



DOI Number: 10.5958/2277-940X.2015.00047.9

Immunomodulatory Activity of *Moringa oleifera* in Albino Rats

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Received: 03 March, 2015

Accepted: 17 May, 2015

ABSTRACT

The study was conducted to evaluate immunomodulatory property of hot aqueous and ethanolic extracts of *Moringa oleifera* in albino rats. This study comprised of six groups containing six rats in each group. Group I served as control, received standard feed and water. Group II and IV received hot aqueous extract of *Moringa oleifera* @ 400 mg/kg b.wt, orally for 45 days. Group III and V received ethanolic extract of *Moringa oleifera* @ 400 mg/kg b.wt, orally for 45 days. Group VI received standard immunomodulatory drug Levamisole @ 50 mg/kg b.wt, orally for 45 days. A significant increase ($P < 0.05$) in HA titre was observed in levamisole treated group VI, ethanolic extract treated group V and hot aqueous extract treated group IV of *Moringa oleifera* as compared to group I. In delayed hypersensitivity test a significant increase ($P < 0.05$) in paw volume was observed in response to Sheep Red Blood Cells (SRBC's) in group II (hot aqueous extract), group III (ethanolic extract) and group VI (levamisole). Phagocytic index was also found to be non-significantly increased in levamisole treated group VI, ethanolic extract treated group III and hot aqueous extract treated group II as compared to group I. Thus the results showed that *Moringa oleifera* possess immunomodulatory property.

Keywords: *Moringa oleifera*, immunomodulation, phagocytic index, extracts

Herbs of immunomodulatory properties are relatively recent concept in phytomedicine. These plants provide alternative approach to conventional chemotherapy for a variety of illness. Immuno-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 entire immune system, but affect primarily cell-mediated immunity, while the humoral immunity may be affected indirectly (Goldsby *et al.*, 2000). *Moringa* leaves have been reported to be a rich source of β -carotene, protein, vitamin C, calcium, potassium and act as good source of natural antioxidants like ascorbic acid,

flavonoids, phenolics and carotenoids that work mutually to strengthen immunity (Akhouri *et al.* 2014a). Hence, the study was undertaken to assess the immunomodulatory activity of *Moringa oleifera* leaf extracts in albino rats.

MATERIALS AND METHODS

Location

The study was conducted in department of Veterinary Pharmacology and Toxicology, Nanaji Deshmukh Veterinary Science University, Jabalpur, India.

Animals

The experiment was conducted on 36 healthy inbred Norwegian strains of albino rats of 6-8 weeks old of either sex, weighing 120 to 150 g. These rats were provided with



ad libitum balanced feed (Baghel, 1999) and water. They were housed under hygienic conditions in polypropylene cages and maintained at $22\pm 1^\circ\text{C}$ under a 12 hour light dark cycle. This study was approved by Institutional Animal Ethical Committee (No.71/IAEC/CWFH/2013)

Indigenous Plants

Fresh leaves of *Moringa oleifera* was procured in bulk around the Veterinary College campus, Jabalpur and authenticated from expert of Government Agriculture College. They were washed, shade-dried and grounded into fine powder and processed to obtain the aqueous and ethanolic extracts. Hot aqueous extract was prepared by boiling one gram of leaf powder in 10 ml sterile distilled water for ten minutes over boiling water bath Akhoury *et al.* (2014b). Ethanolic extracts of *Moringa oleifera* were prepared using Soxhlet apparatus as per the method described by Pandey and Shrivastava (1989).

Experimental Design

This study comprised of six groups containing six rats in each group. Group I served as control received standard feed and tap water. Group II and IV received hot aq. ext. of *Moringa oleifera* @ 400 mg/kg b.wt, orally for 45 days. Group III and V received ethanolic extract of *Moringa oleifera* @ 400 mg/kg b.wt, orally for 45 days. Group VI received standard immunomodulatory drug Levamisole @ 50 mg/kg b.wt, orally for 45 days.

Preparation of Sheep Red Blood Cells (SRBCs) Antigen:

SRBCs were collected from jugular vein of sheep and stored in a cold sterile Alsever's solution for immunization and challenge, at required time schedule. Stored sheep red blood cells were centrifuged and washed three times with pyrogen free sterile normal saline (0.9 % NaCl w/v) and adjusted to a required concentration of 0.5×10^9 and 0.025×10^9 SRBC/ml (Doherty, 1981).

