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ORIGINAL ARTICLE **OPEN ACCESS**

Pharmacological Importance (Immuno-stimulatory embellishment) of Leaf extracts of *Moringa Oleifera* L in Albino Rats

Bilal Ahmad Dar*[1], Mukhtar Ahmad Wani[2], Kawsar Jan[3], Abdul Rashid Teli[4], Shah Khalid[5], Fayaz Ahmad Sheikh[6],Hilal-Ul-Zaman[7],Muzafar Ahmad Sheikh[8]
[1, 6] Department of Chemistry, Government Degree College Ganderbal Kashmir.
[2,7]Department of Chemistry, Government Degree College Handwara Kashmir
[3]Department of Bio-Science, Cluster University Srinagar Kashmir.
[4]Department of Chemistry, Government Degree College Women's Kupwara Kashmir
[5]Department of Botany, Government Degree College Uri Baramulla Kashmir.
[8]P.G.Department of Zoology, SP. College, Cluster University Srinagar.
Corresponding Author: Dr. Bilal Ahmad Dar
ORCID and Email: http://orcid.org/0000-0002-5449-3002
bilaldar87@gmail.com

ABSTRACT
The majority of the secondary metabolites found in medicinal plants have immune-stimulating properties. To examine the immune-stimulatory effects of methanolic Moringa oleifera L. leaf extract in experimental animal models (albino rats).Utilizing the neutrophil adhesion test, neutropenia brought on by cyclophosphamide, and the carbon clearance assay,, the cellular immunity was assessed. Whereas the indirect haemagglutination assay and serum immunoglobulin quantification were also carried out. The outcomes demonstrated that there was considerable (P<0.05) immune-modulatory action in the plant extract.
Key words: *Immuno-modulatory, Moringa oleifera, immunity, neutropenia, haemagglutination.*

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INTRODUCTION

There are many compounds found in medicinal plants that are said to boost immunity [1]. Saguna, Sainjna, and Drumstick Tree are all names for the medicinal plant *Moringa oleifera* L [2-3]. Family Moringaceae. Its leaves have historically been used as heart tonics, and its roots and root bark are said to relieve stomachaches. Asthma, chest cough, pain, ulcer, fever, and other bronchial problems are all treated with leaves [4]. There is, however, a dearth of information on the impact of *Moringa oleifera* L. leaf extract on humoral-immune response, myelo-suppression caused by cyclophosphamide, and phagocytic activity of the cells in the reticulo-endothelial system. The goal of the current investigation is to determine whether the methanolic leaf extract of *Moringa oleifera* L. has any immune-modulating properties. The pathophysiology and genesis of many diseases are both influenced by the immune system. There has long been interest in altering immune responses to treat various diseases. The non-specific immunological regulation of granulocytes, macrophages, natural killer cells, and complement activities is abundant in medicinal plants [5]. The use of natural products as an alternative to conventional medicine in the healing and treatment of many ailments has increased over the past few decades[6] due to worries about the adverse effects of conventional pharmaceuticals. The goal of the current study is to determine whether *Moringa oleifera* L. leaves have immune-modulatory properties.

MATERIAL AND METHODS

A substantial portion of the plant material, *Moringa oleifera* L., was taken from fields in the Alirajpur hamlet of Udaigarh, which is about 45 kilometres from Alirajpur, and from the sides of the roadways in the M.P. district of Alirajpur. Dr. Shah Khalid, a lecturer in botany at the government degree college in Uri Baramulla, later identified and verified the plant, and specimen voucher no. 267 was placed in the department of chemistry at the government degree college in Ganderbal (Jammu and Kashmir).

Selection of doses

According to the OECD standards 423 for acute oral toxicity studies, albino rats dosages of *Moringa oleifera* L. ethanolic extract on body weight with dose of 2000 mg/kg did not result in any deaths or other behavioral or morphological alterations. As a result, the two doses chosen for the test groups were 1/10th and 1/5th of the safe dose. Levamisole has demonstrated good immune-modulatory effect in albino rats with dose of 2.5 mg/kg BW; hence, this dose was chosen for the trial as a reference medication. 200 mg/kg B.W of cyclophosphamide was given as an immunosuppressant (a neutropenia dose).

