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# Preliminary Immunomodulatory Activity of Aqueous and Ethanolic Leaves Extracts of *Ocimum basilicum* Linn in Mice

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**ABSTRACT: OBJECTIVE:** To study the immunomodulatory activity of aqueous and ethanolic extracts of leaves of *Ocimum basilicum* Linn. (Family: Lamiaceae) in mice.
**METHODS:** The aqueous and ethanolic extract of leaves of *Ocimum basilicum* was administered orally at the dosage levels of 400 mg/kg/day body weight in mice. The assessment of immunomodulatory activity on specific and non-specific immunity were studied by haemagglutination antibody (HA) titer, delayed type hypersensitivity (DTH), neutrophil adhesion test and carbon clearance test. In order to induced immunosuppresion in mice by using cyclophosphamide (100 mg/kg/day, p.o.) and levamisole (50 mg/kg/day, p.o.) used as immunostimulating agents.
**RESULTS:** Oral administration of *Ocimum basilicum* (OB) showed a significant increase in the production of circulating antibody titer in response to sheep red blood cells (SRBCs). A significant ($p<0.01$) increase in both primary and secondary HA titer was observed while compared to control group, whereas in cyclophosphamide treated group OB showed significant ($p<0.01$) increase in HA titer. OB showed significantly ($p<0.01$) potentiated the DTH reaction by facilitating the footpad thickness response to SRBCs in sensitized mice. Also OB evoked a significant ($p<0.01$) increase in percentage neutrophil adhesion to nylon fibres and phagocytic activity.
**CONCLUSION:** The study demonstrates that OB triggers both specific and non-specific responses to a greater extent. The study comprised the acute toxicity and preliminary phytochemical screening of OB. From the results obtained and phytochemical studies the immunostimulant effect of OB could be attributed to the flavonoid content.
**KEYWORDS:** *Ocimum basilicum*, Immunomodulation, antibody titre, phagocytic index.

## INTRODUCTION

The immune system is known to be involved in the etiology as well as pathophysiological mechanisms of many diseases.[1-2] It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating variety of foreign invaders.[3]

Immunostimulation constitutes either an alternative to or an adjuvant for conventional chemotherapy and prophylaxis of infection, for tumor, as well autoimmune diseases, especially when the host's immune system is impaired. Immunostimulation is also indicated to counteract immunosuppression or an uneffectively working immune system. [4]

Scientific literatures are continuously reporting herbal drugs having immunomodulatory activity and generally act by stimulating both specific and non-specific immunity. There is need to evaluate the potential of ayurvedic remedies as adjuvants to counteract side effects of modern therapy. [5-6]

Recent screening with plants has revealed many compounds like flavonoids, alkaloids, saponins, terpenoids, monoterpenoids (linalool), glycoproteins, polysaccharides, tannins, essential fatty acids, phenolic compounds and vitamins having pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential.[6]

*Ocimum basilicum* Linn. (Lamiaceae) is commonly known as Sweet basil. The word basil comes from the Greek (basileus), meaning "King", of the royal fragrance of this herb. [7]

The chemical constituents of *Ocimum basilicum* L. are flavonoids, alkaloids, ascorbic acid, terpenoids, tannins, saponin glycosides, and phenolic compounds and leaves samples showed the presence of two major flavone aglycones, which were identified as salvigenin and nevadensin. [8-9]

*Ocimum basilicum* has been used for thousands of years as a medicinal herb. It acts principally on the digestive and nervous systems. [10] According to Ayurveda, *Ocimum basilicum* used as a stomachic, anthelmintic, antipyretic, improves the taste, indigestion, useful in diseases of the heart and blood diseases. Some studies have reported its antioxidant, radical scavenging, antiinflammatory, antiulcer and cardiac stimulant activity. [11, 8]

The present investigation is aimed at studying the immunomodulatory activity of the aqueous and ethanolic extract of leaves of *Ocimum basilicum* in order to justify the traditional claims endowed upon this herbal drug as a rasayana.

