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Immunomodulating Activity of Aqueous Extract of *Leptadaenia Reticulata*

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ABSTRACT

The aim of the present study is to evaluate the effect of aqueous extract of *Leptadaenia reticulata* for immunomodulating activity. The aqueous 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 an increase in carbon clearance, humoral antibody (HA) titre, delayed type hypersensitivity (DTH) and white blood cell (WBC) count in a dose dependent manner. The aqueous extract also enhanced interleukin-2 (IL-2) level in a dose dependent manner while the IL-6 showed almost stable level. The present investigation established pharmacological evidence to support the folklore claim that it is an immunomodulating.

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. It is commonly known as Jivanti, Swarnjivanti or Dori. The botanical identity of the drug made from this plant is highly disputed. *L. reticulata* as the source of the drug and real Jivanti. Ayurvedic formulary of India also accepts this as the true drug plant[1].

Leptadaenia reticulata contains many important phytoconstituents of plants. Previously reported chemical constituents of *Leptadenia reticulata* are α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentriacontanol[2], a triterpene alcohol simiarenol[3] and apigenin[4]. Pregnane glycosides reticulatin, deniculatin and leptaculatin have also been isolated from the aerial parts[5]. Pregnane glycosides reticulin, deniculatin, leptaculatin isolated from aerial parts which on hydrolysis give calogenin tocopherols. Other are acetyl alcohol, lupanol 3-O diglucoside, leptidine 1, saponins, flavonoid, luteolin, diosmtin and tannin. Leaves contain two resins and also a bitter neutral principle, albuminous and colorizing matter, Ca-oxalate, glucose, carbohydrate and tartaric acid[4].

Aqueous extract of the stem of this plant demonstrated vasodilator, transient, inotropic, chronotropic and prolonged hypotensive effect in dogs. A number of studies have been carried out on its galactogogue property. On lactating rats, its ether extract was found to increase lactation[6]. The lactogenic effect of the plant was also studied on Gir cows[7]. It showed an increase in the secretions of the accessory sex organs in the mice[8]. Another formulation Leptaden, has been shown to provide effective treatment in cases of deficient lactation and lack of lactation in humans[9]. The extract of the leaf shows antibacterial and antifungal activities[10]. Therefore this study was carried out with the basic aim of identification of potent immunomodulatory agent.

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.

Aqueous Extract Preparation

Powdered plant material was kept in beaker for 24 hr with water. Filter the content, marc was discarded. Reduce the volume of filtrate in vacuum oven, then dry aqueous extract in Lyophilizer (Heto Drywinner) (Percentage yields 25.57%)[11].

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 aqueous 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 &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. Phagocytic index determined by following formula[12]

$$\text{Phagocytic index} = K\,(\text{sample}) / K\,(\text{control})$$

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 aqueous 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[13].

SRBC Agglutination Test

To study humoral antibody response against antigens SRBC agglutination test was performed [14] 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. aqueous 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.

Cytokine Level Measurement

The mice were divided in the similar four groups with 6 animals 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 .

Drug induced Myelosuppression Test

To determine the effect of drug induced myelosuppression**,** Cyclophosphamide was used to produce myelosuppression 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 aqueous 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[15, 16].

RESULTS AND DISCUSSION

Qualitative chemical tests

The aqueous extracts of *Leptadaenia reticulata* showed the presence of Carbohydrate, Glycosides, Saponin, Phenolic compound, Flavonoids, Steroid, Terpenoids and Tanin.

TLC Studies

Thin layer chromatographic studies of aqueous extracts of *Leptadaenia reticulata* have shown best separation in n-Butanol: Glacial acetic acid: Distilled water (4:1:5) and give ten spots (R_f value-0.10, 0.15, 0.27, 0.31, 0.50, 0.58, 0.64, 0.080, 0.91, 0.97). Spots were visualized by Ninhydrin reagent.

Carbon Clearance Test

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 crude aqueous extract of *Leptadaenia reticulata* treated groups III and IV were elevated as 1.195 ± 0.038 ($P<0.025$) and 1.431 ± 0.054 ($P<0.001$), while group I was observed slightly on lower side as 0.989 ± 0.061 when animals treated with 100, 150 and 50mg/kg b.w. intraperitoneally for seven days.

