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Immunomodulating Activity of Ethanolic Extract of *Leptadaenia Reticulata*

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ABSTRACT

The aim of the present study is to evaluate the effect of ethanolic extract of *Leptadaenia reticulata* for immunomodulating activity. The ethanolic extract of plant of *Leptadaenia reticulata* holds potential as a protective agent against cytotoxic drugs. The extracts when studied on humoral and cell mediated immunity in normal, as well as cyclophosphamide induced immunosuppressed rats. It produced both increase for some parameters and decrease response in some other parameters. The present investigation established pharmacological evidence to support the folklore claim that it is an immunomodulating drug plant.

Keywords: *Leptadaenia reticulata*, Immunostimulant, Cyclophoshamide, Carbon clearance test, Delayed type hypersensitivity, Antibody titre

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INTRODUCTION

Leptadaenia reticulata (Retz.) Wight. & Arn. (family Asclepiadaceae) is a climber having stem with cork-like, deeply cracked bark with numerous branches, among which the younger ones are glabrous[1]. It is considered to be a *Rasayana* (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body[2]. Its medicinal use dates back to about 4500 to 1600 BC, as mentioned in *Atharva Veda*, Kanda eight, Sukta two. The *Atharva Veda* mentioned its uses as a life and strength giver, propagator of milk and useful in many other ailments. Charaka described it as an important rasayana drug, capable of maintaining youthful vigour and strength, and Vagbhata incorporated it among the ten drugs that constitute the Jivaniya gana or the vitalizing group. It promotes health and vigour, improves the voice, alleviates the three dosas–vata, pitta and kapha, and cures eye diseases, haemetemesis, emaciation, cough, dyspnoea, fever, burning sensation, dysentery, night-blindness, poisonous affections and tuberculosis[3]. Anjaria *et al.*[4] mentioned it as a stimulant, galactagogue, eye tonic, astringent, prolapse of uterus, vagina, controlling habitual abortion and maintain pregnancy. Its restorative property makes it an important ingredient in the preparation of 'Chyawanprash' (an Ayurvedic tonic). The leaves, paste and roots are taken orally with water to cure gangrene by the Bhils of southern Rajasthan[5] Kirtikar and Basu[6] mentioned it as a stimulant and tonic.

The alcoholic extracts (50%) of the leaves and roots are reported to be active against *Micrococcus pyrogens, Bacillus megatherium, Escherichia coli, Salmonella typhi, Proteus vulgaris* and *Trychophyton rubrum.* The entire plant has been clinically tested and found useful in treatment of habitual abortion in women. Aqueous extract of the aerial parts has been reported to produce prolonged and pronounced hypotensive effect in dogs[7]. The antioxidant, wound healing, galactagogue and lactogenic activity of the plant has been reported[8,9]. The Plant is in local area people used as tonic but there is paucity of data available on immunomodulatory activity of *L. reticulata* in normal animals. Therefore present work aims at studying effect of ethanolic extract of *L. reticulata* on immune system in normal animals.

MATERIALS AND METHOD

Animals

Swiss balb-c mice (25–30g) of either sex were used for the study. They were purchased from the institute of DRDO, Gwalior, (M.P.) and the animals were housed animal room of department of pharmaceutical sciences under standard conditions of temperature (18-24°C), relative humidity (30%-70%)and 12h-12h light/dark cycle. Food (standard pellet diet) and water were provided ad

libitum. Animal studies were conducted in accordance with the Guidance of animal ethical committee for the Care and Use of Laboratory Animals. Animal experiments were approved by animal ethical committee.

Collection and Identification of Plant Material

The whole plant of *Leptadaenia reticulata* (aerial part) was collected in the summer season from the botanical garden of Department of Botany, Dr. Harisingh Gour University, Sagar, (M.P.) India. The plant was identified and authenticated by the taxonomist of Department of Botany, Dr. Harisingh Gour University, Sagar (M.P.), India and voucher specimen was deposited in institutional herbarium. The herbarium number of *Leptadaenia reticulata* is Bot/Herb/2870. Aerial parts of the plant were shade dried, and then dried plant material was reduced to coarse powder and stored in airtight containers.

