Asian Journal of Pharmaceutical and Clinical Research

INVESTIGATION OF IMMUNOMODULATORY POTENTIAL OF *CLEOME GYNANDRA* LINN.

M. L. KORI[*1], K. GAUR[1], V. K. DIXIT [2]

The aerial parts of *Cleome gynandra* Linn. was studied for immunomodulatory activity in phagocytic activity, cell-mediated and humoral immune system on albino rats. The aqueous extract (125, 250 and 500 mg/kg body weight, orally) and ethanolic extract (50, 100 and 200 mg/kg body weight, orally) were tested in carbon clearance test, delayed type of hypersensitivity, haemagglutination antibody titre, total leucocyte count and estimation of immunoglobulin. Results of present studies suggest that ethanolic extract significantly *(P<0.05)* diminish immune system in dose dependent manner whereas aqueous extract exhibit feeble immunosuppressive effect.

Keywords : Immunosuppression, *Cleome gynandra*, immunomodulatory activity, phagocytic index.

INTRODUCTION

Cleome gynandra Linn. (*Capperdiceae*) is described in Ayurveda and other system of medicine as a curative medicine for neuralgia, headache, cough, wounds, anthelmintic, rubefacient, counterirritant and for snake bite and scorpion sting etc[1]. Various species of cleome are used medicinally in Indo-China, Philippines, Island, North and Central America. The leaf paste of plant has been used in rheumatism, neuralgia, headache and stiff neck. Its warm juice is a popular remedy for ear disease. The leaf juice is applied in skin disease. Juice of fresh leaves is applied externally during pyorrhea and it is also used as a wormicide[2].

Hexacosanol, â-D-glucoside of â sitosterol, free â sitosterol and kaempferol have been isolated from the seed of *Cleome gynandra*[3]. It also contains the minor components 5, 7-dihydroxychromone, 5-hydroxy-3, 7, 4-trimethoxyflavone and luteolin[4]. The present study was undertaken to find out the possible actions of *Cleome gynandra* on immune system in albino rats.

MATERIALS AND METHODS

Aerial parts of *Cleome gynandra* Linn. were collected in the month of August from Pharmacognosy garden of Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M. P.) India. The plants were air dried in shade for 15 days and then the aerial parts of the plants taken for the study.

Preparation of extracts

Powdered aerial parts (500 g) were packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was subjected to ethanolic extraction using two liters of ethanol (95%) in soxhlet apparatus. The ethanolic extract was concentrated under vacuum. The yield of ethanolic extract was 17.6% w/w of crude drug powder. Another 500 g of drug was percolated for 48 hr at room temperature for aqueous extract. The aqueous extract was collected by filtration through muslin cloths. The extract so obtained was concentrated under vacuum and got the yield 8.3 % w/w of crude drug powder.

Animal

Wister albino rats (100-150 g) of either sex were maintained under hygienic husbandry condition in the departmental animal house. The animals fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*. Animals were divided into seven groups and each group has six albino rats.

Administration of drug extract

The albino rats were divided into seven groups. Group I served as a control and received 0.3 ml of 5 % dextrose normal saline (Core Pharmaceuticals Ltd.). Group II received 125 mg/kg body weight of aqueous extract of *Cleome gynandra* (AECG-I) orally, Group III received 250 mg/kg body weight of aqueous extract of *Cleome gynandra* (AECG-II) orally , Group IV received 500 mg/kg body weight of aqueous extract of *Cleome gynandra* (AECG-III) orally, Group V received 50 mg/kg body weight of ethanolic extract of *Cleome gynandra* (EECG-I) orally, Group VI received 100 mg/kg body weight of ethanolic extract of *Cleome gynandra* (EECG-II) orally and Group VII received 200 mg/kg body weight of ethanolic extract of *Cleome gynandra* (EECG-III) orally for seven days.

Determination of toxicity level

Toxicity studies have been performed using a group of six albino rats of either sex were administered graded doses of (100-1000 mg/kg of body weight) of *Cleome gynandra* extract orally. The mortality was observed for 1 to 7 days and data are not shown in this paper.

