 mg/kg bwt of the extract up to 24 h and in the 500 mg/kg bwt group up to 8 h as compared to the Group I that did not receive any cyclophosphamide during the study period.

Effect of extract on hemagglutination antibody titers of SRBC

The mean hemagglutination antibody titer to SRBC showed a dose-dependent increment for the rats dosed with *M. oleifera* leaf extract as compared to the rats that received only cyclophosphamide, with the highest mean titer for the extract concentration of 1000 mg/kg bwt at 26.22 HA units/ μ L. This was followed by the Group III animals that received levamisole then 500 mg/kg bwt of *M. oleifera* leaf extract and least for the 250 mg/kg bwt (Table 4). The group that received normal saline showed a higher mean antibody titer than the cyclophosphamide group at 6.32 HA units/ μ L though not statistically significant.

Discussion

The immune system is the defense mechanism of the body and it helps to protect it from foreign bodies and infection thus playing a part in homeostasis of the body. Modulation of the immune system by way of stimulation or suppression helps in maintaining a disease-free state within an individual. Immunomodulators have therefore been used globally to control disease conditions. The study explored the immunomodulatory activity of the methanolic leaf extract of *M. oleifera* by evaluating its effect on neutrophil adhesion, DTH reactions, hemagglutination antibody titers and on the complete blood count.

Effect on hematological parameters (complete blood count)

Based on these results, the methanolic leaf extract of *M. oleifera* that was administered to the initially immunosuppressed animals had a stimulatory effect on the WBC, neutrophil, and lymphocyte counts with increasing doses close to the already established ED₅₀ of 8.9 g/kg bwt [20]. The observed increment in WBC counts could have been due to the presence of different nutritional elements in the *M. oleifera* methanolic leaf extract and the observed effect was similar to previous studies done elsewhere [14, 15]. Methanol as a solvent has been reported to extract most of the compounds both polar and nonpolar compounds in the plant materials such as leaves, roots, fruits, and stems [21]. Previous studies have reported the nutritional composition of *M. oleifera* as 19 amino acids [12, 22, 23] and 14 fatty acids [22] and these amino acids have been reported to be important in the synthesis of various proteins in the body including the plasma proteins [24] that are important in the defense mechanisms of the body [25]. It also contains various micronutrients such as iron, zinc, copper, calcium, manganese, magnesium, potassium, sodium [26–29], sulfur, vitamin E, beta carotene, thiamine, riboflavin, niacin, pyroxide, biotin, ascorbic acid, cholecalciferol, tocopherol and vitamin K [22, 23, 26, 30], and selenium [31]. It is possible that the methanol extracted almost all these compounds which could contribute to the observed effects on the hematological parameters. These compounds are essential for the development and maturation of the body's immune systems especially the cellular components of hemopoiesis. The micronutrients in *M. oleifera* leaves are essential in the growth, differentiation, and proliferation of immune system cells [32]. Different studies have shown that *M. oleifera* contains various amino acids, vitamins, trace elements, and phytochemical elements such as iron, copper, selenium, zinc, flavonoids, and saponins among others [22, 33–35]. These elements especially vitamins B12, B6, C and E, folic acid, and riboflavin are essential for the synthesis of DNA and in the final maturation of the red blood cell [3, 24]. The amino acids found in *M. oleifera* are also important in the formation of globin which is essential for hemoglobin synthesis [24]. Iron, also a trace element found in *M. oleifera*, is one of the single most important elements in the formation of hemoglobin found in the RBC [24, 36]. The micronutrients also play a key role in balancing the redox state of leukocytes thus protecting them from oxidative stress [24]. These processes are vital in cell proliferation and survival and hence the observed increment of the WBC counts in animals dosed with the extract of *M. oleifera* especially at high doses. The dose-dependent increment in the WBC, lymphocyte, and neutrophil counts could be attributed to the dilution effect of the micronutrients present in *M. oleifera* leaf extract. The findings of the study provided scientific evidence as to why *M. oleifera* is a widely used herb to treat various ailments in Africa and throughout the world in various disease conditions such as HIV/AIDs-related symptoms, syphilis, urinary tract infections, and malaria among others [11, 12]. Also, it was concluded from this study that the herb contains various compounds that increase hemopoiesis and the observed increased production of WBC and RBC, thereby justifying its use widely for the management of anemias and immunodeficiency syndromes [11, 12].