Ethanol extract preparation

Powdered plant material was kept in beaker for 24 hr with Ethanol. Filter the content, marc was discarded. Reduce the volume of filtrate in rotatory evaporator (Percentage yields 20.10%).

TREATMENT

Carbon–Clearance Test for the Determination of Phagocytic Index-

Mice were divided in to four groups having six animals in each. Group I, the control, was given 2ml of 5% normal saline for seven days. Group II, III, IV were administered ethanolic extract of 50mg, 100mg, and 150mg/kg b.w. intraperitonially. After 7 days each mouse was given an intravenous injection of 1ml/30g b.w. of Indian ink. Blood samples from retro-orbital venous plexus were taken at intervals of 0 and 15 minutes., and transferred in to the centrifuge tubes, the blood in these centrifuge tubes were allowed to coagulate at room temperature. It was then centrifuge at 2000 rpm for 10 minutes and clear supernatant was collected. From each centrifuge tube 20 ml of serum was withdrawn using micropipette and transferred to different volumetric flask (25.0 ml) and volume was made up using distilled water absorbance was measured at 650nm. Recorded absorbance was plotted against the time. This absorbance explains us the rate of elimination of carbon from the blood. The phagocytic index was determined by following formula[10]

$$\text{Phagocytic index} = {}^{k}(\text{sample}) / {}^{k}(\text{control})$$

Where $K = (\text{Log}_e OD_1 - \text{Log}_e OD_2) / 15$

OD_1= optical densities at 0 minutes and,

OD_2= optical densities at 15 minutes, respectively.

Delayed Type of Hypersensitivity (DTH)

For the evaluation of delayed type of hypersensitivity (DTH) test animals were divided in to four groups, having six animals in each. Group I, the control, was given 2ml of 5% normal saline and to group II, III, IV was administered of 50mg, 100mg, and 150mg/kg b.w. of ethanolic extract intraperitonially for ten days. On 10th day 0.1ml of SRBC solution was injected subcutaneously in to the right footpad. After 24,48,72,96 hrs, thickness of footpad was measured by plethisometer. Difference in the footpad thickness in control and treated group has been taken as the measure of the DTH reaction[11].

SRBC Agglutination Test

To study humoral antibody response against antigens SRBC agglutination test was performed. Twenty four animals were divided in to four groups having six animals in each group. Group I, was kept as a control and received 2ml of 5% normal saline. Intraperitonially for seven days group II, III, IV were given 50 mg, 100mg, and 150mg/kg b.w. ethanolic extract intraperitonially for ten days.

All the animals were injected with 0.25 ml of 5×10. SRBC/ml on 6th, 8th and 10th days for achieving maximum titre of antibody. On 11th days blood was collected through retro-orbital venous plexus and centrifuge at 2000 rpm for 15 minutes in order to separate serum.100 ml of serum diluted serially with normal saline in separate test tubes, dilution was made up to 20,40,80, 160 and 320 times. To this 50ml of dextrin coated sheep red blood corpuscles added and incubated at 37°C for 18 hrs. All the tubes were subjected to examine agglutination visually and compared with control[12,13].

Cytokine Level Measurement:

The mice were divided in the similar four groups with 6 animal in each group and the levels of IL-2 and IL-6 were determined using BD Opt EIA^{TM} kits according to manufacturer's protocol [PharMingen].