$1/10^{th}$ dose = (200 milligrams per Kg of body weight (200 mg/kg BW)

$1/5^{th}$ dose= (400 milligrams per Kg of body weight (400 mg/kg BW)

Preparation of the test extract

Dimethyl sulphoxide (DMSO) was used to suspend the methanol fraction in order to prepare separate 200 and 400 milligrams of prepared doses to per Kg body weight), which were then given orally with the aid of a gastric cannula. Animals allowed to serve as controls received an appropriate volume of Phosphate Buffer Saline with pH of 7.4.

Antigen

Fresh blood from sheep slain in a nearby slaughterhouse was collected and then used in Alsever's solution. Sheep red blood cells (SRBC) that had been kept in an equal quantities of Alsever's solution during the experiment were extracted and allowed to stand at room temperature. The settling SRBCs were then regulated to a concentration of 5 109 SRBC/ml in normal saline solution for immunization and challenge [7].

Preparation of Alsever's solution

Weight exact quantities of 0.055 grams of Citric acid, 0, 8 grams of sodium citrate, and 2.05 grams of glucose and then pour these chemical substances into small volumes of distilled water so that chemical substances get dissolved then cover the volume up to 100 ml in a volumetric flask and ten stores the stock solution in the refrigerator.

Blood Withdrawal

The selected animals were anaesthetized with ethyl ether to collect the blood samples from the animals. The blood samples collection technique introduces a fine capillary tube inserted in the eye with an angle of 45 ^{0}C and then the sample was collected into micro centrifuge tubes from the retro-orbital plexus

Immunomodulatory Protocols

A) SRBC–Induced Humoral Antibody (HA) Titre

The procedure defined by [8] were used. The right hind footpad of each group of six rats received a subcutaneous injection of 20 ml of SRBC suspension (5 x 10^9 SRBC/ml). They were put to the test seven days later by having 20 ml of intradermal SRBC suspension (5 x 10^9 SRBC/ml) injected into the left hind footpad. The first day was the day of vaccination, or day 0. On day +7 (the day before the challenge) for the main antibody titre and on day +14 for the secondary antibody titre, blood samples were taken from each animal independently using a retro orbital puncture. The procedure described by was used to determine antibody levels [9]**.** All animal's serum was separated into a 25 ml aliquot, which was then put in microtiter plates. 25 l of 1% v/v SRBC suspension (in normal saline) were added to repeated two-fold dilutions of pooled serum produced in normal saline (25 ml). After an hour at room temperature, the microtiter plates were checked for hemagglutination (until control wells showed unequivocally negative pattern). The antibody titre was calculated using the greatest serum dilution to exhibit haemagglutination. Once daily oral doses of isolated ethanolic extract components were administered beginning 7 days before to sensitization and continuing until the challenge.

RESULT AND DISCUSSION

Immuno-stimulatory Activity

The search for chemicals with immuno-simulative or immune-restorative properties may help to keep the immune system healthy. Immune regulation aids in keeping the body in a disease-free state. Using straightforward methods, the immune-stimulating and immunosuppressive characteristics of several plants have been assessed. By examining its impact on both humoral and cell-mediated immunity using various models, including haemagglutination antibody titre, cyclophosphamide-induced myelosuppression, delayed type hypersentivity, and phagocytic index, an effort has been made to evaluate the immunomodulatory activity of the isolated bioactive compounds that showed potent antioxidant activity.