Effect on neutrophil adhesion

Neutrophils are part of the cell-mediated immune responses responsible for the innate immunity that contribute to the clearance of foreign bodies by recognition and migration

toward the foreign body, phagocytosis, and destroying the foreign agent [37]. In the present study, the results showed a dose-dependent increment in the adhesion of neutrophils to the nylon fibers which was an indication of the boosting the neutrophil migration toward foreign bodies [37]. This was in line with findings from previous studies that showed an increment in the neutrophil adhesion when *M. oleifera* was fed to animals [38]. The increased percentage neutrophil adhesion could be attributed to the presence of the various compounds, macronutrients, and micronutrients in *M. oleifera* [12, 22, 34, 35]. Different studies on *M. oleifera* have shown that it contains amino acids, fatty acids [22], vitamins, and trace elements [26] all of which are important in the functioning of neutrophils in the cell-mediated immune response [14]. Poly-unsaturated fatty acids (PUFAs) have been reported to play an important role in priming of neutrophils to be responsive to other immune mediators and play a part in superoxide production. The fluidity of fatty acids gives the cell membrane of the polymorphonuclear neutrophils the ability to undergo diapedesis during migration toward a foreign agent [24]. This greatly contributes to the ability of neutrophils to migrate toward a foreign agent. Micronutrients such as vitamin D play a pivotal role in calcium and bone metabolism, hence being essential in the production of the polymorphonuclear neutrophils. Zinc also found in *M. oleifera* is an essential element for highly proliferating cells and is involved in the activity of cytosolic superoxide dismutase which helps in preventing oxidation reactions within the neutrophils thus prolonging their lifespan [32]. The results from this study suggested that the presence of these various compounds in *M. oleifera* leaf extract may be useful in protection of the body by enhancing phagocytosis. *Moringa oleifera* leaf extract contains vitamins and trace elements that not only facilitate the proliferation and maturation of neutrophils, but also the secretion of cytokines [32, 39] that resulted in an increment in neutrophil migration and adhesion to the nylon fibers as observed in the study. As the extract that was used in the present study was methanolic which contains both the polar and nonpolar compounds [21], it is most probable that these compounds are responsible for the observed improvement in neutrophil adhesion. The plant extract could therefore be used when the immune system is compromised to improve on the cell-mediated immune response as the extract elevates the phagocytic activity of the neutrophils.

Effect on DTH reaction

The DTH reaction is a type IV cell-mediated immune response according to the Coombs and Gell classification of hypersensitivity reactions, 1975 [40]. The test provides a functional in vivo assessment of the cell-mediated immunity. It is often used as a skin test which capitalizes on intradermal inoculation of an antigen. It is therefore used to assess the skin response following intradermal inoculation of the antigen which is dependent on antigen specific memory T-cells and the observed results were due to the recruitment of mononuclear cells and neutrophils. Activation of the T cells leads to the release of lymphokines which causes the activation and accumulation of macrophages, increases vascular permeability, induces vasodilatation and produces inflammation [37, 41]. It also produces a boost in phagocytic activity and increases the concentration of lytic enzymes for more effective killing of microorganisms [37]. This results in the net increase in the thickness of the foot pad in previously immunized animals. This increment in footpad thickness of the Wistar albino rats that were subjected to *M. oleifera* extracts in this study

could be attributed to the ability of the extract to activate lymphocytes and their accessory cell types leading to enhancement in the production of antibodies in the previously immunosuppressed animals thereby increasing cell-mediated immunity. This was in line with other finding from previous studies done using the same plant [39]. *Moringa oleifera* has been found to contain vitamins A, C, and K [12, 25, 30]. These compounds stimulate the immune system by enhancing T-cell proliferation, increasing cytokine production and synthesis of immunoglobulins [42] all of which are important in the inflammatory response that was seen as an increment in the foot pad thickness. Amino acids also present in *M. oleifera* leaf extract are also important in the formation of immunoglobulins and major histocompatibility complexes which are essential in the mediation of the DTH reaction. Trace elements are also essential for the proliferation of the T-cells and Langerhan cells and the activity of the lytic enzymes which are important components of the DTH reaction to antigen. The results of the study therefore showed that the methanolic leaf extract of *M. oleifera* can be used to boost the immune system as there was a dose-dependent increment in paw size in response to antigen.