Drug induced Myelosuppression Test

To determine the effect of drug induced myelosuppression, Cyclophosphamide was used to produce myelo suppression in albino rats. Albino rats were divided in 5 group of six each. Group I was kept as control and given 2ml of 5% normal saline. Group II was treated with Cyclophosphamide 3 mg/kg b.w. for seven days. Group III, IV and V was administered with ethanolic extract of 50 mg, 100 mg, and 150 mg/kg b.w. along with the similar dose of Cyclophosphamide as given to group II intraperitoneally with Cyclophosphamide. On seventh day blood was taken from retro-orbital plexus and subjected to haematological studies, blood

sample of each animal was collected on 15th day, a day after the dose to animal, and again animals were weighed and subjected to haematological studies including haemoglobin count, RBC count, WBC count, Platelet count[14,15].

RESULTS AND DISCUSSION

Qualitative chemical tests

The ethanolic extracts of *Leptadaenia reticulata* showed presence of Protein, Alkaloid, Tanin, Flavonoids, Steroid, Terpenoids, Saponin, and Glycosides.

TLC Studies

Ethanolic extract of the drug best separates in Chloroform: Acetone: Formic acid (74+15+5) and eight spots (R_f value-0.08, 0.19, 0.30, 0.33, 0.79, 0.94, 0.97, 0.99). Spots were visualized by Anisaldehyde-sulphuric acid reagent.

Toxicity Studies

In toxicity test with *Leptadaenia reticulata*, no mortality was recorded with the ethanolic extract.

Carbon Clearance Test (Table 1)

Carbon Clearance depends on time and it was calculated as phagocytic index of time interval between the treated groups of animals compared with the control group. The mean phagocytic index of control (Group I) was found to be 1.007 ± 0.020. The ethanolic extract had given significantly increased phagocytic index as 1.465 ± 0.067 (P<0.001), 1.625 ± 0.055 (P<0.001) and 1.700 ± 0.049 (P<0.001) respectively with 50, 100 and 150mg/kg b.w. intraperitoneally for seven days.

Table 1: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Phagocytic Activity in Carbon Clearance Test

	Groups	Mean absorbance SD		Phagocytic Index(k) ± SD
		0 min	15 min	
I.	Control	0.3361 ± 0.008	0.2413 ± 0.019	1.007 ± 0.020
II.	Crude Ethanolic extract (50mg/kg body wt.)	0.2886 ± 0.013	0.1773 ± 0.011	1.465 ± 0.067***
III.	Crude Ethanolic extract (100 mg/kg body wt.)	0.2794 ± 0.027	0.1629 ± 0.028	1.625 ± 0.055***
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	0.2648 ± 0.029	0.1512 ± 0.015	1.700 ± 0.049***

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

Delayed Type Hypersensitivity Test (Table 2)

Delayed Type Hypersensitivity response to SRBC was calculated as a measure of paw volume (in mm) for each animal and compared with control group I which was injected 2ml of 5% Normal saline intraperitoneally for seven days. Paw volume was calculated after 24, 48, 72 and 96 hrs. Animal treated with crude ethanolic extract showed reduced paw volume after 24, 48, 72 and 96 hrs. The decline in paw volume for group II, III and IV after 24 hrs were found to be 1.41 ± 0.028 ml ($P<0.05$), 1.35 ± 0.019 ml ($P<0.05$) and 1.21 ± 0.013 ml ($P<0.025$) and after 48 hrs there were 0.88 ± 0.024 ml, 0.83 ± 0.011 ml ($P<0.05$) and 0.82 ± 0.025 ml ($P<0.05$) after 72 hrs it was 0.58 ± 0.015 ml, 0.55 ± 0.017 ml and 0.54 ± 0.019 ml and finally after 96 hrs paw volume where paw volume does not showed any significant change and it was 0.29 ± 0.012 ml, 0.28 ± 0.023 ml and 0.28 ± 0.028 ml respectively.

Table 2: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Delayed Type of Hypersensitivity.

