

Immunostimulatory Effect Of Aqueous Extract Of *Leucas aspera* In Cyclophosphamide Induced Immunosuppressive Mice

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Abstract : Immunostimulatory effect of aqueous extract of *Leucas aspera* was evaluated in cyclophosphamide induced immunosuppressive mice. The results clearly show that the aqueous extract of this plant significantly increase the Neutrophil and DTH activity. The extract also elevates the levels of RBC, WBC and Hemoglobin remarkably in immunosuppressive mice ($p < 0.05$). This indicates that *Leucas aspera* provoke both cellular and humoral immune response that is used to treat immunosuppressive disorders.

Key words: Immune stimulation, *Leucas aspera*, leaves.

Introduction

Polyphenols are a group of plant phytochemicals contribute major role for improving human health and maintaining the human life since from ancient days¹. There are several reports indicated that the immune system play vital role for maintain the health in a better way². Plant researchers suggested that plant phytochemicals contribute significant role to improving the immune system there by fighting against numerous infectious diseases^{3,4}. The integrity and different functions of immune system play a vital role for fighting against various diseases⁵. Reports indicated that the plant phytochemicals generally induces the para immunity of the system⁶.

The immune system comprises special cells, proteins, tissues and organs that fight against microorganisms. If a pathogen reaches the barriers, the innate immune system provides immediate, but non-specific responses⁷. Major cells in the immune system are of leukocytes called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from pluripotential hematopoietic stem cells in the bone marrow former involved in humoral immune response, whereas T cells are involved in cell-mediated immune responses^{8,9}. Immunomodulators are substances, which modify the activity of the immune system. They have biphasic effects; some tend to stimulate the immune system, which are low in activity while others inhibit the host parameters, which are normal or already activated. Modulation of the immune system is emerging as a major area in pharmacology especially in cases where undesired immunosuppression is the result of therapy¹⁰.

Indian medicinal plants are claimed to induce paraimmunity, the non-specific immunomodulation of essentially macrophages, granulocytes, NK cells and lymphocytes and complement functions. 'Rasayana' plants are particularly recommended for the treatment of immune disorders. Ayurveda with particular reference to plants may play an important role in modern healthcare, particularly where satisfactory treatment is not available. There is need to evaluate the potential of Ayurvedic remedies as adjuvants to counteract side effects of modern therapy and to compare the cost effectiveness of certain therapies¹¹. Some of the immunomodulators of plant origin are *ocimum sanctum*¹², *Azadirachta indica*¹³, plants from the genus *Alternanthera*, *picrorhiza kurroa*¹⁴, and *Capparis Zeylanica*¹¹.

The *Leucas aspera* is an erect or diffusely branched, annual herb. The leaves are linear or oblong, 2.5 to 7.5 centimeters long, and blunt-tipped, the margins being scalloped. The whorls are large, terminal and auxiliary often 2.5 centimeters in diameter, and crowded with white flowers. The aim of this study to identify this plant has immune enhancement effects which may have great potential to treat cancer and other diseases.

Materials and methods

Sample Collection

The fresh leaves of *Leucas aspera* were collected from in an around kumbakonam during January 2008. The leaves were shade dried at room temperature for 7 days and coarsely powdered.

Aqueous extraction

The powdered leaves sample was soaked in distilled water for 24 hours at 25°C and the filtrate was evaporated under reduced pressure dried *in vacuo*.

Immunomodulatory activity

Animals

Male mice (23-25 gm) were used in the present study was maintained on standard pellet diet. under constant environmental condition (26 ± 2).

Source of chemicals

Cyclophosphamide was purchase from ranbaxy chemical company all the other chemicals used were analyzed grade obtained from mice pharmaceutical company.

Induction of immunosuppression

Finely powdered drug cyclophosphamide (3mg/kg) was suspended into 2% gum acacia solutions as suspending agent were administered orally daily.

Experimental group protocol

The animals were divided into 4 groups each consist of 6 animals. Control mice received similar volume of vehicle, normal saline (2ml/kg) of body weight alone.

Group I - Control

Group II - Cyclophosphamide induced myelosuppressive group

Group III - *Leucas aspera* leaf extract pretreated (200mg/kg)

Group IV - *Leucas aspera* pretreated+cyclophosphamide induced myelosuppression

Treatment

The mice were treated with aqueous extract leucas aspera in cyclophosphamide induced mice and normal for 15 days.