Delayed Type Hypersensitivity Test

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. The decreased value for group II, III and IV after 24 hrs were found to be 1.58 ± 0.027 ml, 1.55 ± 0.016 ml and 1.51 ± 0.011 ml and after 48 hrs there were 0.92 ± 0.019 ml, 0.094 ± 0.028 ml and 0.89 ± 0.018 ml ($P<0.05$) after 72 hrs it was 0.64 ± 0.013 ml, 0.58 ± 0.012 ml and 0.52 ± 0.015 ml ($P<0.05$) and finally after 96 hrs paw volume reduced significantly 0.24 ± 0.015 ml ($P<0.05$), 0.23 ± 0.014 ml ($P<0.05$) and 0.20 ± 0.021 ml ($P<0.025$) respectively.

SRBC Agglutination Test

Agglutination titer to sheep red blood erythrocyte was calculated and compared with Group I (control). Group II, III and IV were treated with crude aqueous 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). An increased was observed at the dose of 50, 100 and 150mg/kg b.w.

Cytokines (IL-2 and IL-6) Assay

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 aqueous extract had given relatively higher IL-2 level as 35.47 ± 3.861, 51.34 ± 6.589 ($P<0.05$), 72.92 ± 7.195pg/ml ($P<0.025$) and IL-6 level was found stable as 31.61 ± 1.837, 34.89 ± 3.453, 33.48 ± 3.007pg/ml respectively

with 50, 100, 150mg/kg b.w.

Drug Induced Myelosuppression Using Cyclophosphamide

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.w.) 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).

Table 1: Effect of Crude Aqueous 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 Aqueous extract (50mg/kg body wt.)	0.3289 ± 0.024	±	0.2366 ± 0.017	0.989 ± 0.061
III.	Crude Aqueous extract (100 mg/kg body wt.)	0.3232 ± 0.012	±	0.2172 ± 0.019	1.195 ± 0.038**
IV.	Crude Aqueous extract (150 mg/kg body wt.)	0.3185 ± 0.022	±	0.1984 ± 0.018	1.431 ± 0.054***

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)

Group III, IV and V were administered crude aqueous extract of (50, 100 and 150mg/kg b.w.) with cyclophosphamide intraperitoneally, in them the mean was found to be 11.02 ± 0.22 (P<0.05), 11.78 ± 0.16 (P<0.05), 12.41 ± 0.19 gms/dl (P<0.05) respectively. Mean RBC count was 3.95 ± 0.094, 4.11 ± 0.173, 4.24 ± 0.117 million/mm^3 (P<0.05), mean WBC count was 11.79 ± 0.151, 12.16 ± 0.114, 12.87 ± 0.512 thousand/mm^3 (P<0.05), Neutrophils count was 57.57 ± 1.12%, 55.43 ± 1.20%, 55.13 ± 0.97%, Lymphocytes count was 35.13 ± 0.99%, 38.43 ± 1.02% (P<0.05), 38.98 ± 0.74% (P<0.05), Monocytes count was 2.14 ± 0.18% (P<0.05), 2.58 ± 0.43%, 2.87 ± 0.23%, Eosinophils count was 2.23 ± 0.56%, 2.32 ± 0.39%, 2.38 ± 0.42% and

platelets count was 2.84 ± 0.215, 2.86 ± 0.483, 2.98 ± 0.381 lacs/mm^3.

Table 2: Effect of Crude Aqueous 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.)	1.58 ± 0.027	0.92 ± 0.019	0.64 ± 0.013	0.24 ± 0.015*
III.	Crude Aqueous extract (100 mg/kg body wt.)	1.55 ± 0.016	0.94 ± 0.028	0.58 ± 0.012	0.23 ± 0.014*
IV.	Crude Aqueous extract (150 mg/kg body wt.)	1.51 ± 0.011	0.89 ± 0.018*	0.52 ± 0.015*	0.20 ± 0.021**

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)

Table 3: Effect of Crude aqueous 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 Aqueous extract (50mg/kg body wt.)	+	+	+	+	-
III.	Crude Aqueous extract (100 mg/kg body wt.)	+	+	+	+	+
IV.	Crude Aqueous 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)

Table 4: Effect of Crude Aqueous Extract of *Leptadaenia reticulata* on Cytokines (IL-2 & 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 Aqueous extract (50mg/kg body wt.)	35.47 ± 3.861	31.61 ± 1.837
III.	Crude Aqueous extract (100 mg/kg body wt.)	51.34 ± 6.589*	34.89 ± 3.453
IV.	Crude Aqueous extract (150 mg/kg body wt.)	72.92 ± 7.195**	33.48 ± 3.007