	Groups	Paw Volume (ml) ± S.D.			
		24 Hrs	48 Hrs	72 Hrs	96 Hrs
I.	Control	1.59 ± 0.010	1.01 ± 0.029	0.64 ± 0.021	0.31 ± 0.016
II.	Crude Aqueous extract (50mg/kg body wt.)	1.41 ± 0.028*	0.88 ± 0.024	0.58 ± 0.015	0.29 ± 0.012
III.	Crude Aqueous extract (100 mg/kg body wt.)	1.35 ± 0.019*	0.83 ± 0.011*	0.55 ± 0.017	0.28 ± 0.023
IV.	Crude Aqueous extract (150 mg/kg body wt.)	1.21 ± 0.013**	0.82 ± 0.025*	0.54 ± 0.019	0.28 ± 0.028

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* $P<0.05$, ** $P<0.025$, and ***$P<0.001$)

SRBC Agglutination Test (Table 3)

Agglutination titer to sheep red blood erythrocyte was calculated and compared with Group I (control). Group II, III and IV were treated with crude ethanolic extract orally for ten days (50, 100, 150mg/kg b.w.) and on 10^{th} day agglutination titer were observed in various serum dilution (X: 20, X: 40, X: 80, X: 160, X: 320). A decrease was observed in the animals of group III and IV which received 100 and 150mg crude ethanolic extract/ kg b.wt. while group II did not showed any change in the activity.

Table 3: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Agglutination Titre to SRBC

	Groups	Serum Dilution in Normal Saline± 50 µl antigen				
		X: 20	X: 40	X: 80	X: 160	X: 320
I.	Control	+	+	+	-	-
II.	Crude Ethanolic extract (50mg/kg body wt.)	+	+	+	-	-

III.	Crude Ethanolic extract (100 mg/kg body wt.)	+	+	-	-	-
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	+	+	-	-	-

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

Cytokines (IL-2 and IL-6) Assay (Table 4)

Cytokines (IL-2 and IL-6) level were observed in all Groups (I, II, III and IV) and treated Groups were compared with control (Group I). The IL-2 and IL-6 levels were observed as 24.21 ± 1.352 and 30.58 ± 2.846pg/ml respectively for control. Crude ethanolic extract had also given higher IL-2 level as 32.15 ± 4.273, 45.24 ± 5.581 (P<0.05), 61.86 ± 5.162pg/ml (P<0.05) and IL-6 level were found stable as 29.91 ± 3.454, 31.07 ± 2.106, 34.83 ± 4.813pg/ml respectively with 50, 100, 150mg/kg b.wt.

Table 4: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Cytokines (IL-2 and IL-6)

	Groups	IL-2 concentration in mice serum (pg/ml) Mean ± S.D.	IL-6 concentration in mice serum (pg/ml) Mean ± S.D.
I.	Control	24.21 ± 1.352	30.58 ± 2.846
II.	Crude Ethanolic extract (50mg/kg body wt.)	32.15 ± 4.273	29.91 ± 3.454
III.	Crude Ethanolic extract (100 mg/kg body wt.)	45.24 ± 5.581*	31.07 ± 2.106
IV.	Crude Ethanolic extract (150 mg/kg body wt.)	61.86 ± 5.162*	34.83 ± 4.813

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

Drug Induced Myelosuppression Using Cyclophosphamide (Table 5)

The Group I was control and received as usual 2 ml of 5% of normal saline and various hematological observations were taken. In them the mean haemoglobin was 13.11 ± 0.12 gms/dl, mean RBC count was 4.65 ± 0.154 million/mm^3 and mean WBC count was 13.27 ± 0.425 thousand/mm^3. Neutrophils count was 53.10 ± 1.51%, Lymphocytes count was 40.82 ± 1.08%, Monocytes count was 2.45 ± 0.38%, Eosinophil count was 2.35 ± 0.51% and platelets count was 3.23 ± 0.243 lacs/mm^3. In Group II cyclophosphamide (3mg/kg b.wt) were administered and there was a significant decrease in all hematological parameters studied except Neutrophils and Monocytes count which were slightly increased. Mean haemoglobin was 8.25 ± 0.29 gms/dl (P<0.025), mean RBC count was 3.72 ± 0.121 million/mm^3 (P<0.05) and WBC count was 11.16

