Global Journal of Pharmacology, 3 (2): 85-89, 2009
ISSN 1992-0075

Comparative Screening of Immunomodulatory Activity of Hydro-alcoholic Extract of *Hibiscus rosa sinensis* Linn. and Ethanolic Extract of *Cleome gynandra* Linn

[1]Kalpesh Gaur, [1]M.L. Kori and [2]R.K. Nema

[1]Geetanjali College of Pharmaceutical Studies, Manwa Khera, Udaipur, Rajasthan, India
[2]Rishiraj College of Pharmacy, Indore, Madhya Pradesh, India

Abstract: The assesement of immunomodulatory activity of hydro-alcoholic extract of flowers of *Hibiscus rosa sinensis* Linn. (75, 150 and 300 mg/kg, p.o.) and ethanolic extracts of aerial parts of *Cleome gynandra* Linn. (50, 100 and 200 mg/kg, p.o.) were done by carbon clearance method for non-specific immunity, haemagglutination antibody titre method for humoral immunity and footpad swelling method for cell mediated immunity on wistar albino rats. Results of present studies suggest that the hydro-alcoholic extract of *Hibiscus rosa sinensis* Linn. was found to possess significant immunostimulatory action on immune system but ethanolic extract of *Cleome gynandra* Linn. exhibited significant immunosuppression effect in dose dependent manner when compare with control group.

Key words: Carbon clearance method · Cellular mediated immunity · Haemagglutination antibody titre · Immunostimulatory action

INTRODUCTION

The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years and the concept of 'rasayana' in Ayurveda is based on related principles [1]. Indian medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions [2]. Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health concept of strengthening host defences against different diseases [3]. These plants, labelled as 'rasayana', have been endowed with multiple properties like delaying the onset of senescence and improving mental functions by strengthening the psycho-neuro-immune axis [4].

Hibiscus rosa sinensis Linn. (Family: *Malvaceae*) known in Sanskrit as Japa or Rudhrapushpa and the flowers have been reported in the ancient Indian medicinal literature with beneficial effects in heart diseases [5]. The flowers are refrigerant, emollient, demulcent and aphrodisiac; also emmenagogue. Petals are used to stimulate thicker hair growth and to prevent premature graying, hair loss and scalp disorders. It acts as a natural emollient hair conditioner and can be used in hair washes, treatments and vinegar rinses for the hair. Leaves are emollient, anodyne and aperient or laxative. Hibiscus is useful in menorrhagia, strangury, cystitis and other conditions of the genito-urinary tract [6]. These chemical constituents were reported in plant i.e. cyanidin, quercetin, flavonoids, hentriacontane, thiamine, riboflavin, niacin and ascorbic acid [7].

Cleome gynandra Linn. (Family: *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 [8]. 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 [9]. Hexacosanol, β-D-glucoside of β sitosterol, free β sitosterol and kaempferol have been isolated from the seed of *Cleome gynandra* [10]. It also contains the minor components 5-7-dihydroxychromone, 5-hydroxy-3, 7, 4-trimethoxyflavone and luteolin [11].

Corresponding Author: Kalpesh Gaur, Geetanjali College of Pharmaceutical Studies, Manwa Khera, Udaipur, Rajasthan, India
E-mail: kalpeshgaur@gmail.com

MATERIALS AND METHODS

Plant Materials: The flowers of *Hibiscus rosa sinensis* Linn. and aerial parts of *Cleome gynandra* Linn were collected from Udaipur, Rajasthan in the month of July 2007. The plant was identified with the help of available literature and authenticated by Prof. V.K. Dixit, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M. P.) 470003. The plants were dried in shade for 15 days prior to study.

Preparation of Crude Extracts

Preparation of Hydro-alcoholic Extract of Hibiscus Rosa Sinensis: Powdered flowers (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 extracted with two liters of alcoholic: water (7:3) mixture in soxhlet apparatus. The extract was concentrated under vacuum to get solid crude mass. This dried crude extract of ethanolic: water (7:3) were stored in a desiccators and used for further experiment after suspending in sodium carboxymethyl cellulose (CMC) 2 % w/w. The yield of ethanolic: water (7:3) mixture or hydro-alcoholic extract was 15.76% w/w of crude drug power.

Preparation of Ethanolic Extract of Cleome Gynandra: 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 7.6% w/w of crude drug power.

Toxicity Studies: The doses of *Hibiscus rosa sinensis* Linn. (75, 150 and 300 mg/kg, p.o.) and *Cleome gynandra* Linn. (50, 100 and 200 mg/kg, p.o.) and were selected range from 1/6 to 1/15 of LD_{50} based on the preliminary study conducted at our laboratory and data are not shown in this paper.