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

Table 5: Effect of Crude Aqueous 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/m^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.w.)	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 Aqueous Extract (50mg/kg body wt.) + Cyclophosphamide	11.02 ± 0.22*	3.95 ± 0.094	11.79 ± 0.151	57.57 ± 1.12	35.13 ± 0.99	2.14 ± 0.18*	2.23 ± 0.56	2.84 ± 0.215
IV.	Crude Aqueous Extract (100mg/kg body wt.) + Cyclophosphamide	11.78 ± 0.16*	4.11 ± 0.173	12.16 ± 0.114	55.43 ± 1.20	38.43 ± 1.02*	2.58 ± 0.43	2.32 ± 0.39	2.86 ± 0.483
V.	Crude Aqueous Extract (150mg/kg body wt.) + Cyclophosphamide	12.41 ± 0.19*	4.24 ± 0.117*	12.87 ± 0.512*	55.13 ± 0.97	38.98 ± 0.74*	2.87 ± 0.23	2.38 ± 0.42	2.98 ± 0.381

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)

Crude aqueous extract of *Laptadaenia reticulata* showed significant immunostimulant activity in carbon clearance test by increasing phagocytic index in a dose dependent manner. Crude aqueous extract, increased the phagocytic index significantly as 1.195 ± 0.038 ($P<0.025$) and 1.431 ± 0.054 ($P<0.001$) (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[17]. In the same experiment a dose of 50mg/kg b.w. of crude aqueous extract did not show any significant increase or decrease in the phagocytic effect. This suggests that the active substance, which stimulates the immune system, either is absent or present in such a low concentration that no invocation to phagocytes is generated significantly. Crude aqueous extract have the phytocontents for chemostimulation 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[18]. 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 pre existing 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[19].

Delayed Type Hypersensitivity Test was done to study the effect at crude aqueous 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 aqueous extract contain amines and multiple hormonal substances like lymphokines. These hypersensitive responses particularly were by attracting and activating macrophages[19]. Aqueous extract of plant contains saponins and saponins are immunostimulatory agents[18].

An increase in humoral immune response was observed. Agglutination titer to SRBC fraction also showed agglutination titer up to the same level (Table 3). 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, other compounds are equally potent for the synthesis of immunoglobulins. 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. From the above results it is possible that there is an enhancement in the level of IgM and IgG because antibody tire against SRBC were raised. In many plants similar activities and increase titer of IgM etc. are observed[21, 22]. The responsible chemicals were alkaloids flavonoids, polysaccharides and polypeptides[23].

Cytokines are essential mediators of cell-to-cell signals in physiological and pathological immune responses and in the inflammatory response. Under normal conditions, these cyto-kines act as crucial signals in the development of appropriate defenses. However, exaggerated or prolonged release can lead to pathological conditions. Crude aqueous 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[24, 25]. 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[26]. The suppressive effect of cyclophosphamide was protected by the administration of aqueous extract. 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. Crude aqueous extract of 100, 150 mg/kg b.w. showed significant increase in the haemoglobin, RBC count, WBC count; Lymphocytes and platelet count in a dose dependent manner (Table 5). This suggests that the constituent of the plant preventing the access of cyclophosphamide to the stem cells so that synthesis of haemoglobin, WBC and RBC is not inhibited. Another point is that the compound as are reutilizing this immunosuppressant before it could act upon haemopoeitic and myeloid tissue and its effective amount is present in 100/150 mg of extract. The crude aqueous extract also enhanced the number and activities of various immune cells and protected the animal form the adverse effect of cyclophosphamide. In addition to carbohydrates, glycosides and saponins, proteins also contribute to a larger extent to immuno stimulation more activity that was observed with aqueous extract[27].

The results of the paw edema assay prompted the hypothesis that crude aqueous extract has components with immunosuppressive activity alongside others, which can be immunostimulatory. Several other plant extracts have also been shown to have simultaneous immunosuppressive and immunostimulatory effects[28, 29]. Overall results with the *Leptedaenia reticulata* showed its immunostimulant as well as immunosuppressant nature.

CONCLUSION

Finding of these studies suggest that the crude aqueous extract is capable to strengthen the immune system. The aqueous 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. It also increase the antibody titer, which means modulation of humoral immunity.

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