± 0.241 thousand/mm^3 (P<0.05). Mean Neutrophils count was 59.84 ± 0.89%, Lymphocytes count was 33.59 ± 0.85% (P<0.05), Monocytes count was 3.01 ± 0.26%, Eosinophil count was 2.41 ± 0.24% and platelets count was 2.40 ± 0.456 lacs/mm^3 (P<0.05). Group III, IV and V were administered crude ethanolic extract of (50, 100 and 150mg/kg b.wt) with cyclophosphamide intraperitoneally, in them the mean haemoglobin was found to be 9.84 ± 0.15, 10.05 ± 0.50, 10.13 ± 0.38 gms/dl respectively. Mean RBC count was 3.79 ± 0.142, 3.75 ± 0.219, 3.68 ± 0.087 million/mm^3, mean WBC count was 11.54 ± 0.326, 12.03 ± 0.257, 12.38 ± 0.249 thousand/mm^3 (P<0.05), Neutrophils count was 55.52 ± 0.72%, 57.67 ± 1.17%, 57.91 ± 0.76%, Lymphocytes count was 33.81 ± 0.57%, 34.66 ± 0.41%, 35.27 ± 0.39%, Monocytes count was 2.37 ± 0.33% (P<0.05), 2.49 ± 0.48%, 2.64 ± 0.28%, Eosinophils count was 2.61 ± 0.37%, 2.65 ± 0.42%, 2.77 ± 0.36% and platelets count was 2.74 ± 0.511, 2.82 ± 0.349, 2.91 ± 0.410 lacs/mm^3.

Table 5: Effect of Crude Ethanolic Extract of *Leptadaenia reticulata* on Drug Induced Myelosuppression using cyclophosphamide for 7 days

	Groups	Hb Gms/dl Mean±SEM	RBC Mean±SEM Million/mm^3	WBC Mean±SEM Thausand/mm^3	Neutrophills % Mean±SEM	Lymphocytes % Mean±SEM	Monocyte % Mean±SEM	Eosinophill Count % Mean±SEM	Platelets Lacs/mm^3 Mean±SEM
I.	Control	13.11 ± 0.12	4.65 ± 0.154	13.27 ± 0.425	53.10 ± 1.51	40.82 ± 1.08	2.45 ± 0.38	2.35 ± 0.51	3.23±0.243
II.	Cyclophosphamide (3mg/kg b.wt.)	8.25±0.29**	3.72 ± 0.121*	11.16 ± 0.241*	59.84 ± 0.89	33.59 ± 0.85*	3.01 ± 0.26*	2.41 ± 0.24	2.40±0.456*
III.	Crude Ethanolic Extract (50mg/kg body wt.) + Cyclophosphamide	9.84 ± 0.15	3.79 ± 0.142	11.54 ± 0.326	55.52 ± 0.72	33.81 ± 0.57	2.37 ± 0.33*	2.61 ± 0.37	2.74±0.511
IV.	Crude Ethanolic Extract (100mg/kg body wt.) + Cyclophosphamide	10.05 ± 0.50	3.75 ± 0.219	12.03 ± 0.257	57.67 ± 1.17	34.66 ± 0.41	2.49 ± 0.48	2.65 ± 0.42	2.82±0.349
V.	Crude Ethanolic Extract (150mg/kg body wt.) + Cyclophosphamide	10.13 ± 0.38	3.68 ± 0.087	12.38 ± 0.249*	57.91 ± 0.76	35.27 ± 0.39	2.64 ± 0.28	2.77 ± 0.36	2.91±0.410

Where, n = 6 swiss balb-c mice per group, tabular value represents mean ± S.D.