Effect on hemagglutination antibody titer

A hemagglutination test was performed to determine the effect of methanolic leaf extract of *M. oleifera* on the humoral immune response. Humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into plasma cells that secretes antibodies. Antibodies thus function as the effectors of the humoral response by binding to the antigens and neutralizing them or facilitating their elimination by cross linking to form clusters that are then ingested by phagocytic cells [38]. The study results demonstrated that methanolic leaf extract of *M. oleifera* had a stimulatory effect on the humoral immune response. This was evidenced by the mean hemagglutination antibody titer to SRBC that showed a dose-dependent increment for the rats dosed with *M. oleifera* leaf extract as compared to the rats that received only cyclophosphamide. Immunoglobulins and antigen-binding fragments are essential in the humoral immune responses that are products of amino acid chains and glycoproteins [24], most of which are present in *M. oleifera* leaf extract. Copper, which is also present in *M. oleifera*, is essential for the functioning of the enzyme ceruloplasmin which plays part in the humoral immune response. Other compounds such as fatty acids, zinc, vitamin C, vitamin B6, vitamin B12, manganese, and selenium are also essential for the maturation of the B-lymphocytes in the bone marrow [37]. Results from this study therefore demonstrated that the *M. oleifera* leaf extract contains compounds that can stimulate the production of antibodies in an immunocompromised animal. This may justify the common usage of the herb as an immune stimulant.

Conclusions

Based on the findings from the study, the methanolic leaf extract of *M. oleifera* increased both the cell-mediated and humoral immune responses in rats. This could be attributed to the different macronutrients, micronutrients and phytochemicals present in the plant. The methanolic leaf extract of *M. oleifera* therefore has a potential therapeutic value in several immunosuppressing clinical conditions and hence the reason for its use in local communities to alleviate various disease conditions.

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Figure 1.

Effect of different concentrations of *M. oleifera* methanolic leaf extract on the delayed hypersensitivity reactions in Wistar albino rats by measuring paw size.

N/S, normal saline; CYP 200, cyclophosphamide 200 mg/kg bwt; LEV 50, levamisole 50 mg/kg bwt; MO 250, *M. oleifera* leaf extract 250 mg/kg bwt; MO 500, *M. oleifera* leaf extract 500 mg/kg bwt; MO 1000, *M. oleifera* leaf extract 1000 mg/kg bwt.

Table 1

Group treatment of experimental animals for the study.

Group	Treatments		Study procedures		
	Day 0 treatment, mg/kg bwt	Route	Day 1–14 dosing, mg/kg bwt	Route	
I	–		–	–	CBC, DTH, HA titers, NA
II	CYP	200 s.c.	–	–	
III	CYP	200 s.c.	50 Lev	i.g.	
IV	CYP	200 s.c.	250 MO	i.g.	CBC
V	CYP	200 s.c.	500 MO	i.g.	DTH
VI	CYP	200 s.c.	1000 MO	i.g.	Neutrophil adhesion test
VII	CYP	200 s.c.	250 MO	i.g.	Hemagglutination antibody titer test
VIII	CYP	200 s.c.	500 MO	i.g.	
IX	CYP	200 s.c.	1000 MO	i.g.	

MO, *M. oleifera* methanolic leaf extract; CYP, cyclophosphamide; Lev, levamisole; CBC, complete blood count; DTH, delayed-type hypersensitivity test; NA, neutrophil adhesion test; s.c., subcutaneous injection; i.g., intragastric route.

Table 2

Effect of varying doses of methanolic leaf extract of *M. oleifera* on hematological parameters of Wistar albino rats taken on day 14 of the study.