Standard Drug: Septilin (Dabur India Ltd., Baddi, H.P.) was used as a standard drug at a dose of 500 mg/kg, p.o. Septilin, a proprietary herbal preparaton has been reported to produce wound healing and immunomodulatory activities which contains *Balsamodendron mukul Tinospora cordifolia, Emblica offiinalis, Rubia cordifolia, Moringa pterygosperma, Glycyrrhiza glabra*, Shankh bhasma and maharasnadi quath [12].

Animal: Wister albino rats (120-150 g) of either sex were used. The animals housed under standard laboratory conditions maintained at 25 ± 1°C and under 12 / 12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee.

Albino rats were divided into eight groups viz. Group A; received 0.5 ml of 2 % w/v sodium carboxy methyl cellulose suspension p.o. for 14 days as a control group, Group B; received 500 mg/kg, p.o. of Septilin 14 days, Group C; received 75 mg/kg, p.o. of hydro-alcoholic extract of *Hibiscus rosa sinensis* (HAEHRS-1) for 14 days, Group D; received 150 mg/kg, p.o. of hydro-alcoholic extract of *Hibiscus rosa sinensis* (HAEHRS-2) for 14 days, Group E; received 300 mg/kg, p.o. of hydro-alcoholic extract of *Hibiscus rosa sinensis* (HAEHRS-3) for 14 days, Group F; received 50 mg/kg, p.o. of ethanolic extract of *Cleome gynandra* (EECG-1) daily for 14 days, Group G; received 100 mg/kg, p.o. of ethanolic extract of *Cleome gynandra* (EECG-2) daily for 14 days and Group H; received 200 mg/kg, p.o. of ethanolic extract of *Cleome gynandra* (EECG-3) daily for 14 days.

Determination of Phagocytic Index: All groups were administered with 0.2 ml/animal of carbon suspension (Pelikan Tuschea Ink, Germany) intravenously through tail vein on seventh day. Blood samples were collected from retro-orbital plexuses immediately before and 5, 10, 15 and 20 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. The graph were plotted between absorbance against time for each animal and its respective test groups. The phagocytic index was calculated by the slope of time concentration curve [13].

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 7. Antibody levels were determined by the haemagglutination technique [14-15]. Two-fold dilutions sera in saline (0.025 ml) were mixed with 0.025 ml of 0.1% v/v SRBC suspension in 96 well microtitre plates. The plates were incubated at 37±1°C for 1 h 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 7 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 24 and 48 h after challenge using Mitutoyo Dail Caliper (Mitutoyo Manufacturing Company, Japan). The difference in the thickness of the right hind paw and left hind paw was used as a measure of DTH reaction [16]. Statistical analysis- Values expressed are mean ± SEM (Standard error of mean); using Student's t-test. $P < 0.05$ were considered as significant.

RESULTS

The results of phagocytic index determination method; clearly indicates that the rate of elimination of carbon particles by HAEHRS-1, HAEHRS-2, HAEHRS-3, EECG-1, EECG-2, EECG-3 and septilin were found to be 0.811, 0.723, 0.701,0.481, 0.422, 0.290 and 0.679 respetively (Table 1).

In haemagglutination antibody titre method; the values of primary and secondary antibody titre by HAEHRS-1, HAEHRS-2, HAEHRS-3, EECG-1, EECG-2, EECG-3 and septilin were summrised in Table 1 and Fig. 1.

In cell mediated immune response determination method; the footpad thickness after 24 h and 48 h by HAEHRS-1, HAEHRS-2, HAEHRS-3, EECG-1, EECG-2, EECG-3 and septilin were summrised in Table 2 and Fig. 2.

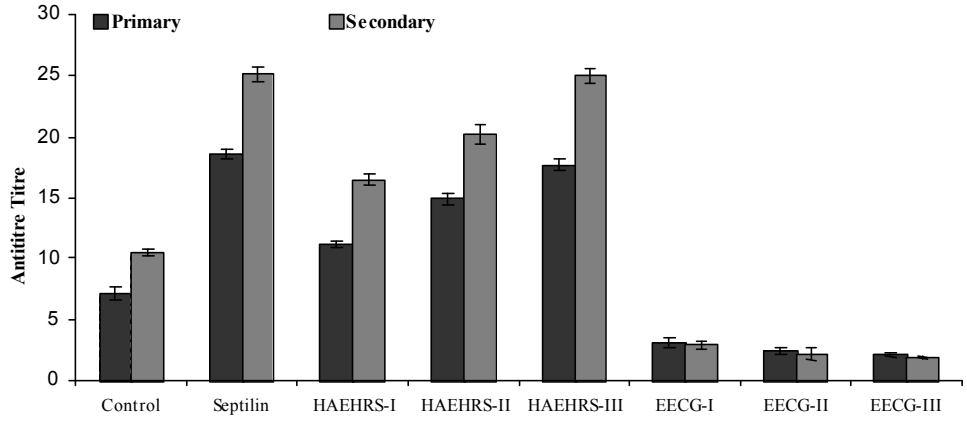

Fig. 1: Effects of hydro-alcoholic extract of *Hibiscus rosa sinensis* and ethanolic extract of *Cleome gynandra* on haemagglutination antibody titre