Mode of sacrifice

After the treatment animals were fasted overnight and were sacrificed under mild chloroform anesthesia. Blood was collected by jugular vein puncture and liver was quickly. Excised of washed in saline. Bottled dry and stored at 4°C.

Results and discussion

Immunomodulatory activity

Immunomodulation in a drug induced immunosuppression in an experimental hyper-reactivity model by the some preparation can be said to be true immunomodulation. Most of this chemotherapeutic agent available today are immunosuppressant, cytotoxic and exert a variety of side effects that are particularly evident in cancer

chemotherapy. A Botanical based immunomodulators are often employed as supportive or adjuvant therapy to overcome the undesired effects of cytotoxic chemotherapeutic agents and to restore health to normal¹⁵.

Haemoglobin

There is significant decrease in the amount of haemoglobin have been found out in G-II compare to normal ($P < 0.05$) and also no significant difference in between G I and G III. The amount of haemoglobin have been increased in Group IV treated groups compared to G-II ($P < 0.05$). This indicates the leaves of *Leucas aspera* alleviate the effect of cyclophosphamide induced RBC destruction. The increase in the hemoglobin content in G- IV is the indication of immunostimulant phenomenon of aqueous extract *Leucas aspera*. Some of the herbs such as *O. basilicum*, *Cinnamomum zeylanicum*, *Juglans regia*, *Mentha piperita* increases the hemoglobin content in copper acetate induced rats. Like wise, aqueous extract of *Leucas aspera* also increases the hemoglobin content compared to cyclophosphamide induced immunosuppressive rats¹⁷. (Table 1).

Table : 1 Effects of aqueous extract of leaves of *Leucas aspera* on haemoglobin (g/dl) cyclophosphamide Induced in Myelosuppression in mice.

S.No.	Groups	Haemoglobin
1	G I	11.5±1.0
2	G II	8.2±0.3*
3	G III	11.1±0.51***
4	G IV	9.41±0.93**

The data were expressed as mean ± S.D. (N=6 mice)

* P < 0.05 Group II Vs Group I, **P < 0.05 Group IV Vs Group II

***P < 0.05 Group III Vs Group II (not significant)

Effect on a number of RBC, WBC, Absolute Eosinophil and Blood platelets

The immunosuppressive agents seriously reduce the levels of various biochemical markers such as RBC, WBC and platelets. Some of the earlier reports of immunomodulatory effects of plant extracts is used to increase the level of above mentioned biochemical parameters. Cyclophosphamide at the dose of 2 mg/kg Intra-peritoneally caused a significant reduction ($P < 0.05$) in the RBCs, WBCs, and Platelets Count compared to normal mice which received normal saline. There is no significant difference have been found out in between group-I and Group – III mice ($P > 0.5$). Combined treatment of Cyclophosphamide and Aqueous extract of *Leucas aspera* (200mg/kg) resulted in a restoration bone marrow activity ($P < 0.05$) as compared with cyclophosphamide treatment alone (Table 2) but in contrast cyclophosphamide induced the number of Eosinophil count which is showed in cyclophosphamide treatment group. This allergic reactions can be reverted in cyclophosphamide along with aqueous extract of *Leucas aspera* treated groups. There is no significant difference have been noticed in between Group III and Group I ($P > 0.5$) (Table4). The significant increase in the levels of RBC, WBC and platelets in G-IV confirms the immunostimulatory effects *Leucas aspera*. Our findings are also comparable with the previous research of immunomodulatory effects certain plant extracts¹⁷.

