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RESEARCH ARTICLE

Ethonolic Leaf extract of Moringa oleifera L. has Immuno-stimulatory Action in Albino Rats

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ABSTRACT

Medicinal plants are important sources of different secondary metabolites most of which have immune-stimulatory action. To investigate the immune-stimulatory action of ethanolic leaf extract of Moringa oleifera L. in an experimental animal models (albino rats). The cellular immunity was evaluated using Neutrophil Adhesion Test, Cyclophosphamide-Induced neutropenia and carbon clearance assay. Whereas, serum immunoglobulin estimation and Indirect haemagglutination assay was also performed. The results showed that the plant extract possessed significant (P<0.05) immune-modulatory activity.

Keywords: *Immuno-modulatory, Moringa oleifera, immunity, neutropenia, haemagglutination.*

INTRODUCTION

Medicinal plants are a rich source of substances that are claimed to induce immunity [**1**]. *Moringa oleifera* L. family Moringaceae commonly known as Saguna, Sainjna (Hindi) and Drum stick tree (English) is a medicinal plant [2] and leaf of this plant are traditionally used as cardiac tonic; root and root bark are considered carminative, stomach ache [3]. Leaves are also used in the treatment of pain, ulcer, fever, pectoral cough, asthma and other bronchial disorders [4]. However, there is a paucity of data available on the effect of the extract of *Moringa oleifera* L. leaf on humoral-immune response, cyclophosphamide-induced myelo-suppression and phagocytic function of the cells of the reticulo-endothelial system. Therefore, the present study was undertaken to investigate the immune-modulatory effect of ethanolic leaf extract of *Moringa oleifera* L. The immune system is involved in the etiology, as well as pathophysiology mechanism of many diseases. Modulation of immune responses to alleviate various diseases has been of interest for many years. Medicinal plants are rich sources of substances which are non-specific immune modulation of essentially granulocytes macrophages, natural killer cells and complement functions [5]. Because of the concerns about the side effect of conventional drugs, the use of natural products as an alternative to conventional treatment in the healing and treatment of various diseases has been on the rise in the last few decades [6]. The present study is an attempt to find out the immune-modulatory activity of *Moringa oleifera* L. leaves.

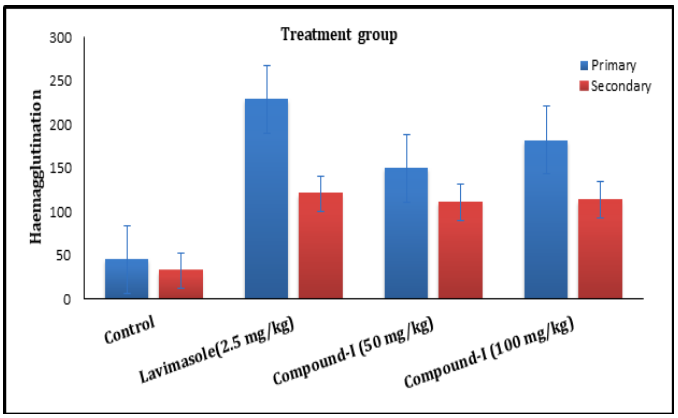
MATERIAL AND METHODS

The Plant material *Moringa oleifera* L. was collected from the roadsides of Alirajpur District M.P. and a major quantity of the plant material was collected from fields of the local village of Alirajpur namely Udaigarh some about 45 km from Alirajpur in the Month of December – January 2017. The plant was then identified and authenticated by Dr. Shah Khalid

Table 1: Effect of Isolated Compound-I on Haemagglutination Antibody Titer

Group[n]	Treatment	Haemagglutination Antibody Titer	
		Primary (1^0)	Secondary (2^0)
I	Control (PBS pH 7.4)	44.66 ± 6.74	32.0 ± 0.0
II	Levamisole (2.5 mg/kg b.w.)	228.23 ±28.62***	120.12 ± 12.11***
III	Compound-I (50 mg/kg b.w.)	149.59 ± 23.34*	110.31 ± 32.01*
IV	Compound-I (100 mg/kg b.w.)	181.43 ± 34.72**	113.21 ± 13.71**

Values are expressed as Mean ± SEM; * P<0.05 as compared to control.



Graph 1: Showing the effect of Compound-I on Haemagglutination Antibody Titre (Primary and Secondary).

Lecturer of Botany Government Degree College Uri Baramulla and Specimen Voucher No. 267 was put in the Department of Chemistry, Government Degree College Ganderbal (Jammu and Kashmir).