(* P<0.05, ** P<0.025, and ***P<0.001)

The crude ethanolic extract enhanced the phagocytic index in dose depended manner which was 1.465 ± 0.067 (P<0.001), 1.625 ± 0.055 (P<0.001) and 1.700 ± 0.049 (P<0.001) respectively with 50, 100 and 150mg/kg b.wt. (Table 1). Increase in phagocytic index indicates that phagocytosis is increasing. Stimulation of phagocytosis is influenced by the activation of macrophages, the activated macrophages secrete a number of cytokines, which in turn stimulate other immune cells[16]. Crude ethanolic extract have the phytocontents for chemo stimulation of phagocytosis. The component(s) of the extract activate the receptors to remove antigen (here the carbon particles) through pinocytosis as the antigen is very small. In case of mouse CRI, CRI2, CR3, CR3b and CR3bi are the main receptors. The phenols, flavonoids, terpennes and saponins are responsible to incite them, which in turn eliminate carbon particles or phagosite. Neutrophils or monocytes, which are main phagocytic leucocytes, take up particles through minimum 40 receptors expressed on their surface[17]. These receptors are for IgG complement, mannose and galactose terminated oligosaccharides. It is supposed that many of the receptors become active due to the exposure of the extracts.

The preexisting and newly formed IgG may be playing their role in the identification of the antigen, activation of MoRC. IgG receptors, and the attachment of the receptors to facilitate phagocytosis. Many Flavones increase phagocytosis through complement C3 and C1. Flavonoids are present in the extract of the plants. Besides them some other compounds are also there which work in association of flavonoids to activate CR3b and CR3bi receptor of phagocytes and ligation of complements with the receptors[18].

Delayed Type Hypersensitivity Test was done to study the effect at crude ethanolic extract on cell-mediated immune response to paw edema in 24, 48 hrs and then after 72 and 96 hrs paw volume significantly decreased when compared with control (Table 2).

The reduction in paw volume may be because of a quick action of various enzymes, hormones etc on the invader, simultaneously phagocytosis increased because of activated macrophages and hence reduction in paw volume was observed. Reduction in paw volume after 24 hr. and onwards point to the fact that saponins and similar compounds increase the metabolic activity of the neighboring cells to release metabolites and activated macrophages eliminate the causative agents hence the edema gradually reduces. The increase in paw volume, in response to infiltration of CD4 line of T-lymphocytes and as usual diapedesis of mononuclear macrophages and liberation of edema causing substances for example serotonin, prostaglanddulin E, cytokines etc. The infiltration of lymphocytes is possibly because of the compounds, which perhaps observed the cell-mediated immune response. Extract of *Laptadaenia* having potent activity to

involve cell- immune response. This indicates that ethanolic extract contain amines and multiple hormonal substances like lymphokines. These hypersensitive responses particularly by attracting and activating macrophages[19].

The crude ethanolic extract caused a decrease in the agglutination titer. Crude ethanolic extract at the doses of 100, 150mg/kg b. wt. showed agglutination titer only up to X: 40 and the ethanolic extract suppresses humoral immune response and interfere with antibody formation so antibody is formed insignificantly, affects agglutination titer against SRBC titer (Table 3). The study of the results depict that the ethanolic extract surprisingly show almost no change in agglutination titer, perhaps the amount of the compounds that can invoke antibody synthesis is not enough to incite T4 and B lymphocytes or such compounds are not in the extract. This cannot be ignored that immunosuppression may be caused by the other contents of the extract. Aqueous extract of the plant contained proteins, oligosaccharides and their conjugated compound besides β-sterols, saponins, flavonoids, flavones etc. the antigenicity to elicit antibodies of first two compounds is well known, but ethanol soluble fraction is devoid of some compounds. Red blood cell at neutral pH possesses negative ions that form cloud, which repel one another Immunoglobulins like IgM can overcome the electric barrier and get cross-link with red blood cells, this leads to subsequent agglutination. In many plants similar activities and increase titer of IgM etc. are observed[20,21].