## MATERIALS AND METHODS

### PLANT MATERIAL

The leaves of *Ocimum basilicum* were collected at in the month of September, 2007 from local area of Sangli, Maharashtra state, India and authenticated by Dr. U. S. Yadav of the Department of Botany, Willingdon College, Sangli, where a voucher specimen has been preserved for future identification.

### EXTRACTION

The leaves were separated from the fresh stems and dried on filter paper sheets under shade at room temperature until with changing of color of filter papers. The shade-dried, coarsely powdered leaves (500g) were successively extracted with petroleum ether (60-80$^{o}$C) for 8 hr. to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% ethanol to obtain ethanolic extract. The dried marc was cold macerated with distilled water and chloroform (9:1) for 7 days to obtain aqueous extract. The both ethanolic and aqueous extracts were evaporated under reduced pressure at low temperature (30$^{o}$C) to dryness to yield yellowish and brown color extracts of *Ocimum basilicum* respectively, stored in an airtight container in refrigerator for further experimental studies.

### PRELIMINARY PHYTOCHEMICAL SCREENING

Aqueous and ethanolic extracts of *Ocimum basilicum* were subjected to preliminary phytochemical screening for the detection of various plants constituents. [12-13]

### ANIMALS

Inbred colony of swiss albino mice of weighing between 20-25 gm were housed in groups of 5 to 6. All mice were feed with pelleted diet (Pranav Agro Industries Ltd, Sangli, India) and water ad libitum. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

### DRUGS AND CHEMICALS

All the drugs and chemicals were of analytical grade. Drugs were procured from Levamisole (Khandelwal Pharmaceutical Ltd. Mumbai), Cyclophosphamide (Biochem pharmaceutical, Mumbai), Colloidal carbon (Indian ink, camel India Pvt. Ltd.).

### TEST COMPOUND FORMULATIONS

The dilution of aqueous extract of leaves of *ocimum basilicum* (AEOB) was prepared in distilled water and the aqueous suspension of ethanolic extract of leaves of *ocimum basilicum* (EEOB) was prepared in 0.5 % carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within 7 days and stored at 80$^{o}$C while for further use, freshly prepared solution was used. The vehicle alone served as control.

### ACUTE TOXICITY STUDIES

Acute toxicity studies were performed according to organization for economic cooperation and development (OECD) guidelines. [14] Mice weighing between 20-25 gm in groups of five were used (n=5). The animals were fasted for 4 hr. with free access to water only. The both EEOB and AEOB extracts was administered orally in doses of 2000 and 5000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

## EXPERIMENTAL PROCEDURE

### ANTIGENIC MATERIAL: PREPARATION OF SHEEP RBCs (SRBCs)

Sheep blood was collected in sterile Alsevere's solution in 1:1 proportion of Alsevere's solution (freshly prepared). Blood was kept in the refrigerator and processed, for the preparation of SRBCs batch, by centrifugating at 2000 rpm for 10 minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use. [2]

### CARBON INK SUSPENSION

Pelican AG, Germany, ink was diluted eight times with saline and used for carbon clearance test in a dose of 10 µl/gm BW of mice. [15]

## HAEMAGGLUTINATION ANTIBODY (HA) TITER [16]

The mice were divided into eight groups consisting of five animals each. Mice in group I received vehicle only for 21 days. Group II received cyclophosphamide (negative control) 100 mg/kg, p.o. on 9th and 16th day as a single dose. Mice in treatment group III and IV were given AEOB (400 mg/kg/day/p.o) and EEOB (400 mg/kg/day/p.o.) daily for 21 days respectively. Immunosuppresed animals in group V and VI were given AEOB (400 mg/kg/day/p.o) and EEOB (400 mg/kg/day/p.o.) plus cyclophosphamide (100 mg/kg/p.o.) on 9th and 16th day as a single dose respectively. Group VII received standard drug levimasole (50 mg/kg/p.o.) as an established immunostimulant agent for 21 days and group VIII received levimasole (50 mg/kg/p.o.) for 21 days plus cyclophosphamide (100 mg/kg/p.o.) on 9th and 16th day as a single dose.