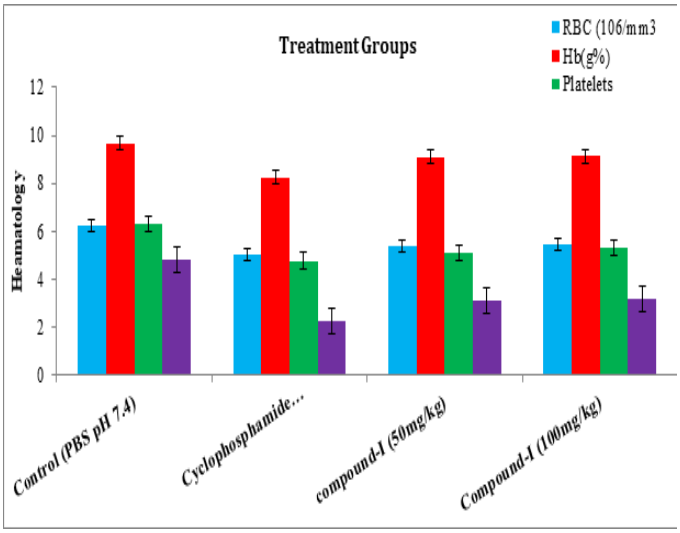
Selection of doses

From the acute oral toxicity studies as per the OECD guidelines 423, no mortality or other behavioral and morphological changes were observed at all doses up to a level of 2000 mg/kg b.w. of ethanolic extract *Moringa oleifera* L. in albino rats. Thus the two different doses selected for test groups were 1/10th (200mg/kg BW) and 1/5th (400 mg/kg BW) of the safe dose. Levamisole has shown good immunomodulatory activity at a dose of 2.5 mg/kg BW in albino rats, the same dose was selected for study as a standard drug. Cyclophosphamide was used as an immunosuppressant drug at a dose of 200 mg/kg b.w. (a neutropenia dose).

Table 2: Effect of isolated Compound-I on Cyclophosphamide induced myelosuppression (Hematology)

Group[n]	Treatment	RBC (10^6/ mm^3)	Hb (g%)	Platelets	WBC (10^3/ mm^3)
I	Control (PBS pH 7.4)	6.232 ± 0.070	9.683 ± 0.101	6.300 ± 0.057	4.800 ± 0.096
II	Cyclophos-phamide (30 mg/kg)	5.023 ± 0.056	8.250 ± 0.136	4.767 ± 0.088	2.267 ± 0.244
III	Compound-I (50 mg/kg)	5.390 ± 0.183	9.100 ± 0.068	5.083 ± 0.068	3.117 ± 0.075
IV	Compound-I (100 mg/kg)	5.433 ± 0.169	9.117 ± 0.070	5.317 ± 0.124	3.150 ± 0.076

Values are expressed as Mean ± SEM; * P<0.05 as compared to control.



Graph 2: Showing the effect of Compound-I on Cyclophosphamide Induced Myelosuppression (Hematology)

Preparation of the test extract

Ethanol fraction was suspended in dimethyl sulphoxide (DMSO) to prepare different doses (200 and 400 mg/kg body weight) and administered orally with the help of gastric cannula. The control animals were given an equivalent volume of Phosphate buffer Saline (PBS pH 7.4) vehicle.

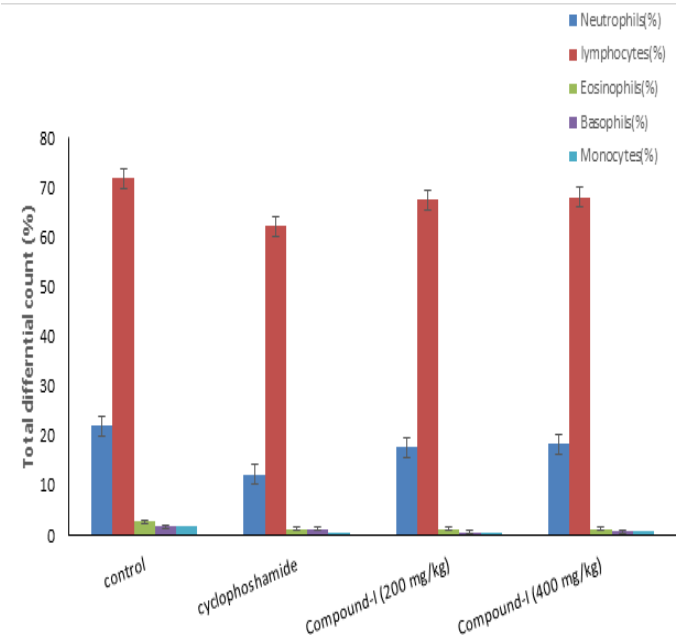
Antigen

Fresh blood was collected from sheep sacrificed in a local slaughter house in Alsever's solution (the formula is mentioned ahead). During the experiment, an adequate amount of stock solution (sheep red blood cells (SRBC) stored in Alsever's solution) was