Determination of Phagocytic index

After schedule treatment of drugs as per earlier discussion,

***Corresponding author:** [*1] Geetanjali College of Pharmaceutical Studies, Udaipur, Rajasthan,
[2] Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M. P.)
e-mail: mohanlalkori@indiatimes.com

on 8th day, all groups were administered with 1 ml per animal of carbon suspension (Pelikan Tuschea Ink, Germany) intravenously through tail vein. Blood samples were collected from retro-orbital plexuses immediately before and 3, 6, 9 and 12 min after the injection of carbon suspension. An aliquot of each and 25 µl of blood sample lysed with 2 ml of 0.1 % acetic acid and absorbance was observed at 675 nm (Shimadzu DB-UV-VIS Spectro photometer 1601). The graph of absorbance against time was plotted for each animal and its respective test groups. The phagocytic index was determined by the slope of time concentration curve[5-8].

Determination of humoral immune response

The animals were immunised with 0.1 ml of 1×10^8 SRBC, intraperitoneally on day 0. Blood samples were collected from individual animals from the retro-orbital plexuses on day 8th (before secondary challenge) for primary antibody titre and on 15th for secondary antibody titre. Antibody levels were determined by the haemagglutination technique[9-11]. Two-fold dilutions sera in saline (0.025 ml) were mixed with 0.025 ml of 0.1% v/v SRBC suspension in microtitre plates. The plates were incubated at 37°C for an hour and then inspected for haemagglutination. The highest dilution giving rise to macroscopic haemagglutination was taken as antibody titre. Antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significance.

Determination of cell mediated immune response

The animals were immunized by injecting 0.1 ml of SRBC suspension containing 1×10^8 cells, intraperitoneally, on day 0 and challenged on day 10 with 0.05 ml of 2×10^8 SRBC in the right hind foot pad. The contra lateral paw received an equal volume of saline. The foot thickness was measured at 0, 24 and 48 hr after challenge using Mitutoyo Dail Caliper (Mitutoyo Manufacturing Company, Japan) [12-13]. The difference in the thickness of the right hind paw and left hind paw was used as a measure of delayed type of hypersensitivity (DTH) reaction.

Determination of albumin and globulin

Albumin and globulin levels were measured on 8th day. Blood samples were collected from retro orbital plexus and concentration of albumin and total protein were determined by colorimetrically at 540 and 630 nm respectively[6]. A difference in the concentration of total protein and albumin was recorded as a globulin concentration.

Total Leucocyte count

Total leucocytes were counted using haemocytometer on 8th day. 0.5 ml of blood was withdrawn from retro orbital plexus immediately using WBC pipette and then diluted up to the mark with WBC dilution fluid (Qualigens Fine Chemical, New Delhi, INDIA) and was shaken on rotary shaker. A drop of sample was put on a naeuber's chamber and number of leucocytes per cubic mm was determined by observing under the microscope[11].

Estimation of immunoglobulin

Estimation of immunoglobulin was carried out after 7 days treatment with extract, 1ml of 1% w/v bovine serum albumin (Sigma, USA) was administered intravenously to all the groups. After 24 hr the levels were estimated by immunoturbidometric method in each sample [14].

Statistical analysis

Results were expressed as mean ± SEM, difference between control and treated groups were tested for significance by

TABLE- 1 Effect of *Cleome gynandra* on cellular immune response.

Extract (mg/kg)	Mean Phagocytic index	Percent Inhibition		Total Leucocyte count	Protein levels (g/dl)	
		24 hr	48 hr		Albumin	Globulin
Control	---	---	3.4±0.07	10481.16±463.5	4.38±0.19	3.16±0.28
AECG-I	0.788±0.04	27.06±4.02	61.07±7.10	7833.33±282.97	3.55±0.55	1.30±0.12
AECG-II	0.753±0.08	31.52±5.01	69.39±8.00	7083.33±306.25	4.13±0.68	1.24±0.07
AECG-III	0.674±0.05	33.02±6.00	75.16±7.6	6533.33±326.59	4.46±0.24	1.10±0.07
EECG-I	0.481±0.04	35.02±6.00	92.74±8.90	6700.00±260.76	4.20±0.46	0.99±0.06
EECG-II	0.422±0.05	44.41±5.99	94.08±9.01	6683.33±231.33	4.40±0.26	0.93±0.03
EECG-III	0.290±0.07	67.15±6.30	97.43±9.10	6483.33±222.86	4.40±0.41	0.88±0.04

n=6, Values are Mean ± SEM, student t test *P<0.05* (significant)

applying Student t-test *(P<0.05)*.