Cytokines are essential mediators of cell-to-cell signals in physiological and pathological immune responses and in the inflammatory response. Crude ethanolic extract enhanced IL-2 levels in a dose dependent manner while the IL-6 showed almost stable levels (Table 4).

The qualitative analysis of IL-2 in both control and experimental animals was assessed and correlated with significant increase in WBCs count/lymphocyte count in experimental animals. So for IL-6 is concerned its increase can be correlated with increase paw volume. The cytokines whenever increase in very low concentration a definite effect is produced. IL-2 is formed to increase with many plants extracts as reported by Ganguli *et al.*[22] and Bone[23]. These low molecular weight proteins activate the receptors on lymphocytes to cure these sensitivity a growth and activity. IL-2 also stimulates other cellular effectors like GCFs and GMCSF although these factors are not estimated but an indirect conclusion about their increase can be made which inhibits WBCs count and paw edema test. Probably the glucosteroids and flavones which are present in good quantity in the extracts, they are directly or indirectly responsible to elevate the cytokines.

Cyclophosphamide suppresses humoral, cellular, non-specific and specific cellular immune response. When animal was treated with cyclophosphamide then haemoglobin (Hb), RBC

counts, WBC count, Lymphocyte% and Platelet count all are reduced significantly[24]. The suppressive effect of cyclophosphamide was protected by the administration of aqueous extract and their ethanol soluble and ethanol insoluble fraction. Flavonoids in biological systems tend to adhere with the molecules of cyclophosphamide this causes to increase the size of the molecules and prevent its entry to the stem cells. As already stated that such compounds are detected in the plant extract besides this some more compounds are there which are not only negating the effect of cyclophosphamide, but also accelerating the total WBC and haemoglobin count. The crude ethanol extract did not make any significant elevation in the hematological parameters taken for study. Crude ethanolic extract of *Leptedaenia* showed a mixed effect, sometimes the values of blood parameters increase or decrease (Table 5). The ethanolic extract showed a dual nature, stimulatory as well as suppressive effect. In some case extract protected the animal from the effect of cyclophosphamide but at certain doses of the extract only it showed a slight reduction in the given values. Several other plant extracts have also been shown to have simultaneous immunosuppressive and immunostimulatory effects[25,26]. Overall results with the *Leptedaenia reticulata* showed its immunostimulant as well as immunosuppressant nature.

CONCLUSION

Finding of these studies suggest that the crude ethanolic extract is capable to strengthen the immune system. The ethanolic extract modulate immune responses significantly as it increased the phagocytic index, modulate the phagocytic functions of macrophages and phagocytes, which means it has a profound effect over the innate immunity. It also modulate the function of cytotoxic T-cell that produces delayed type hypersensitivity immune response, which gives a better protection against viruses and tumors.

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REFERENCES

1. Chemexcil, Selected Medicinal Plants of India. A Monograph of Identity, Safety and Clinical Usage, Pharmaceuticals and Cosmetics Export Promotion Council, Bombay. 1992; 196–198.
2. Daniel M. Medicinal Plants-Chemistry and properties. New Delhi: Oxford and IBH Publishing Co.Pvt.Ltd; 2006.
3. Sivarajan V.V. and Balachandran I. Ayurvedic Drugs and Their Plant Sources, Oxford and IBH, New Delhi. 1999; 195–200.