On 7th and 14th day of the study, mice from all the groups (i.e. group I to VIII) were immunized and challenged respectively, with SRBCs in normal saline (0.1ml of 20% SRBCs) intraperitoneally. Blood was withdrawn on 14th and 21st day from retro-orbital plexus under mild ether anaesthesia from all antigenically sensitised and challenged mice respectively. Blood was centrifuged to obtain serum, normal saline was used as a diluent and SRBCs count was adjusted to (0.1% of SRBCs). Each well of a microtitre plate was filled initially with 20 µl of saline and 20 µl of serum was mixed in the first well of micro titer plate. Subsequently the 20 µl diluted serum was removed from first well and added to the next well to get twofold dilutions of the antibodies present in the serum. Further twofold dilutions of this diluted serum were similarly carried out till the last well of the second row (24th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 20 µl SRBCs (0.1% of SRBCs) were added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titers were expressed in the graded manner, the minimum dilution (1/2) being ranked as 1, and mean ranks of different groups were compared for statistical significance. Antibody titer obtained on 14th day after immunization (on 7th day) and on 21st day after challenge (on 14th day) with SRBCs was considered as primary and secondary humoral immune response respectively.

## DELAYED TYPE HYPERSENSITIVITY (DTH) RESPONSE [17-18]

The drug treatment was exactly the same as described above for HA titer. On 14th day of the study, all the groups I to VIII were immunized with SRBCs (0.1ml of 20% SRBC i.p.) in normal saline. On day 21st all animals from all the groups were challenged with 0.03 ml of 20% SRBCs in subplantar region of right hind paw. Foot pad oedema in mice was used for detection of cellular immune response. On 21st day, injection of 0.1ml of 20% SRBCs in the subplantar region of right hind paw in the volume of 0.03 ml and normal saline in left hind paw in same volume. Foot pad reaction was assessed after 24 hr. on 22nd day, in terms of increase in the thickness of footpad as a result of hypersensitivity reaction due to oedema, the thickness of the right hind footpad was measured using vernier calliper. The footpad reaction was expressed as the difference in the thickness (m.m.) between the right foot pad injected with SRBCs and the left footpad injected with normal saline.

## CARBON CLEARANCE TEST [19]

The drug treatment was exactly the same as described above for HA titer. On 14th day, 3 hours after the last dose all the animals of each group were given colloidal carbon intravenously in a volume of 1 ml/100 g. Blood samples were then withdrawn (25 µl) from retro-orbital plexus at 0 and 15 minutes after injection of colloidal carbon ink and lysed in sodium carbonate solution (3 ml). The optical density was measured spectrophotometrically at 650 nm. The phagocytic index (K) was calculated using the formula:

$$K = \frac{(\text{In } OD_1 - \text{In } OD_2)}{(t_2 - t_1)}$$

Where, $OD_1$ and $OD_2$ are the optical densities at time $t_1$ and $t_2$ respectively.

## NEUTROPHIL ADHESION TEST [1]

Mice were divided into four groups of five animals each. The control group I received vehicle, while animals of treatment group II and III were given AEOB (400 mg/kg/day/p.o.) and EEOB (400 mg/kg/day/p.o.) daily for 14 days respectively. Group IV received levimasole (50 mg/kg/p.o.) for 14 days. On the 14th day of the treatment, blood samples from all the groups were collected by puncturing retro-orbital plexus under mild ether anaesthesia. Blood was collected in vials pre-treated by disodium EDTA and analysed for total leukocyte count (TLC) and differential leukocyte count (DLC) by fixing blood smears and staining with Leishman's stain. After initial counts, blood samples were incubated with nylon fiber (80 mg/ml of blood sample) for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as follows,

$$\text{Neutrophil adhesion (\%)} = \frac{NIu - NIt}{NIu} \times 100$$

Where,
NIu: Neutrophil Index before incubation with nylon fiber.
NIt: Neutrophil Index after incubation with nylon fiber.

