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RESEARCH ARTICLE

ANTI-IMMUNOMODULATORY ACTIVITY OF *BORRERIA ARTICULARIS* ON CYCLOPHOSPHAMIDE INDUCED ALBINO WISTAR RATS

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ABSTRACT

In the present investigation immunomodulatory activity was studied for the plant *Borreria articularis* leaf extract using rat model. Borreria articularis is an Indian medicinal system and has been used in ayurvedic preparation for the treatment of various ailments throughout the countries. Immunomodulation is a process, which alters the immune system of an organism by inferring with its function. Cyclophosphamide was used as a standard immunosuppressant agent. Sodium Carboxy Methyl Cellulose (SCMC) is being a non-immunogenic and tolerogenic substance, it has been considered as control group. In order to know their effectiveness on humoral antibody production against SRBC, haemagglutination and delayed type hypersensitivity (DTH) were studied. The results of immunomodulatory activity for 7 day pretreatment and 15 days post treatment are tabulated respectively. The antibody titer has been measured using haemagglutination (HA) test and DTH, it has been increasing in proportionate to the concentration of extract dose up to 1200 mg/kg.

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INTRODUCTION

The modulation of immune responses to alleviate diseases has been of immense interest for many years. The concept of immunomodulation relates to a non-specific activation of the immune system. It primarily implies a non-antigen dependent stimulation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells, lymphocytes and also the production of various effector molecules by activated cells (Para immunity). Being non-specific, it is expected to provide protection against different pathogens including bacteria, fungi, viruses etc., (Sai Ram *et al.,* 1997). There is a growing interest in identifying and characterizing natural compounds with immunomodulatory activity ever since their possible use in modern medicine has been suggested (Lee *et al*., 1995). A large number of plants and their isolated constituents have been shown to potentiate immunity (Savnur, 1950). Medicinal plants have been shown to exert anti-inflammatory, anti-gout, anti-stress and anti-cancer effects by modulating the immune functions (Singh 1986). The protective effect of *Borreria articularis* against cyclophosphamide (CP) - induced suppression of humoral immunity in mice was reported (Bin Hafeez *et al*., 2001). It was also found that *Borreria articularis* had antimutagenic effects against benzo-a-pyrene and CP-induced mutagenicity.

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In the present study an attempt has been made to evaluate the Immunomodulatory activity of *Borreria articularis* plant extract.

MATERIALS AND METHODS

Male Albino Wistar Rats were divided into seven groups (I - VII) each comprising of male rats weighing in the range of 150-200g were selected.

Group I: Control rat (1% Sodium carboxy methyl cellulose).
Group II-VI: Test extracts (I/II/III) (2.8% Plant leaves extract + 1% Sodium carboxy methyl cellulose) (5 dose level 75-1200 mg/kg).
Group VII : 75 mg/kg of Cyclophosphamide drug induced rat.

Immunomodulation study: To study the immunomodulatory activity, 2.8% plant extract was suspended in 1% sodium carboxy methyl cellulose (SCMC) to prepare suitable dosage forms. The control animals were given an equivalent volume of the sodium carboxy methylcellulose vehicle without plant extract. Cyclophosphamide was used as a standard immunosuppressant agent.

Antigen: Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep Red Blood Cells (SRBCs) were washed three times in normal saline and adjusted to a concentration of 0.1 mL containing 1×10^8 cells for immunization and challenge.

Table 1. Effect of Borreria articularis and *cyclophosphamide* on HA titre and DTH response using SRBCs as an antigen in rat 7 days pretreatment

Borreria articularis Groups/dose mg/kg	Haemagglutination titre	DTH response mean paw edema in mm
I-Control	16.4±1.57	0.30±0.03
II-75	32.5±2.28	3.15±0.46
III-150	64.6±3.12	4.24±0.35
IV-300	128.5±3.35	5.12±0.49
V-600	512.4±3.52	5.65±0.65
VI-1200	1024.5±4.25	4.54±0.54

Values are mean ±SD of Six individual observations. Values are significant at P< 0.05

Table 2. Effect of Borreria articularis and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in rat 15 day pretreatment

Borreria articularis Groups/dose mg/kg	Heamagglutination titre	DTH response mean paw edema in mm
I-Control	8.5 ±0.68	0.28±0.03
II-75	16.3 ±1.45	2.85±0.36
III-150	32.4±1.76	3.15±0.45
IV-300	64.5±2.58	4.86±0.48
V-600	256.6 ±2.84	5.12±0.35
VI-1200	512.4±3.58	4.76±0.94
VII- Induced control – 50	4.00±0.68	0.56±0.09

Values are mean ±SD of Six individual observations. Values are significant at P< 0.05

Humoral Antibody (HA) response

Humoral Antibody (HA) response was identified using the method described by Puri *et al.* (1994) was adopted. Rats were divided into seven groups, each group containing six rats. Drugs were administered in various groups, i.e. Group I – Control (Sodium carboxy methyl cellulose (SCMC) 1%), Group II – VI test extracts I (7 dose levels 75 – 1200 mg/kg p.o.) and Group VII- standard drug (Cyclophosphamide 75mg/kg, p.o.). The animals were immunized by injecting 0.1 ml of SRBCs suspension, containing $1X10^8$ cells, intraperitoneally, on day 0. Blood samples were collected in micro centrifuge tubes from individual animals of all the groups by retro orbital vein puncture on day 7^{th} and 14^{th}. The blood samples were centrifuged and the serum separated. Antibody levels were determined by the hemagglutination technique.