Delayed type hypersensitivity (DTH) response to SRBC:

After administration of the extract for 30 days, CMI was seen on 30th, 37th and 44th day and were studied by

delayed type hypersensitivity (DTH) reaction to Sheep Red Blood Cells (SRBC) following the method of Doherty (1981) with some modification. Six rats from each group were immunized by injecting 0.5ml of 5×10^9 SRBC, intraperitoneally on 30th day. The rats were then challenged by subcutaneous administration of 0.025ml of 5×10^9 SRBC/ml into the left hind footpad on 37th day and 44th day for primary and secondary immune response respectively. DTH was measured at 24 hour after the SRBC challenge and expressed as mean per cent increase in paw volume with the help of plethysmometer.

Carbon Clearance Assay (Mononuclear Phagocytic System Function Assay):

The Phagocytic ability of albino rats was determined by Carbon Clearance Assay (CCA) based on method of Jayathirtha and Mishra (2004). Supernatant fraction of Black India Ink was obtained through centrifugation (5000 rpm) for 30 minutes. Animals of group II and III were given extract daily for 7 days. After administration of extract for 7 days, the rats were injected via tail vein with carbon ink suspension (10 μ l/g body weight) on 9th day. Blood samples were drawn (in EDTA solution, 5 μ l) from the retro orbital plexus at 0 and 15 minutes. A 25 μ l sample was mixed with 0.1% sodium carbonate solution (2 ml) and its optical density was measured at 680 nm. The phagocytic index (K) was calculated using the equation:

$K = (\log OD_1 - \log OD_2) / 15$ where, OD1 and OD2 are optical densities at 0 and 15 minutes.

Haemagglutination test

Humoral immune response was evaluated by determining the HA titer of rats against SRBC as per the method described by Fulzele *et al.* (2002). The rats were immunized with SRBC on day 30th and 37th. Two ml blood was collected on day 38th and 45th without any anticoagulant for separation of serum.

Statistical Analysis

Statistical analysis was performed according to the method described by Snedecor and Cochran (1994). Data was analyzed by Factorial Design. Significant differences between means of group and interval was compared by DMRT (Duncan's Multiple Range Test).

Table 1. Effect of *Moringa oleifera* leaf extracts on paw volume (%)

Days	Group I (control)	Group II (aqueous)	Group III (ethanolic)	Group VI (levamisole)
7	4.3 ^c ± 0.33	7.8 ^{bc} ± 0.83	8.0 ^{bc} ± 0.82	7.8 ^{bc} ± 0.91
14	6.2 ^c ± 0.60	10.5 ^b ± 1.36	11.3 ^{ab} ± 0.80	14.3 ^a ± 2.52

Mean ± SE with different superscript differ significantly (P <0.05) among both the group and interval

RESULTS AND DISCUSSION

Delayed hypersensitivity was assessed, using SRBC as allergen after administration of the extracts for 30 days. The mean values of paw volume are shown in table 01. The values of paw volume showed significant variation in all groups and intervals. The paw volume increased in a time dependent manner in group II and III as compared to control group. On day 14 significantly higher value (P <0.05) was found in group VI as compared to day 7, denoting increase in the cell mediated immunity.

The phagocytic ability of albino rats was determined by carbon clearance assay. The mean values of phagocytic index are shown in table 02. The phagocytic index was higher in levamisole treated group VI as compared to control and other groups. However, increase in values were non significant.

Table 2. Effect of *Moringa oleifera* leaf extracts on phagocytic index

Group	Phagocytic index
I (control)	0.018 ± 0.0022
II (aqueous)	0.023 ± 0.0017
III (ethanolic)	0.042 ± 0.0026
VI (levamisole)	0.088 ± 0.0143

The mean values of HA titer are exhibited in table 03. The values of HA titer showed significant variation in all groups and intervals. The HA titer increased in a time dependent manner (P <0.05) in group IV (aqueous) and group V (ethanolic). However, group VI (levamisole), which was considered as standard immunomodulator showed significantly higher HA titer on day 14 as compared to control group.