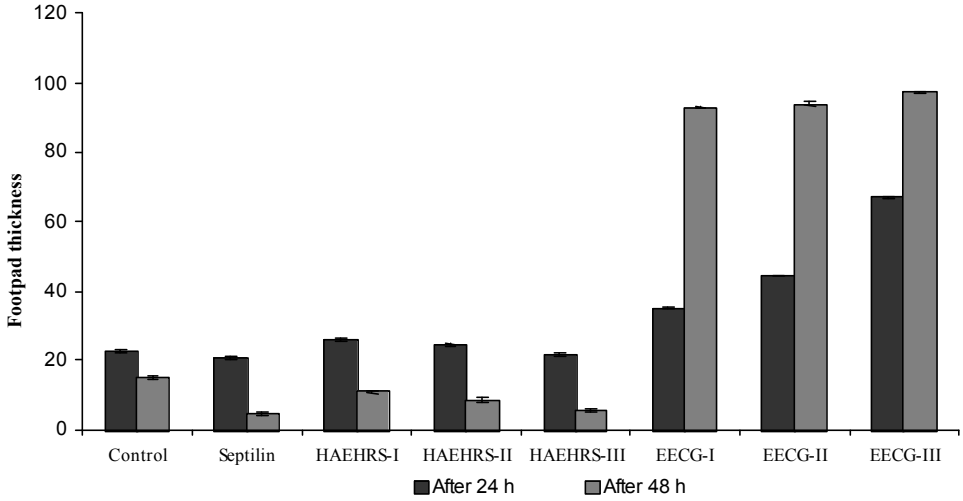

Fig. 2: Effects of hydro-alcoholic extract of *Hibiscus rosa sinensis* and ethanolic extract of *Cleome gynandra* on Delayed Type Hypersensitivity

Table 1: Effects of hydro-alcoholic extract of *Hibiscus rosa sinensis* and ethanolic extract of *Cleome gynandra* on phagocytic index, haemagglutination antibody titre and delayed type hypersensitivity

		Haemagglutination Antibody Titre		Delayed Type Hypersensitivity (footpad thickness)	
Treatment	Phagocytic Index	Primary	Secondary	24 h	48 h
Control	-	7.14±0.50	10.5±0.32	22.51±0.05	15.2±0.03
Septilin	0.679	18.47±0.40^{c}	25.13±0.60^{c}	20.56±0.80^{c}	4.5±0.70^{c}
HAEHRS-1	0.811	11.22±0.30^{b}	16.45±0.45^{a}	26.13±0.01^{b}	11.2±0.03^{c}
HAEHRS-2	0.723	14.87±0.54^{c}	20.22±0.74^{c}	24.21±0.08^{c}	8.3±0.07^{c}
HAEHRS-3	0.701	17.67±0.45^{c}	24.98±0.55^{c}	21.33±0.07^{c}	5.6±0.04^{c}
EECG-1	0.481	3.14±0.41^{a}	2.97±0.33^{a}	35.02±0.03^{a}	92.74±0.12^{a}
EECG-2	0.422	2.49±0.21^{b}	2.21±0.52^{a}	44.41±0.06^{a}	94.08±0.05^{a}
EECG-3	0.290	2.17±0.22^{a}	1.89±0.12^{b}	67.15±0.02^{a}	97.43±0.02^{a}

Values are expressed as the mean ± SEM; (n = 6). Statistics significant vs Control, $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$

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 [17]. 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 [18]. 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 products, if 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 [19].

In the present study; phagocytic index were determined by carbon clearance method. 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. This study demonstrates that *Hibiscus rosa sinensis* treatment is potentiated more the phagocytosis of reticulo endothelial system and *Cleome gynandra* increase in clearance response or decrease in phagocytic index reveals decrease in macrophages activity.

The haemagglutination antibody titre was used to assess humoral immune response. At the selected dose; both primary and secondary antibody titre were observed in rats treated with extracts of *Hibiscus rosa sinensis* and *Cleome gynandra.* The augmentation of the humoral immune response to SRBCs by extract of *Hibiscus rosa sinensis* evidenced by increase in the antibody titres in the blood of rats. In case of *Cleome gynandra* extract suppressed humoral antibody immune response as evidanced by decreased antibody titre in albino rats challanged with SRBC. 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, neutralization of toxins, etc [20].