Parameters	Extract, mg/kg bwt					
	Group I (N/S)	Group II (CYP)	Group III (Lev 50 mg/kg)	Group IV (250 mg/kg)	Group V (500 mg/kg)	Group VI (1000 mg/kg)
WBC ($10^3/\mu\text{L}$)	12.04 $\pm$ 1.30	10.76 $\pm$ 1.34	22.53 $\pm$ 0.6 ^a	7.663 $\pm$ 2.19	16.36 $\pm$ 0.21	21.43 $\pm$ 0.90 ^a
NEUT ($10^3/\mu\text{L}$)	2.10 $\pm$ 0.23	3.17 $\pm$ 0.43	11.20 $\pm$ 0.04 ^a	2.86 $\pm$ 0.86	0.26 $\pm$ 0.02	10.37 $\pm$ 0.49 ^a
LYMP ($10^3/\mu\text{L}$)	8.31 $\pm$ 1.39	0.45 $\pm$ 0.04	9.13 $\pm$ 0.29 ^a	6.50 $\pm$ 0.95	3.76 $\pm$ 1.05	9.26 $\pm$ 0.34 ^a
MON ($10^3/\mu\text{L}$)	1.04 $\pm$ 0.12	1.61 $\pm$ 0.33	1.53 $\pm$ 0.01	1.01 $\pm$ 0.36	0.89 $\pm$ 0.02	1.20 $\pm$ 0.09
EO ($10^3/\mu\text{L}$)	0.43 $\pm$ 0.09	0.11 $\pm$ 0.10	0.63 $\pm$ 0.00	0.03 $\pm$ 0.01 ^a	0.01 $\pm$ 0.00 ^a	0.51 $\pm$ 0.09
BAS ($10^3/\mu\text{L}$)	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.04 $\pm$ 0.01 ^a	0.01 $\pm$ 0.00	0.02 $\pm$ 0.00	0.04 $\pm$ 0.01 ^a
RBC ($10^3/\mu\text{L}$)	6.94 $\pm$ 0.48	6.45 $\pm$ 0.18	7.38 $\pm$ 0.72 ^a	6.22 $\pm$ 0.67	6.38 $\pm$ 0.23	5.05 $\pm$ 0.69
HGB (g/dL)	12.28 $\pm$ 0.71	11.06 $\pm$ 0.19	12.12 $\pm$ 0.19 ^a	11.38 $\pm$ 0.62 ^a	10.95 $\pm$ 0.31	8.15 $\pm$ 0.95
HCT (%)	37.48 $\pm$ 2.54	34.13 $\pm$ 0.23	36.27 $\pm$ 1.11 ^a	34.80 $\pm$ 1.81 ^a	35.35 $\pm$ 0.55 ^a	27.00 $\pm$ 2.65
PLT ($10^3/\mu\text{L}$)	809.0 $\pm$ 63.39	502.50 $\pm$ 80.52	1102 $\pm$ 128 ^a	1418 $\pm$ 141.8 ^a	1040 $\pm$ 259.9	1157 $\pm$ 300.0 ^a

Values expressed as mean $\pm$ SEM.

^a p 0.05, p values is in comparison to the means of the control Group II (cyclophosphamide only). CYP, Cyclophosphamide; Lev, levamisole; WBC, white blood cell count; NEUT, neutrophils; LYM, lymphocytes; MON, monocytes; EO, eosinophil; BAS, basophils; RBC, red blood cell count; PLT, platelet count. Group I, Normal control group; Group II, immunosuppressed, untreated group; Group III, immunosuppressed, intra-gastric levamisole 50 mg/kg bwt; Group IV, immunosuppressed, daily intragastric 250 mg/kg bwt of extract; Group V, immunosuppressed, daily intra-gastric 500 mg/kg bwt of extract; Group VI, immunosuppressed, daily intragastric 1000 mg/kg bwt of extract.

Table 3

Effect of varying concentrations of *M. oleifera* methanolic leaf extracts on the neutrophil adhesion of Wistar albino rats.

Group	Dose, mg/kg	Mean, % Neutrophil		Neutrophil adhesion (%)
		Before treatment	After treatment	
Group I	NS	18.73±2.60	17.55±2.82	55.24 ^a
Group II	CYP 200	27.73±1.26	26.85±1.61	-12.71
Group III	LEV 50	49.71±0.31	40.13±2.8	30.78 ^a
Group IV	MOE 250	33.18±0.92	39.28±3.53	-7.10
Group V	MOE 500	35.65±0.94	29.90±1.28	17.28 ^a
Group VI	MOE 1000	48.58±0.60	42.05±0.41	29.94 ^a

Values are presented as mean±SEM (standard error of the mean);

^a p < 0.05; CYP, cyclophosphamide; LEV, levamisole; MOE, *M. oleifera* leaf extract; NS, normal saline; comparisons were done with the means of control Group II.

Table 4

Effect of different doses of *M. oleifera* methanolic leaf extracts on the of humoral antibody response to SRBC as determined by hemagglutination antibody titers in Wistar albino rats.

Groups	Treatment	Dose	Mean hemagglutination antibody titer ($\pm$ SEM) HA units/ μ L
I	Normal saline	10 mL/kg bwt	6.32 $\pm$ 0.73
II	Cyclophosphamide	200 mg/kg bwt	4.21 $\pm$ 0.35
III	Levamisole	50 mg/kg bwt	24.79 $\pm$ 1.18 ^a
VII	Methanolic extract of <i>M. oleifera</i>	250 mg/kg bwt	10.73 $\pm$ 0.57 ^a
VIII	Methanolic extract of <i>M. oleifera</i>	500 mg/kg bwt	18.25 $\pm$ 1.24 ^a
IX	Methanolic extract of <i>M. oleifera</i>	1000 mg/kg bwt	26.22 $\pm$ 1.70 ^a

Values expressed as mean $\pm$ SEM;

^a p = 0.05 compared with the cyclophosphamide only group (Group II).