4. Anjaria, J. V., Parabia, M., Bhatt, G. and Khamar, R. Nature Heals: A Glossary of Selected Indigenous Medicinal Plants of India, Sristi Innovations, Ahmedabad. 1997; 50.
5. Singh V. and Pandey R.P. *Ethnobotany of Rajasthan*, *India*, Scientific Publishers (India), Jodhpur. 1998; 367.
6. Kirtikar K.R. and Basu B.D. *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehradun. 1994; 2(2), 1629–1630.
7. Rastogi R.P. and Mehrotra B.N. Compendium of Indian Medicinal Plants, CDRI, Lucknow. 1999; 243-244.
8. Diallo C., Samake F.B., Paulsen B.S., Michaelsen T.E. and Keita A. Wound healing plants in Mali, the Bamaco region. An ethnobotanical survey and complement fixation of water extracts from selected plants, *Pharm. Biol*. 2002; 40(2), 117-28.
9. Anjaria J.V., Varia M.R., Janakiraman K. and Gulati O.D. Studies on *Leptadenia reticulata*. Lactogenic effects on rats. *Indian J Expt Biol*. 1975; 13: 448-449.
10. Das M, Dasgupta SC, Gomes A. Immunomodulatory and antineoplastic activity of common Indian Toad (*Bufo melanostictus*, Schneider) skin extract. Ind. J. Pharmacology. 1998; 30:311.
11. Dikshit V, Damre AS, Kulkarni KR et al. Preliminary screening of immunocin for immunomodulatory activity. Ind J Pharma Sci. 2000; 62 (4):257-260.
12. Nelson DA, Mildenhall P. Studies on cytophillic antibodies. The production by mice of macrophage cytophillic antibodies to sheep erythrocytes; relationship the production of other antibodies and development of delayed like hypersensitivity. Aust J Exp Biol Medi Sci. 1967; 45:113.
13. Weir DM. "Handbook of Experimental Immunology, Blackwell Scientific Publication", 3rd Edn, 1979; 3.
14. Gokhale AB, Damre AS, Saraf MN. Investigations into the immunomodulatory activity of *Argyreia speciosa*. J. Ethnopharmacol. 2003; 84: 109-114.
15. Joshi VD. Practical Physiology. Vikash publishers, New Delhi, 1994; 15-34.
16. Nose M, Terawaki K, Oguri K, Ogihara Y, Yoshimatsuk A, Shimomura K. Activation of macrophages by crude polysaccharide fraction obtained from shoots of *Glycyrrhiza glabra* and hairy roots of *Glycyrrhiza uralensis* In vitro. Biol. Pharm. Bull. 1998; 21(10): 1110-1112.

17. Verotta L, Guerrini M, EI-Sebakhy Asaad AM, Toaima SM, Abou-Sheer ME, Luo YD, Pezzuto JM. Cycloartane saponins from *Astragalus peregrinus* as modulators of lymphocyte proliferation. Fitoterapia. 2001; 72: 1894-1905.
18. Estrada A, Katsclis GS, Laarveld B, Barl B. Isolation and evaluation of immunological adjuvant activities of saponins from *Polygala senega* L.Comp. Immunol. Microbial. Infect. Dis. 2000; 23: 27-43.
19. Kulkarni S, Desai S. Immunostimulant activity of Inulin from *Saussurea lappa* roots. Ind.J. Pharm.Sci. 2001; 63 (4) 292-294.
20. Rezaeipoor R, Sanei Ziaei L, Kamalinejad M. Effect of *Isatis cappadocica* on humoral immune responses. Fitoterapia. 2000; 71: 14-18.
21. Freier DO, Wright K, Klein K, Voll D, Dabiri K. *Echinacea purpurea* in female Swiss mice. Immunopharmacol. Immunotoxicol. 2003; 25(4): 551-560.
22. Ganguly T, Badheka LP, Sainis KB. Immunomodulatory effect of *Tylophora indica* on Con A induced lymphoproliferation. Phytomedicine. 2001; 8:431-437.
23. Bone K. Clinical Applications of Ayurvedic and Chinese Herbs, Monographs for the Western Herbal Practitioner. Warwick, Australia: Phytotherapy Press. 1996.
24. Doherty NS. Selective effect of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cell in mice. Agents Action. 1981; 11, 237.
25. Yamaguchi H. Immunomodulation by medicinal plants. Adv Exp Med Biol. 1992; 319: 287-297.
26. Mediratta PK, Sharma KK, Singh S. Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. J Ethnopharmacol.2002; *80*: 15-20.