Table 3. Effect of *Moringa oleifera* leaf extracts on haemagglutination titer

Days	Group I (control)	Group IV (aqueous)	Group V (ethanolic)	Group VI (levamisole)
7	2.7 ^c ± 0.42	4.3 ^c ± 0.80	6.0 ^c ± 0.89	6.7 ^{bc} ± 0.84
14	3.3 ^c ± 0.42	10.7 ^{ab} ± 1.69	10.7 ^{ab} ± 2.46	12.0 ^a ± 1.79

Mean ± SE with different superscript differ significantly (P <0.05) among both the group and interval.

Cell mediated immunity is modulated by thymus-derived lymphocytes (T lymphocytes) which are sensitized by antigen and on subsequent contact they responded with a delayed type hypersensitivity reaction. Activation of T cells released lymphokines, which lead to activation and accumulation of macrophages and thus increased concentration of lytic enzymes for more effective killing and this ultimately resulted in increase in paw volume in immunized animals. Significantly higher values were observed in group VI (levamisole) followed by similar values in group III (ethanolic extract) group II (hot aq. ext.).

The highest HA titer was shown by standard levamisole group VI on 14th day followed by similar HA titer in group II (hot aq. ext.) and group III (ethanolic ext.). This potentiation of haemagglutinating antibody titer indicated that drugs used in the experiment induced immunostimulation through humoral immunity.

The immunomodulatory activity of *Moringa oleifera* may be attributed to the presence of an array of phytochemicals. The tropane alkaloids in the leaf extracts of *Moringa oleifera* are metabolised in the liver into dimethylxanthine and finally methyl uric acid by cytochrome P450



oxygenase systems. Methyl uric acid in liver stimulates the expression of tumour necrosis factor (in the endothelial cells of the liver by macrophages), which modulates the immune system (Yoshida, 1997). Also, saponins are implicated in the modulation of 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 antigen-presenting mast cells. (Oda *et al.*, 2000; Zahid *et al.*, 2007). Nutrients affect immune function by directly acting within the lymphoid system, or indirectly by affecting cellular material or other organ systems that act as immune regulators. The presence of minerals such as selenium, zinc, iron, manganese and magnesium in the leaf extract 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.

Moringa oleifera contains phenylalanine, lysine, arginine, methionine and histidine etc. Arginine is known to augment cellular immunity, enhance lymphocyte and macrophage proliferation (Ferreria *et al.*, 2008). Vitamin A present in *Moringa oleifera* could improve the competence of immune system as it has been documented that vitamin A facilitates lymphocyte proliferation and increases antibody production (Park *et al.*, 1992). The presence of flavonoids in leaf extracts may be a contributing factor to its immune boosting qualities as they are particularly known to activate lymphocytes.

The highest phagocytic index was found in group VI (levamisole) followed by group III (ethanolic) and group II (aqueous). Phagocytes play an integral role in host defence and produces reactive oxygen species by macrophages reaction to foreign agents in the body. Phagocytic cells are sensitive to oxidative damage thus affecting the balance between prooxidant production and antioxidant defence in cell function. Disturbance in this balance in oxidants represents an oxidative stress. The presence of phenolic compounds in leaf extract may help in preventing oxidative stress by scavenging free radicals and bio-activation of carcinogens for excretion in liver (Khanna *et al.*, 2002).

Phenolic compounds are implicated to scavenge nitric oxide molecule directly, thereby preventing the oxidation of low density lipoprotein cholesterol (LDL-C) and tissue oxidative damage. 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 the immobilization and adhesion of leukocytes to endothelial wall and degranulation of neutrophils without affecting superoxide production, thereby regulating inflammatory responses in tissue injury and immune responses (Ferrandiz *et al.*, 1996). This study revealed a marked effect on immunomodulatory activity of levamisole (group VI), ethanolic extract (group III) and aqueous extract (group II) of *Moringa oleifera*. Increased in HA titer was exhibited by ethanolic and aqueous extract treated group on day 7 and 14. Delayed hypersensitivity test also marked a similar trend in cell mediated immune response. Phagocytic index was found to be increased in levamisole, ethanolic and aqueous extract treated group.

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