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Studies on the Immunomodulatory Effects of *Cleome viscosa*

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The immunomodulatory effect of aqueous and ethanolic extracts of the aerial parts of *Cleome viscosa* Linn (Capparidacea) was investigated in mice. The assessment of immunomodulatory activity was carried out by various hematological and serological tests. Extract of the plant showed significant immunosuppressant activity and administration of extract remarkably decreased the number of WBC and spleenic lymphocytes. It also decreased phagocytic index and both cellular and humoral antibody response. It is concluded that the test extract possessed promising immunosuppressant properties.

The immune system is known to be involved in the etiology as well as pathophysiologic mechanism of many diseases. Immunology is thus probably one of the most rapidly developing areas of biomedical research and has great promises with regard to the prevention and treatment of a wide range of disorders, inflammatory diseases of skin, gut, respiratory tract, joints and central organs. In addition infectious diseases are now primarily considered immunological disorders, while neoplastic diseases and organ transplantation and several autoimmune disease may involve an immunosuppressive state[1]. Modulation of immune responses to alleviate the disease has been of interest for many years and the concept of *Rasayana* in Ayurveda is based on related principles. The function and efficiency of the immune system may be influenced by many exogenous factors like food, pharmaceuticals, physical and psychological stress and hormones etc. resulting in either immunostimulation or immunosuppression. The healthy state is believed to be based on a sophisticated fine-tuning of immunoregulatory mechanisms.

Suppressive and cytotoxic activity affecting the function of immune system has been reported for a many of synthetic and natural therapeutic agents. Among the synthetic substance, azathioprin and cyclophosphamide are used but these drug have a number of side effects in long-term treatment. Cyclophosphamide is an alkylating agent resulting in the cross linking of DNA and causes inhibition of DNA synthesis. The major drawbacks of this drug are myelosuppression, which is undesirable[2]. Immunomodulator of herbal origin appear to be a better alternative to overcome the above problem.

Cleome viscosa is described in Ayurveda and other systems of medicine to cure many diseases such as headache and cough. *Cleome viscosa*, a member of the family Capparidacea, occurs in tropical and sub-tropical area. The family comprises nearly 275 species in 12 genera. Various species of Cleome are used medicinally in India, Indonesia, Philippines, North and Central America. The leaves are rubefacient and vesicant. The juice of the leaves mixed with *ghee* (clarified butter) is used in the treatment of inflammations of the middle ear. The leaves are also used for external applications for wounds and ulcers. Seeds too are reported to have rubefacient, vesicant and antihelmentic properties. Poultice made from seeds are said to be counter irritants in chronically painful joints. Seeds are used to treat roundworm infections. The decoction of roots is administered as a febrifuge[3-7].

In our previous study we evaluated the hepatoprotective

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properties of the *Cleome viscosa*[8]. The effect of *Cleome viscosa* on the immune system at various levels i.e. haemotopoiesis, nonspecific mechanism and cellular response has not been extensively studied. Therefore the object of present study was to investigate the immunomodulatory activity of the aqueous and alcoholic extract of *cleome viscosa.*

MATERIALS AND METHODS

Cleome viscosa is grown on the sides of the agriculture fields by the villagers. It is an erect herbaceous annual herb. The healthy plant material was collected from Panth Nagar locality, Sagar in the month of August-September. Specimen at voucher no. 3814 was deposited in the herbarium of the Botany Department. Samples were dried in shade for 15 days and reduced to moderately coarse powder mechanically.

Extraction procedure:

Powdered plant material (aerial parts, 1 kg) was packed in a Soxhlet extractor. The drug was defatted with petroleum ether (60-70°) for about 25-30 cycles. The defatted material was subjected to ethanolic extraction using 95% ethanol for 48 h in a Soxhlet extractor. Aqueous extraction was prepared after treating the material with methanol and successively washed vigorously with 2% saline and distilled water. The extract was finally prepared in distilled water and concentrated under vacuum (Vacuum oven, Jyoti Scientific Ltd., Gwalior). After drying the extract was weighed and yield was calculated.

Experimental protocols:

Healthy BALB/C mice (8-12 W; 80-100 g) of either sex were selected for the study. The animals were fed on a commercial diet (Hindustan Lever Pellets Bangalore), and water *ad libitum.* The mice were acclimated to laboratory hygienic conditions for ten days before starting the experiment. Permission of the Institutional Animal Ethical Committee was obtained for all animal experimentation as per the approved protocol.