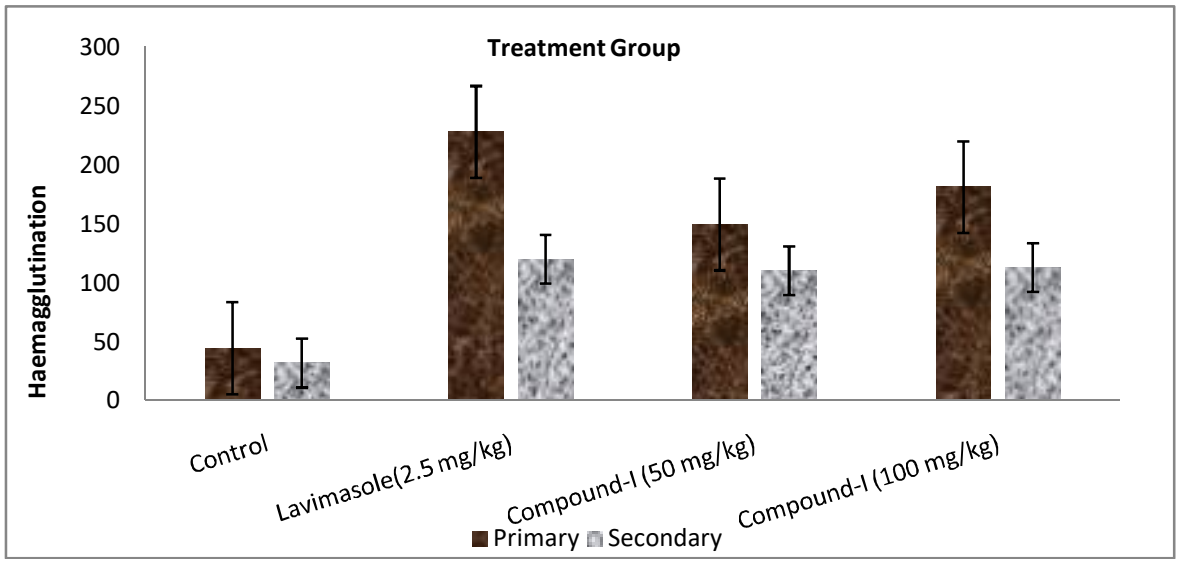
Haemagglutination Antibody Titre

The outcomes of injecting the methanolic extract of *Moringa oleifera* L. are presented in (Table 1 and Graph 1). The humoral reaction against SRBC was established using the haemagglutination antibody. At a dose of 100 mg/kg b.w., the extracted purified compounds significantly (P<0.05) increased the HA titer value compared to the control.

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).

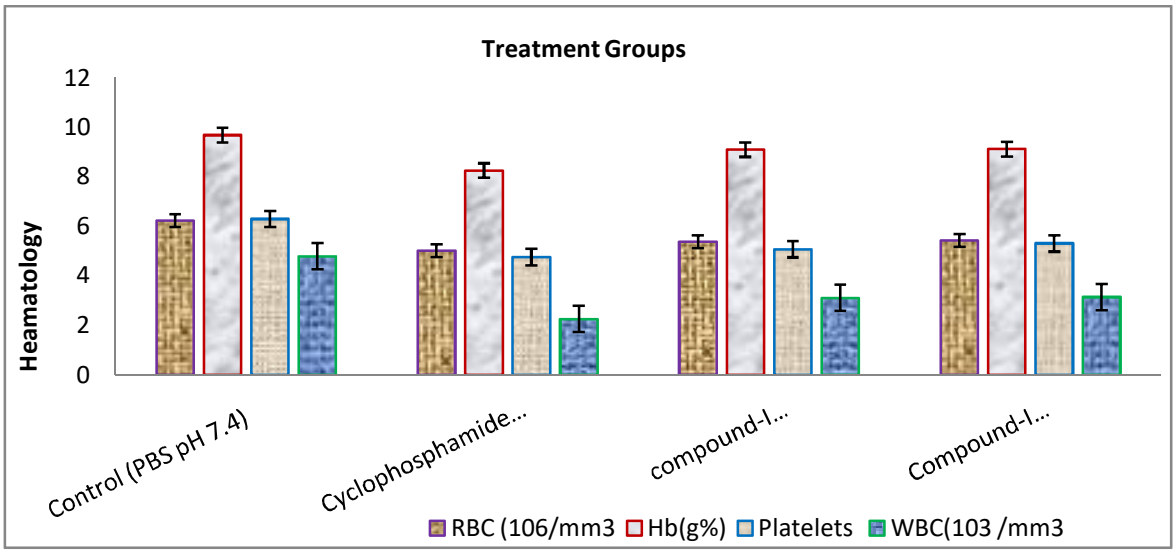
Cyclophosphamide Induced Myelosuppression