## STATISTICAL ANALYSIS

All the results were expressed as mean ± Standard Error (SEM). Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison test. *P*-values <0.01 were considered as statistically significant.

## RESULTS

## ACUTE ORAL TOXICITY STUDY

Acute oral toxicity was carried out by up-down regulation method. It is found that AEOB and EEOB were safe at limit dose 4000 mg/kg with no mortality in studied subjects respectively. 1/10th of this dose i.e. 400 mg/kg for AEOB and EEOB was used in the subsequent study respectively.

## PRELIMINARY PHYTOCHEMICAL SCREENING

The AEOB found to contain carbohydrates, proteins, amino acids and flavonoids. The EEOB were found to contain carbohydrates, proteins, amino acids, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds. TLC analysis showed the presence of flavonoids (Rf × 100) value for the EEOB was found to be as 65 which is the near the Rf value for flavanol and flavones). [20]

## HAEMAGGLUTINATING ANTIBODY [HA] TITER

Effect of AEOB and EEOB on primary and secondary antibody response on HA titer are shown in [Table 1].
Primary antibody response on day 14th in AEOB and EEOB (400 mg/kg/p.o.) treated group with normal immune status showed significant increase (P<0.01) in HA titer when compared with the control group. A significant decrease (P<0.01) in the antibody titer was observed in the cyclophosphamide-treated group when compared with the control group. In immunosuppressed groups, where the immunity was suppressed by administration of cyclophosphamide on day nine, EEOB (400 mg/kg/p.o.) administration produced a significant (P<0.01) rise in the antibody titer when compared with the cyclophosphamide-treated group. Secondary antibody titer on twenty-first day in EEOB-treated group with normal immune status group showed a significant rise (P<0.01) in the antibody titer when compared with the control group. In the immunosuppresed groups where the immunity was suppressed by administration of cyclophosphamide on day sixteenth EEOB showed a significant rise (P<0.01) in HA titer when compared with the cyclophosphamide group. Both AEOB (400 mg/kg/p.o.) and EEOB (400 mg/kg/p.o.) extract results are comparable with LMS (50 mg/kg/p.o.) standard.

## DELAYED TYPE HYPERSENSITIVITY

Effect of AEOB and EEOB on cell mediated immune response by DTH induced footpad oedema is shown in [Table 2]. On 21st day cyclophosphamide-treated group showed significant (p<0.01) decrease in the mean difference of paw thickness when compared to control group. In the all groups of mice with normal immune status, of AEOB (400 mg/kg/p.o.) and EEOB (400 mg/kg/p.o.) showed significant (p<0.01) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with control group. In the all groups of mice treated with cyclophosphamide i.e. an immunosuppressed groups are, AEOB and EEOB showed significant (p<0.01) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with cyclophosphamide (negative control) group. Heightened delayed type hypersensitivity reaction suggests activation of cellular immune system.

## NEUTROPHIL ADHESION TEST

Effect of AEOB and EEOB on neutrophil activation by the neutrophil adhesion test is shown in [Table 3]. Cytokines are secreted by activated immune cells for margination and extravasation of the phagocytes mainly polymorphonuclear neutrophils. The % neutrophil adhesion was significantly (p<0.01) increased by AEOB (400 mg/kg/p.o.) and EEOB (400 mg/kg/p.o.) when compared with the control group, showed possible immunostimulant effect. OB significantly evoked increase in the adhesion of neutrophils to nylon fibers which correlates to the process of margination of cells in blood vessels.