Administration of test extract:

Animals were randomly divided into seven groups, each group was consisting of six animals. Animals in the treatment groups were given the test extracts in 0.1% (w/v) sodium carboxymethyl cellulose (CMC) daily for seven days. Group I animals served as control and received equivalent volume of sodium carboxymethyl cellulose (0.1 % w/v) as vehicle. Aqueous extract in doses of 50, 100 and 150 mg/kg of body weight was administered intraperitoneally to animals in groups II, III and IV, respectively, whereas ethanolic extract in doses of 50, 100 and 150 mg/kg of body weight was administered intraperitoneally to animals of group V, VI and VII, respectively for 7 d. Blood samples were collected from retro-orbital plexus through a capillary and spleenectomy was performed on some animals only. Both the aforementioned processes were done 24 h after the last dose of the extract was administered.

Blood and spleenic leukocyte count:

After 7 d of administration of the extracts blood was collected from retro-orbital plexus of each animal for white blood cells (WBC) count. The animals were then killed by cervical dislocation and their spleen were removed, weighed, macerated and washed with phosphate buffer saline (pH-7.2) and pellets obtained after centrifugation at 3000 rpm (Remi centrifuge, Mumbai) for 4 min was re-suspended in PBS. After staining with tryphan blue the leukocyte counting was done with haemocytometer (Fein-Optik, Blankenburg, Germany). The results of these analyses were compared with that of control group of mice.

Determination of phagocytic index:

The mice treated with the extract were injected 7 days after the last dose of the extract, with 0.1 ml of carbon suspension i.v. through the caudal vein. Blood samples (50 µl) were collected form retro-orbital plexus of individual animals immediately before and at 3, 6, 9 and 12 min after the injection of carbon suspension, lysed with 2 ml of 0.1% glacial acetic acid and the absorbance was measured spectrophotometrically at 675 nm. The rate of carbon clearance termed as phagocytic index, was calculated as the slope of time vs. concentration curve[9].

Delayed type hypersensitivity reaction using SRBC as an antigen:

The method described by Doherty[10] was used. Mice of either sex were divided into seven groups of six each. Aqueous and ethanolic extract in three different doses (50, 100 and 150 mg/kg, i.p.) were administered a single dose on the day 0 and administration was continued once daily up to seven days. After seven days the mice were primed with 0.1 ml of SRBC suspension containing $1x10^8$ cells, i.p., and challenged on the day 14 with 0.05 ml of $2x10^8$ SRBC in the right hind food pad. The contralateral paw received equal volume of 0.1 % CMC. The thickness of the food pad was measured at 0, 24 and 48 h after challenge using Vernier calipers. The difference in the thickness of the right hind

paw and the left hind paw was used as a measure of delayed type hypersensitivity (DTH) reaction.

Humoral antibody response to SRBC:

Mice of either sex were divided into seven groups of six each. Drug extract in three different doses (50, 100 and 150 mg/kg, i.p.) was administered a single dose on the day 0 and administration was continued once daily up to seven days. On day 7 the mice were immunized with 0.1 ml of $1x10^8$ SRBC, i.p. Five days later, blood samples were collected from the retro-orbital plexus and antibody level was determined by haemagglutination techniques as described by Puri *et al.*[11]. The blood samples were centrifuged to collect serum and equal volume of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25 µl volume of normal saline in microtitration plates was added 25 µl of 1% suspension of SRBC in saline. After mixing, the plates were incubated at 37° for 1 h and examined for haemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer. After 7 d of administration of the test extract the hemoglobin content, total RBC count and haematocrit value was also determined.

Statistical Analysis:

Experiments were repeated twice and results are expressed as mean±S.D. Data was analyzed by Student's t-test using software Prism (Graphpad, USA) and considered significant at $P<0.005$.

RESULTS

Total WBC and viable spleenic lymphocyte count:

A significant, dose related decrease in the WBC count was observed in mice treated with aqueous and ethanolic extract (50-150 mg/kg, i.p.) for 7 d (Table 1). No significant changes were observed in hemoglobin content, RBC count and haematocrit value. Both aqueous and ethanolic extract of *C. viscosa* exhibited a slight decrease in spleenic lymphocyte count at lower dose (50 mg/kg) and at the higher dose (150 mg/kg) significantly decrease in the spleenic lymphocytes count in comparison to the control was observed (Table 1).