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 defence against infectious organisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions [20]. Therefore, increase in DTH reaction in rats response to T cell dependent antigen revealed the stimulatory effect of hydro-alcoholic extracts of *Hibiscus rosa sinensis* on T cells. In case of *Cleome gynandra* showed the less protective activity against impaired DTH conditions. Thus, the immunostimulatory effect produced by hydro-alcoholic extracts of *Hibiscus rosa sinensis* may be due to cell mediated and humoral antibody mediated activation of T and B cells but *Cleome gynandra* exhibited immunosuppressive action. It can therefore be concluded that the hydro alcohalic extract of *Hibiscus rosa sinensis* is a potent immunostimulant and can be used as a complimentary therapeutic agent.

ACKNOWLEDGMENT

The authors are grateful to Mr. J.P. Agarwal, Chairman and Managing Trustee , Geetanjali University Trust, Udaipur (Rajasthan) for providing laboratory facilities throughout the study.

REFERENCES

1. Sharma, P., 1983. Charaka Samhita, Chikitasasthana. Chaukhamba Orientalia, Varanasi, India, pp: 54.
2. Sainis, K.B., P.F. Sumariwalla, A. Goel, G.J. Chintalwar, A.T. Sipahimalani and A. Banerji, 1997. Immunomodulatory Properties of Stem Extracts of *Tinospora cordifolia*: Cell Targets and Active Principles. (Eds.) Narosa Publishing House, New Delhi, India, pp: 95.
3. Thatte, U.M. and S.A. Dhanukar, 1986. Ayurveda and contemporary scientific thought. Trends in Pharmacological Sci., 7: 247.
4. Katiyar, C.K., Brindavanam, N.B., P. Tiwari and D.B.A. Narayana, 1997. Immunomodulation. (Eds.). Narosa Publishing House, New Delhi. pp: 163-187.
5. Nadkarni, A.K., 1976. Indian Materia Medica. Vol-1 Popular Prakashan (Pvt) Ltd, Bombay, India, pp: 1199.
6. Kirtikar, K.R. and B.D. Basu, 2005. Indian Medicinal Plants. 2nd ed. International Book Distributors, Dehradun, pp: 335.
7. Nair, R., T. Kalariya and S. Chanda, 2005. Antibacterial activity of some selected indian medicinal flora. Turk J. Biol., 29: 41-47.
8. Chopra, R.N., S.L. Nair and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi, pp: 70.
9. Kirtikar, K.R. and B.D. Basu, 1976. Indian Medicinal Plants. II International Book Distributors, Dehradun, pp: 181.
10. Gupta, R.K., S. Chandra and V. Mahadevan, 1968. Chemical examination of the seed of *Gynandropsis petaphylla*. The Indian J. Pharmacy, 30(5): 127-128.
11. Jain, A.C. and S.M. Gupta, 1985. Minor phenolic components of the seeds of *Gynandropsis gynandra.* J. Natural Products, 48(2): 332-333.
12. Rao, C.S., C. Raju, S. Gopumadhavan, B.L. Chauhan, R.D. Kulkarni and S.K. Mitra, 1994. Immunotherapeutic modification by an ayurvedic formulation Septilin., 32(8): 553-558.
13. Biozzi, G., B. Benacerraf and B.N. Halpern, 1953. Quantitative study of the granulopectic activity of the reticulo-endothelial system 1: the effect of ingredient present in India ink and of substances affecting blood clotting in vivo on fate of carbon particles administration intravenously in rats, mice and rabbits. British J. Experimental Pathology, 34: 426-457.
14. Ray, A., P.K. Mediratta, S. Puri and P. Sen, 1991. Effects of stress on immune responsiveness, gastric ulcerogenesis and plasma corticosterone in rats: modulation by diazepam and naltrexone. J. Experimental Biology, 29: 233.
15. Puri, A., R. Saxena, R.P. Saxena and K.C. Saxena, 1993. Immunostimulant agents from *Andrographis peniculata*. J. Natural Product, 56: 995-999.
16. Doherty, N.S., 1971. Selective effect of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cells in mice. Agents and Actions, 11: 237-242.
17. Wagner, H. and A. Proksch, 1983. Immunomodulatory drugs of fungi and higher plants in economic and medicinal plant research. Academic Press, London., 1: 113.
18. Bamunnarachi, A. and K.T. De Silva, 1989. An ethanopharmacognostic approach to the search for immunomodulators of plant origin. Planta Medica, 55: 339-348.
19. Rossi-Bergmann, B., S.S. Costa, M.R.S. Borges, S.A. Da Silva, G.R. Noleto and M.L.M. Souza, *et al.,* 1994. Studies on Kalanchoe pinnata. Phytotherapy Res., 8: 399.
20. Miller, L.E., In: H.R. Ludke, J.E. Peacock and R.H. Tomar, 1991. Manual of Laboratory Immunology. (Eds.), Lea and Febiger, London, pp: 1-18.