Table 3: Effect of isolated Compound-I on Cyclophosphamide induced myelosuppression (Total differential count %)

Group[n]	Treatment Group	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)
I	Control (PBS pH 7.4)	22.00 ± 0.577	71.83 ± 0.654	2.667 ± 0.210	1.500 ± 0.223	1.667 ± 0.210
II	Cyclophosphamide (30 mg/kg)	12.17 ± 0.703	62.17 ± 0.477	0.666 ± 0.210	0.166 ± 0.166	0.167 ± 0.167
III	Compound-I (50 mg/kg)	17.67 ± 1.085	67.50 ± 0.845	1.167 ± 0.401	0.333 ± 0.211	0.333 ± 0.211
IV	Compound-I (100 mg/kg)	18.33 ± 1.054	68.00 ± 0.365	1.267 ± 0.401	0.500 ± 0.223	0.661 ± 0.210

Values are expressed as Mean ± SEM; * P<0.05 as compared to control.



Graph 3: Showing the effect of Compound-I on Cyclophosphamide induced myelosuppression (Total differential count %).

taken and allowed to stand at room temperature. It was washed three times with normal saline. The settled SRBCs were then suspended in normal saline and RBCs of this suspension were adjusted to a concentration of 5×10^9 SRBC/ml for immunization and challenge [7].

Preparation of Alsever's solution

Formula:
Citric acid 0.055gm
Sodium citrate 0.8gm
Glucose 2.05gm
Sodium chloride 0.42gm
Distilled water to make volume up to 100 ml
All the above solids were weighed and dissolved in distilled water in a conical flask and made the volume up to 100 ml. It was then stored in the refrigerator.

Blood Withdrawal

For withdrawing the blood samples, the animals were lightly anesthetized using ethyl ether. A fine capillary was gently inserted into the lower angle of the eye at 45°C and blood was withdrawn from retro-orbital plexus into micro centrifuge tubes.

Immunomodulatory Protocols

A) SRBC–Induced Humoral Antibody (HA) Titre

The method described by [8] was followed. Groups of six rats per treatment were immunized by injecting 20µl of SRBC suspension (5×10^9 SRBC /ml) subcutaneously into the right hind footpad. Seven days later they were challenged by injecting 20µl of SRBC suspension (5×10^9SRBC /ml) intradermal into the left hind footpad. The day of immunization was referred to as day 0. Blood samples were collected from all the animals separately by a retro orbital puncture on day +7 (before challenge) for primary antibody titre and on day +14 for secondary antibody titre. Antibody levels were determined by the method described by [9-16]. Briefly 25µl aliquot of serum of each animal was taken in microtitre plates. To serial two-fold dilutions of pooled serum (made in 25µl normal saline), 25µl of 1% v/v SRBC suspension (in normal saline) was added. The microtitre plates were kept at room temperature for 1 hour and then observed for haemagglutination (until control wells showed unequivocally negative pattern). The value of the highest serum dilution showing haemagglutination was taken as the antibody titre. Ethanolic extract isolated compounds were fed orally once daily, starting with 7 days before sensitization till the challenge.

RESULT AND DISCUSSION

Immuno-stimulatory Activity

Searching of substances with immune-simulative or immune-restorative effects could contribute to the maintenance of the immune system. Immune modulation helps in maintain disease Free State of the body. Many plants have been evaluated for immune-stimulant and immunosuppressive properties using simple techniques. An attempt has been made to evaluatetheimmunomodulatoryactivityoftheisolated bioactive compounds that showed potent antioxidant activity using different by investigating its effect on both humoral as well as cell-mediated immunity using different models as: Haemagglutination antibody titre, cyclophosphamide induced myelo-supression, delayed type hypersentivity and phagocytic index.

Haemagglutination Antibody Titre

The effect of ethanolic extract of *Moringa oleifera* L. administration as such is shown in **(**Table 1 and Graph 1**)** Haemagglutination antibody was determined to establish the humoral response against SRBC. The purified isolated compounds showed a significant increase ($P<0.05$) in HA titer value compared to control at a dose of 100 mg/kg b.w.

Cyclophosphamide Induced Myelosuppression

Cyclophosphamide at the dose of 30 mg/kg (intraperitoneal) caused a significant reduction in total WBC count, differential leukocyte counts and platelets and a marginal reduction in RBC and Hb% as compared to the control group (Group-I), the results are represented in (Table 2, 3 and Graph 2, 3**).**

CONCLUSION

The results of the present study showed that *Moringa oleifera* L. leaf extracts a potent immunostimulant, stimulating specific and non-specific immune mechanisms. It may be due to the presence of various phytoconstituents present in *Moringa oleifera* L.like phenolics, flavonoids, tannins, and alkaloids, which are already reported to possess immunomodulatory activity.

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