When compared to the control group (Group-I), cyclophosphamide at a dose of 30 mg/kg (intraperitoneal) strongly reduced the entire WBC count, selective leukocyte counts, and platelets, and only gradually reduced the RBC and Hb%. The results are shown in the table below **(Table 2, 3 and Graph 2, 3)**.

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	Cyclophosphamide (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.

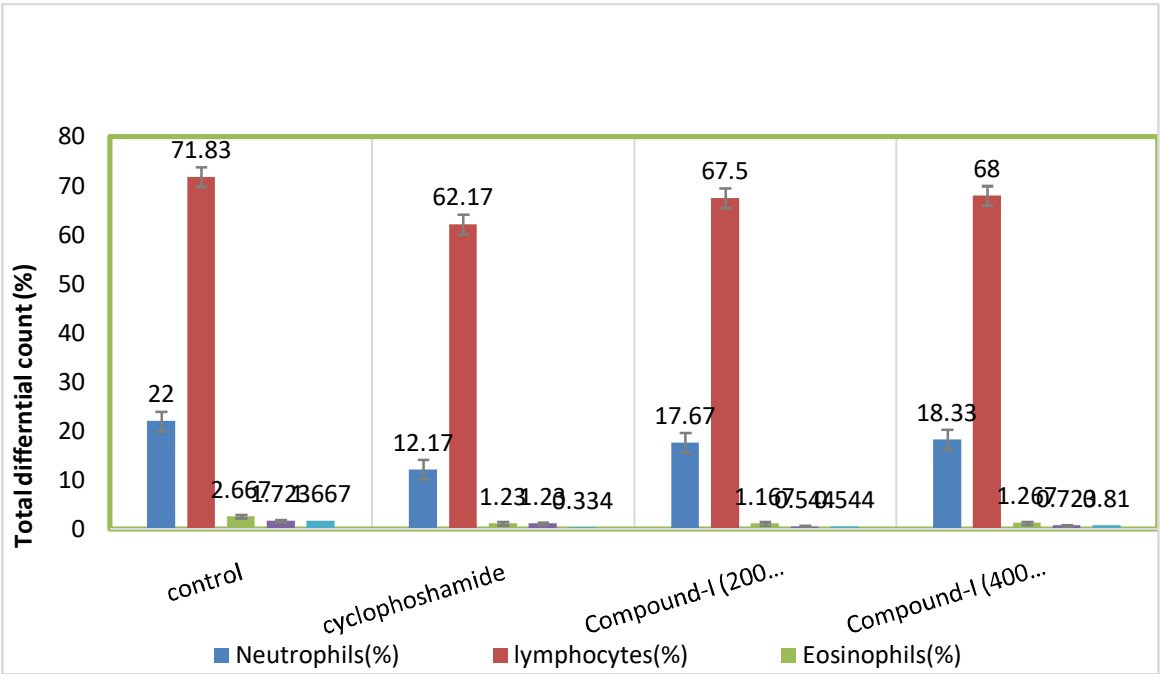


Graph 2: Showing the effect of Compound-I on Cyclophosphamide Induced Myelosuppression (*Hematology*)

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 %).*

CONCLUSION

According to the findings of the current investigation, *Moringa oleifera* L. leaf extract in methanol is a powerful immunostimulant that boosts both particular and general immune processes. It might be because *Moringa oleifera* L. contains a number of phytoconstituents, such as phenolics, flavonoids, tannins, and alkaloids, which have already been shown to have immunomodulatory effect.

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