## CARBON CLEARANCE TEST

Effect of AEOB and EEOB on the phagocytic activity by the carbon clearance test is shown in [Table 3]. The phagocytic activity of the reticuloendothelial system is generally measured by the rate of removal of carbon particles from the blood stream. In carbon clearance test, OB treated all groups, exhibited significantly high phagocytic index. The phagocytic index of AEOB (400 mg/kg/p.o.) and EEOB (400 mg/kg/p.o.) showed significant (p<0.01) increased in phagocytic index when compared to control group. This indicates stimulation of the reticuloendothelial system.

## Assessment of Humoral immune response

**Table No. 1 Effect of *O. basilicum* treatment on primary and secondary antibody titre.**

| Sr. No. | Groups (n=5) | Treatment, Dose and Route | Primary Antibody titre | Secondary Antibody titre |
|---|---|---|---|---|
| I. | Vehicle control | Distilled water, 10 ml/kg, (p.o.) | 4.00 ± 0.577 | 5.66 ± 0.88 |
| II. | Negative control | Cyclophosphamide (Cyp.), 100 mg/kg, (p.o.) | 3.00 ± 0.577** a | 4.66 ± 0.33** a |
| III. | AEOB treated | *O.basilicum,* 400 mg/kg, (p.o.) | 6.33 ± 0.33** a | 7.66 ± 0.66 |
| IV. | EEOB treated | *O.basilicum,* 400 mg/kg, (p.o.) | 6.33 ± 0.33** a | 8.00 ± 0.57** a |
| V. | LMS treated | Levamisole, 50 mg/kg, (p.o.) | 8.00 ± 0.57** a | 8.66 ± 0.88**a |
| VI. | AEOB + Cyp.treated | *O.basilicum,* 400 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 4.33 ± 0.88 | 6.66 ± 0.33 |
| VII. | EEOB + Cyp.treated | *O.basilicum,* 400 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 6.00 ± 0.57** b | 8.00 ± 0.57** b |
| VIII. | LMS + Cyp.treated | Levamisole, 50 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 7.33 ± 0.57** b | 8.33 ± 0.33** b |

Values are expressed as (Mean ± S.E.M.), n= 5, ** p< 0.01
a: Negative control (Cyclophosphamide treatment alone) and only normal immune status groups *i.e,* AEOB, EEOB and LMS treated groups were compared with vehicle control (Group I).
b: Immunosuppressed test extracts of AEOB, EEOB and LMS + Cyp. treated groups were compared with negative control (Group II).
(Statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.)

## Assessment of Cell Mediated Immune Response.

**Table No. 2 Effect of *O. basilicum* treatment on cell mediated immune response by delayed type hypersensitivity induced footpad oedema.**

| Sr. No. | Groups (n=5) | Treatment, Dose and Route | Mean diff.of paw oedema in (mm) |
|---|---|---|---|
| I. | Vehicle control | Distilled water, 10 ml/kg, (p.o.) | 0.293 ± 0.088 |
| II. | Negative control | Cyclophosphamide (Cyp.), 100 mg/kg, (p.o.) | 0.445 ± 0.0035** a |
| III. | AEOB treated | *O. basilicum,* 400 mg/kg, (p.o.) | 0.756 ± 0.003** a |
| IV. | EEOB treated | *O. basilicum,* 400 mg/kg, (p.o.) | 0.767 ± 0.011** a |
| V. | LMS treated | Levamisole 50 mg/kg, (p.o.) | 0.952 ± 0.003** a |
| VI. | AEOB + Cyp.treated | *O. basilicum,*400 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 0.769 ± 0.010** b |
| VII. | EEOB + Cyp.treated | *O. basilicum,*400 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 0.823 ± 0.002**b |
| VIII. | LMS + Cyp.treated | Levamisole, 50 mg/kg, (p.o.) + Cyp.100 mg/kg, (p.o.) | 0.956 ± 0.004** b |

Values are expressed as (Mean ± S.E.M.), n= 5, ** p< 0.01
a: Negative control (Cyclophosphamide treatment alone) and only normal immune status groups *i.e,* AEOB, EEOB and LMS treated groups were compared with vehicle control (Group I).
b: Immunosuppressed test extracts of AEOB, EEOB and LMS + Cyp. treated groups were compared with negative control (Group II).
(Statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.)