Phagocytic index:

Phagocytic Index was determined by measuring the concentration of Indian ink at different time intervals. The rate of carbon clearance is a measure of phagocytic activity. The results suggest that in the case of control animals, the concentration of Indian ink obtained after 12 minutes of experimental time was decreased nearly 54.3% from its initial values. On the other hand, the ethanolic extract (dose 150 mg/kg) showed significant immunosuppressive activity as reflected by higher recovery of the carbon particles (92.6 %) as compared against that obtained by the control (54.3%) (Table 2). The same trend was followed even after increasing the dose and the results were found as directly influenced by the increase in dose. Administration of aqueous and ethanolic extract of *Cleome viscosa* brought significant changes in phagocytic index. Different doses of the extracts also influenced the phagocytic index. Mean phagocytic index of 0.496, 0.437 and 0.305 were recorded with 50, 100 and 150 mg/kg dose of ethanolic extract, respectively. The results were statistically significant. However ethanolic extract shown even better results in terms of carbon clearance at the dose levels lower than that of aqueous extract (Table 2).

TABLE 1: EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME VISCOSA* ON SPLEEN WEIGHT, WBC AND BLOOD LEUKOCYTE COUNT

Group	Substance	Dose (mg/kg)	Spleen weight (g)	WBC counts per mm	Spleenic leukocyte count
I	Control	0.1% CMC	0.142±0.022	10.280±425	45.400±225
II	Aq. Extract	50	0.138±0.016	9325±370	43.530±220
III	Aq. Extract	100	0.132±0.018	8745±360	41.350±225
IV	Aq. Extract	150	0.128±0.020	8420±325	40.500±250
V	Eth. Extract	50	0.126±0.022	7850±320	38.200±260
VI	Eth. Extract	100	0.118±0.018	7010±285	34.230±310
VII	Eth. Extract	150	0.112±0.014	6530±270	26.550±210

Value are represent as mean ± SD (n =6)

TABLE 2: EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME VISCOSA* ON PHAGOCYTIC ACTIVITY AS DETERMINED BY CARBON CLEARANCE METHOD

Group	Substance	Dose	Concentration of Indian Ink (nl/ml)				Phagocytic
		(mg/kg)	3 min	6 min	9 min	12 min	index
I	Control	0.1 % CMC	2188±52	1969±49	1658±40	1136±35	1.00
II	Aq. Extract	50	2295±50	2061±51	1796±42	1628±38	0.891
III	Aq. Extract	100	2385±63	2224±54	1868±46	1734±40	0.768
IV	Aq. Extract	150	2455±65	2316±60	2072±50	1951±43	0.689
V	Eth. Extract	50	2671±66	2562±63	2546±53	2464±45	0.496
VI	Eth. Extract	100	2825±68	2810±67	2589±53	2534±50	0.437
VII	Eth. Extract	150	2949±72	2890±68	2783±55	2730±53	0.305

Value are represent as mean ± SD (n =6)

Delayed hypersensitivity test:

Both ethanolic and aqueous extract produced a significant, dose related decrease in DTH reactivity in mice. The edema achieved a peak at 24 h. The percent edema being 34.0±0.9 for the control group in comparison to 15.5±0.4 and 25.6±0.4 % for ethanolic and aqueous extract respectively. Results showed that aqueous and ethanolic extract showed a significantly decrease in paw edema in compared to control (fig. 1). The activities suggest possible decrease in cell mediate immunity of the extract.

Humoral antibody response:

The humoral antibody response was determined after immunization of the animals with SRBC. This was performed by SRBC haemagglutination test. It was found that after immunization with SRBC, the ethanolic extract caused statistically significant decrease (in all the doses levels) in the antibody titer in the SRBC agglutination test. At the lower dose of aqueous extract (50 mg/kg) did not causes significant decrease in the antibody titer on the other hand at the higher doses a satisfactory decrease was observed (Table 3).

DISCUSSION

Intraperitoneal administration of aqueous and ethanolic extracts of drug brought significant change in both non-specific and specific immunity. Ethanolic extract of *Cleome viscosa* showed significant decrease in leukocytes, blood and spleenic lymphocytes count. Incitement of immune system was again confirmed by decreased phagocytic index as determined by carbon clearance, which directly indicates the decreased phagocytic activity of certain immune cells.