Results and Discussion

Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy[15]. Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation[16]. The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such drugs are well tolerated by the patient, may be developed into alternative coadjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc[17].

The aqueous as well as the ethanolic extract demonstrated reduction in phagocytic activity in animals of treated groups. The phagocytic index of 0.788±0.04, 0.753±0.08 and 0.674±0.05 recorded respectively with AECG-I, AECG-II and AECG-III whereas with EECG-I, EECG-II and EECG-III the phagocytic index were 0.481±0.04, 0.422±0.05 and 0.290±0.07, as an evidence of ethanolic extract suppressed the carbon clearance to a greater extant (Table 1). In view of the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to B-cells, the extracts evaluated for its effect on macrophage phagocytic activity. When the carbon suspension is injected intravenously, the rate of clearance of carbon from blood by macrophage is governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. The increase in clearance response or decrease in phagocytic index reveals that treated animals decrease the macrophages activity.

SRBC agglutination test was performed to know humoral antibody response of the extracts. It was observed that after immunization with SRBC, the ethanolic extract caused significant decrease in the antibody titre. At lower dose level, the aqueous extract did not cause significant decrease in the antibody titre. However, at higher dose (AECG-III), a significant decrease was recorded (Table 2). Ethanolic extract exhibited better result in this experiment also and all the administered doses caused significant reduction in antibody titre. Thus the drug extracts suppressed humoral antibody immune response as evidenced by decreased antibody titre in albino rats challenged with SRBC. Decrease in the humoral response as evidenced by an decrement of antibody responsiveness to SRBC in albino rats as consequence of both pre and post-immunization drug treatment indicates the reduced the responsiveness of macrophages and B-lymphocyte subsets involved less in antibody synthesis[18]. Thus the decrement of the humoral response to SRBC *in vivo* with no effect on B-cell proliferative responses in metabolized to its active form in vivo or acts indirectly to produce its effect.

In the present investigation, SRBC-induced delayed-type hypersensitivity was used to assess the effect of the fraction on cell-mediated immunity. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defense against infectious

TABLE- 2 Effect of *Cleome gynandra* on humoral immune response.

Extract (mg/kg)	Antibody Titre				Immunoglobulin	
	Antibody titre range	7 days Primary	Antibody titre range	14 days Secondary	IgM	IgG
Control	192-384	352±84	192-384	334±78	197.16±5.2	1816.66±23.6
AECG-I	192-384	269±60	192-384	240±41	95.56±3.8	952.33±6.5
AECG-II	192-384	240±39	192-384	231±29	93.33±3.0	907.16±6.9
AECG-III	192-384	201±28	96-192	185±35	90.60±3.7	881.83±9.2
EECG-I	96-192	183±41	96-192	164±42	89.16±4.3	853.16±4.3
EECG-II	96-192	150±31	96-192	125±27	84.50±7.1	819.00±36.2
EECG-III	96-192	124±25	48-96	94±22	70.66±4.1	803.83±25.5

n=6, Values are Mean ± SEM, *P<0.05* (significant)

organisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions[18]. Therefore, increase in DTH reaction in rats response to T cell dependent antigen revealed the inhibitory effect of aqueous and ethanolic extracts of *Cleome gynandra* on T cells. The ethanolic extract of *Cleome gynandra* (EECG-I) showed better activity i.e. cause 92.74 % inhibition (Table 1).

Albumin to globulin ratio was determined for both the extracts. Albumin and globulin are the serum components where the later also take part in immune system. The ratio was determined in all the groups, the concentration of albumin was found normal but globulin concentration was significantly decreased (Table 2) and this reduction was directly proportional to the dose of both extract ($P<0.05$ as calculated in all groups by t-test).

Both aqueous and ethanolic extracts caused decrease in total leukocyte count in treated animals. The leukocyte count was affected in aqueous extract but was statistically insignificant. Again ethanolic extract showed greater activity than aqueous extract. Maximum reduction was seen with the EECG-III. Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization and neutralization of toxins etc[19].