Table : 2 Effects Of Aqueous Extract Of Leaves Of *Leucas Aspera* On Numbers Of Red Blood Cells (RBC X 10⁶/Mm³) Absolute Eosinophil Count (Mean SD (%)) Blood Platelets In 1000/Mm³ On Day 15 After Cyclophosphamide Treatment

S.No.	Groups	WBC x 10 ³ mm ³	RBC x 10 ⁶ mm ³	Abolute Eosinophil Count Mean SD (%)	Blood Plateletes in 1000/mm ³
1	G I	13.0±2.1	10.1±0.3	0.65±0.58	457.3±0.31
2	G II	8.6±3.4*	6.7±1.0*	4.97±0.29*	280.6±0.09*
3	G III	13±0.3.1.5***	8.75.0.49***	4.36±0.81*	485.01±0.76***
4	G IV	12.74±1.7**	9.7±0.92**	4.57±0.28	310.2±0.08**

The data were expressed as mean ± S.D. (N=6 mice)

* P < 0.05 Group II Vs Group I, **P < 0.05 Group IV Vs Group II

***P < 0.05 Group III Vs Group II (not significant)

The administration of *Leucas aspera* extract significantly ameliorated the total WBCs count, RBCs count, haemoglobin and platelets count and also restored the myelosuppressive effects induced by cyclophosphamide (Table 4).

Neutrophil Adhesion Test

Pretreatment of Aqueous extract of (200mg/kg, oral), evoked a significant ($P < 0.05$) increase in the invitro Neutrophil adhesion to nylon fibres, which correlates the increase in & Neutrophil (Table 3).

Table 3 : Effects of Aqueous extract of leaves of *Leucas aspera* on Neutrophil Adhesion Test.

S.No.	Groups	% Neutrophil		Neutrophil Index		% Neutrophil Adhesion
		UTB	FTB	UTB	FTB	
1	G I control	44±2.5	37±3.5	282.8±35.0	218±25.6	24.0±1.8
2	G III pretreated	47±2.1	38±1.0	294.73±35.2	222.6±28.9	25.7±1.4

The data were expressed as mean ± S.D. (N=6 mice)

* $P < 0.05$ Group III Vs Group I

The neutrophil, an end cell unable to divide and with limited capacity for protein synthesis is, nevertheless, capable of a wide range of responses, in particular chemotaxis, phagocytosis, exocytosis and both extra cellular killing.

DTH Reactions

The cell mediated immune response was assessed by DTH reaction (Foot pad reaction) The aqueous extract of *Leucas aspera* produced a significant increase in DTH reactivity in mice ($P < 0.05$). Increase in DTH reaction in mice in response to cell dependent antigen revealed the stimulatory effect of Aqueous extract of *Leucas aspera* on T cells (Table 4).

Table 4: Effects of Aqueous Extract Of Leaves Of *Leucas Aspera* On Numbers Of Delayed Hypersensitive Response (% Increase In Foot Pad Thickness)

S.No.	Groups	DTH response (mm)
1	G I	0.89±0.04
2	G III	0.93±0.06**

The data were expressed as mean ± S.D. (N=6 mice)

* $P < 0.05$ Group III Vs Group I

In the present study aqueous extract (200mg/kg, oral) of *Leucas aspera* produced significant increase in % neutrophil. This may help in increasing immunity of body against microbial infections (Benacerraf, 1978). Cell mediated immunity (CMI) involves effector mechanisms carried out by lymphocytes and their products. CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity and delayed type hypersensitivity reactions¹⁶. Therefore, increase in DTH reaction in mice in response to T cell dependent antigen revealed the stimulatory effect of aqueous extract of *Leucas aspera* on T cells. (Table 6). The results obtained in the present study indicate that aqueous extract of *Leucas aspera* is potent immunostimulant, stimulating both the specific and non-specific immune mechanisms¹⁷.

All the above results, indicated that the aqueous extract of *Leucas aspera* potentially increases the level of immune cells in chyclophosphamide induced immunosuppressive mice. These activities of the *Leucas aspera* may be attributed to the presence o different poyphenols and flavonoids present the leaves.

Conclusion

The present study investigate immunomodulatory effect of *Leucas aspera* in cyclophosphamide induced immunosuppressive mice. The results indicate that there is significant increased level of blood components such as RBCs, WBCs, and Blood platelets, when compared to cyclophosphamide treated groups. The restoration of normal histology has been observed in aqueous extract treated groups. Compared to cyclophosphamide induced myelosuppressive mice. Stimulatory effect of DTH reaction in the Aqueous extract of *Leucas aspera* treated group indicates that the extract stimulate cell mediated immunity in a specific manner. These results suggest that *Leucas aspera* may stimulate both cellular and humoral immune response that is used to treat immunosuppressive disorders. This study will have a way for future investigators to elucidate a suitable component for stimulate the immune system to make an individual healthy and normal.

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