DTH is a part of the process of graft rejection, tumor immunity, and, most important, immunity to many intracellular infectious microorganism, especially those causing chronic disease such as tuberculosis. 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 and promoting increased phagocytic activity and increased concentration of lytic enzymes for more effective killing. Delayed type hypersensitivity reaction exhibited by the aqueous extract was not significant while the percent edema of footpad treated with ethanolic extract was significantly decreased,

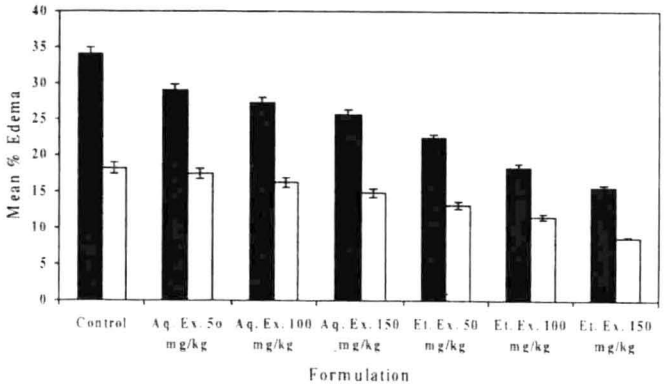

Fig. 1: Effect of aqueous and ethanolic extract on SRBC-induced DTH.

Effect of 50, 100 and 150 mg/kg doses of aqueous and ethanolic extract administered i.p. on DTH induced by intraperitoneally given SRBC ($1x10^8$ cells) at 24 h (■) and 48 h (□) . Value are represent as mean SD (n=6)

showed the decreased in the DTH reaction which indicate the immunosuppressant nature of drug (fig 1). The immunosuppressant nature of the ethanolic extract was further confirmed by decrease in the total WBC count. Change in WBC count is indicative of immune strength of the body.

One of the species of Cleome i.e. *Cleome droserifolia* has been also found to possesses immunomodulatory activities; its administration reduces the production of NO from activated macrophages. Nitric oxide is the gas produced by activated macrophages, dilates the blood capillaries to develop inflammation and to kill the bacteria as well[12,13].

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody function as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to from clusters that are readily ingested by phagocytic cells. Aqueous and ethanolic extract of the *C. viscosa* showed the significant inhibition in production of circulating antibody titer when tested on sheep erythrocyte specific haemagglutination antibody titer assay on mice. This indicates the decreased responsiveness of macrophage and T and B lymphocyte subsets involved in antibody synthesis.

It is evident from the results that the extracts modulate the immunological responses. The aqueous extract, which exhibits relatively weak immunosuppression properties, can be accredited to the antigens present in the form of soluble proteins, oligosaccharides and/or a glycoprotein that decrease its immunosuppressant properties. The preliminary examination for chemical composition confirms the presence of such compounds in the extract. This also suggests that ethanolic extract does not contain them and the ethanolic extract has such water insoluble components causing immunosuppression. The presence of alkaloids and saponins in this ethanolic extract can be assigned to immunosuppression in mice. Alkaloid isolated from *sedum sarmentosum* is reported to be immunosuppressant[14]. Although saponins from a large class of organic compound, a some of the saponins are observed to be immunostimulant still many of such compound are immunosuppressant. The use of plant products as immunomodulators is still in a developing stage. Some plant products as *Viscum album* extract, *Withania somnifera* extract[15] and herbal preparations like *Rasayanas*[16] are highly promising remedies in immunosuppressive conditions. Alkaloid isolated from *Sedum sarmentosum* possesses immunosuppressive

TABLE 3: EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *CLEOME VISCOSA* HUMORAL AND DELAYED TYPE HYPERSENSITIVITY

Group	Substance	Dose (mg/kg)	Antibody titer
I	Control	0.1 % CMC	276±6.8
II	Aq. Extract	50	241±5.2
III	Aq. Extract	100	196±4.8
IV	Aq. Extract	150	175±4.2
V	Eth. Extract	50	148±2.5
VI	Eth. Extract	100	114±2.0
VII	Eth. Extract	150	088±1.5

Value are represent as mean ± SD (n =6)

activities[11] and presence of such compounds in the ethanolic extract of *C. viscosa* is qualitatively confirmed. These compounds could be responsible for its immunosuppressant properties.

The overall pharmacological investigation conclusively demonstrated immunosuppressant activity in ethanolic extract of *Cleome viscosa* Linn. It may be rewarding to take the plant for detailed immunopharmacological studies with a view to take the plant for detailed immunopharmacological studies with a view to explore its potential as alternate herbal medicine for overcome the problem of toxicity of allophatic immunosuppressant e.g. cyclophosphamide for treatment of different autoimmune disease and in organ transplantation.

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