Immunoglobulin was estimated by immunoturbidometric assay. It was observed that the IgG levels were reduced significantly by ethanolic extract at all doses, but the aqueous extract could produce the statistically significant results only at higher dose (Table 2). IgG and IgM levels were influenced by both aqueous extract and ethanolic extract but to the different magnitude at different dose levels. Amongst the extracts tested ethanolic extract showed better activity even with lower doses. The level of IgG in serum of treated and control animals show the experimentation is presented in Table 2. Ethanolic extract treated and aqueous extracts statistically significantly decreased the level of serum IgG in comparisons to the level of IgG before the immunization.

The overall pharmacological investigations conclusively demonstrate immunosuppression activity in the ethanolic extracts of *Cleome gynandra* Linn. The aqueous extract revealed relatively week activity as compared against the ethanolic extract. This may be due to the biphasic solubility profile of the ethanol as an extraction solvent, which can extract both polar and non-polar components of the plant *Cleome gynandra* Linn.

Acknowledgments

One of the author Mohan Lal Kori, gratefully acknowledges University Grant Commission (UGC), New Delhi, India, for providing financial assistance.

REFERENCES

1. Chopra RN, Nair SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: CSIR, 1956, 70.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, II. Dehradun: International Book Distributors; 1976, 1, 181.
3. Gupta RK, Chandra S, Mahadevan V. Chemical examination of the seed of *Gynandropsis petaphylla*. The Indian Journal of Pharmacy, 1968, 30, 5, 127-128.
4. Jain AC, Gupta SM. Minor phenolic components of the seeds of *Gynandropsis gynandra.* Journal of Natural Products, 1985, 48, 2, 332-333.
5. Atal CK, Sharma ML, Kaul A, Khajuria A. Immunom odulating agents of plant origin I: preliminary screening. J. Ethnopharmacol., 1986; 18: 133-141.
6. Pallabi DE, Dasgupta SC, Gomes A. Immunopotentiating and immunoprophylactic activities of immune 21, a polyherbal product. Indian J. Pharmacol., 1998; 30: 163-168.
7. Kulkarni SR, Karande VS. Study of the immunostimulant activity of naphthoquinone extract of leaves of *Lawsonia alba* Linn. Indian Drugs, 1998; 35, 427.
8. Biozzi G, Benacerraf B, Halpern BN. Br. J. Exp Pathol. 1953; 34:426.
9. Ray A, Mediratta PK, Puri S, Sen P. J. Exp. Biol., 1991; 29: 233.
10. Puri A, Saxena R, Saxena RP, Saxena KC. J. Nat. Prod. 1993; 56 : 995-999.
11. Subramonium A, Rajasekharan S, Latha PG, Evans DA, Pushpangadan P. Immuomodulatory and antitumor activites of Janakia arayalpathra root. Fitoterapia, 1996; 67, 140.
12. Doherty, NS. Agents and Actions, 1971; 11: 237–242.
13. Puri A, Saxena R, Saxena RP, Saxena KC. Macrophage migration as an index of immune status. Immunol. Invest, 1991; 20: 431.
14. Ray A, Banerjee BD, Sen P. Modulation of humoral and cell mediated immune response by *Azadirachta indica* in mice. Ind. J. Exp. Biol., 1996; 34: 698.
15. Wagner H, Proksch A. Immunomodulatory drugs of fungi and higher plants in economic and medicinal plant research. Academic Press, London. 1983; 1:113.
16. Bamunnarachi A, De Silva KT. An ethanopharmacognostic approach to the search for immunomodulators of plant origin. Planta. Med. 1989; 55: 339-348.
17. Rossi-Bergmann B, Costa SS, Borges MRS, Da Silva SA, Noleto GR, Souza MLM. Phyther. Res., 1994; 8: 399.

18.Banacerraf B. A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and B lymphocytes. J. Immunol. 1978; 120: 1809-1812.

19. Miller LE. In: Ludke, H.R., Peacock, J.E., Tomar, R.H. (Eds.), Manual of Laboratory Immunology. Lea and Febiger, London, 1991.