**Assessment of Non-specific immune response**

**Table No. 3 Effect of *O. basilicum* treatment on neutrophil activation by neutrophil adhesion test and phagocytic activity by carbon clearance test.**

| Sr. No. | Groups (n=5) | Treatment, Dose and Route | % Neutrophil Adhesion (Mean ± S.E.M.) | Phagocytic Index |
|---|---|---|---|---|
| I. | Control | Distilled water 10 ml/kg, (p.o.) | 29.25± 0.57 | 0.029 ± 0.0004 |
| II. | AEOB treated | *O. basilicum* 400 mg/kg (p.o.) | 45.02±1.01** | 0.010 ± 0.0008 ** |
| III. | EEOB treated | *O. basilicum* 400 mg/kg (p.o.) | 46.26±0.34** | 0.082 ± 0.0009** |
| IV. | LMS treated | Levamisole 50 mg/kg (p.o.) | 73.24±0.06** | 0.083 ± 0.001** |

Values are expressed as ( Mean ± S.E.M.), n= 5, **p<0.01

AEOB, EEOB and LMS treated groups werer compared with control group. (Statistically analysed by one- way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.)

**DISCUSSION**

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor. [21] The results obtained in the present study indicate that *Ocimum basilicum* is a potent immunostimulant, stimulating specific and non-specific immune mechanisms.

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells. To evaluate the effect of OB on humoral response, its influence was tested on sheep erythrocyte specific HA titre in mice. [22] Cyclophosphamide showed significant inhibition in antibody titre response, while AEOB and EEOB counteract the suppression of both primary and secondary humoral responses induced by cyclophosphamide. [4] This indicates the enhanced responsiveness of macrophages, T and B lymphocyte subsets involved in antibody synthesis.

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). [23] DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilatation, macrophage accumulation, and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. When activated TH1 cells encounter certain antigens, viz. SRBCs. They secrete cytokines that induce a localised inflammatory reaction called delayed type hypersensitivity. [24] DTH comprises of two phases, an initial sensitisation phase after the primary contact with SRBCs antigen. During this period TH1 cells are activated and clonally expanded by APC (antigen presenting cells) with class II MHC molecule (eg. langerhans cells and macrophages are APC involved in DTH response). A subsequent exposure to the SRBCs antigen induces the effector phase of the DTH response, where TH1 cells secrete a variety of cytokines that recruits and activates macrophages and other non specific inflammatory mediators. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages. [25]

Therefore, increase in DTH reaction in mice in response to T cell dependent antigen revealed the stimulatory effect of AEOB and EEOB on T cells.

Cytokines are secreted by activated immune cells for margination and extravasation of the phagocytes mainly polymorphonuclear neutrophils. Significantly evoked increase in the adhesion of neutrophils to nylon fibers which correlates to the process of margination of cells in blood vessels. [17] In the present study, OB evoked a significant increase in percent of neutrophils. This may help in increasing immunity of body against microbial infections.

The role of phagocytosis is the removal of microorganisms and foreign bodies, dead or injured cells. The increase in the carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by the opsonisation of parasites with antibodies and complementing C3b, leading to a more rapid clearance of parasites from the blood. [26] AI appeared to enhance the phagocytic function by exhibiting a clearance rate of carbon by the cells of the reticulo-endothelium system.

OB has a significant immunostimulatory activity on both the specific and non-specific immune mechanisms. The immunostimulatory activity of OB could be attributed to the presence of flavonoids (quercetin), alkaloids, tannins, saponin glycosides and phenolic compounds. Therefore, the plant holds promise for being used as an immunostimulating agent and an in-depth study on various fractions of the extract effective as immunomodulating entities from the plant is warranted to determine the most potent immunostimulating fraction from OB. Thus, the study validates the traditional use of OB as a 'Rasayana' in Ayurvedic system of medicine.

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