Delayed Type Hypersensitivity (DTH)

Delayed type hypersensitivity was assessed using rat**.** On day 7, the thickness of the right hind foot pad was measured using vernier caliper. The rats were then challenged by injection of 1×10^8 SRBCs in right hind foot pad. Foot thickness was measured again 24 h after this challenge. The difference between the pre and post challenge footpad thickness, expressed in mm was taken as a measure of the DTH response. The plant extract and Cyclophosphamide was administered orally on day 0 and continued till day 7^{th} and 14^{th} days of challenge. The procedure of immunization by injecting SRBCs suspension, collection of blood sample for haemagglutination and measurement of inflammation above was followed as described.

Statistical Analysis

All the data were analyzed as per the method of Pillai and Sinha (1968).

RESULTS AND DISCUSSION

Immunomodulation is a process, which alters the immune system of an organism by interfering with its functions. This interference results in either immunostimulation or immunosuppression. An immunomodulator is substance that helps to regulate the immune system. This "regulation" is a normalization process, so that an immunomodulator helps to optimise immune response. Immunomodulators are becoming very popular in the worldwide natural health were as these do not tend to boost immunity, but to normalize it. Keeping this in view, efforts have to be directed to modulate the immune responses, to permit effective treatment of various ailments associated with immune system and thus the development of a safe and effective immunomodulator for clinical use. Immunomodulators are biological response modifiers; exert their effects by improving host defense mechanisms against diseases. Immune regulation is a complex balance between regulatory and effector cells and any imbalance in immunological mechanism can lead to pathogenesis (Sehar *et al.,* 2008). Herbal medicine has become an integral part of standard healthcare, based on a combination of time honored traditional usage and ongoing scientific research. Increased interest in medicinal herbs has prompted for scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections (Atal *et al.,* 1986). There is growing interest in identifying and characterizing natural compounds with immunomodulatory activity ever since their possible use in modern medicine has been suggested (Sai Ram *et al*, 1997). The modulation of immune responses to alleviate diseases has been of interest for many years. The concept of immunomodulation relates to a non-specific activation of the immune system. It primarily implies a non-antigen dependent stimulation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells, lymphocytes and also the production of various effector molecules by activated cells (para immunity).

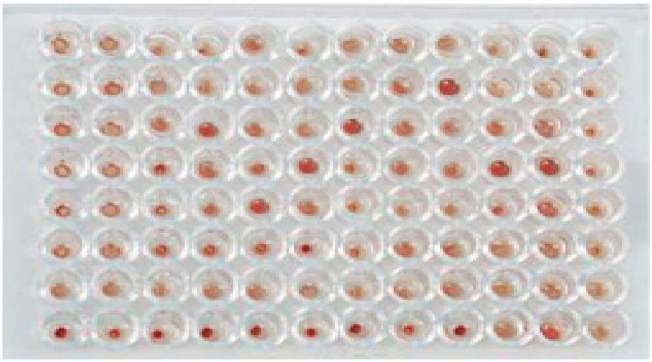


Plate 1. Effect of Haemagglutintion test for 7th days serum collected from animal induced with SRBC and treated with *Borreria articularis*

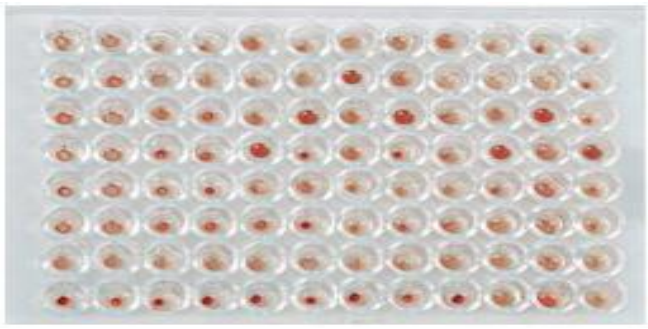
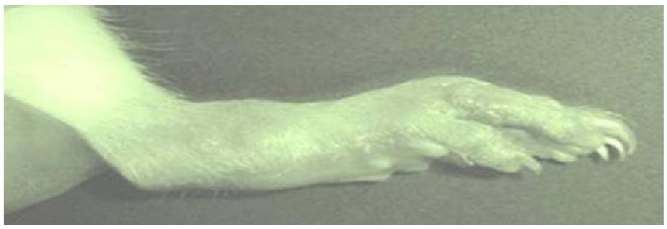


Plate 2. Effect of Haemagglutintion test for 15th days serum collected from animal induced with SRBC and treated with *Borreria articularis*

(A)



(B)

Plate 3. Immunomodulation study- Delayed type hypersensitivity (DTH) response in paw of rats (A- Normal; B-Strong response)

Being non-specific, it is expected to provide protection against different pathogens including bacteria, fungi, viruses etc (Lee Gi *et al*., 1995). In the present investigation immunomodulatory activity was studied for all the plant extract using rat model. In order to know their effectiveness on humoral antibody production against SRBC was studied. In order to know the effectiveness on cell mediated immunity, delayed type hypersensitivity (DTH) were also analyzed. Sodium Carboxy Methyl Cellulose (SCMC) is being a non-immunogenic and tolerogenic substance, it has been considered as control group. Cyclophosphamide given for the VII group showed a very weak response because of its immunosuppressive nature. The results of immunomodulatory activity done with 7 day pretreatment are presented in table 1 and Plate 1 the same experiment but down with 15 days pretreatment represented in Table 2 and Plate 2 respectively. The result for *Borreria articularis* as immunomodulatory agent has been analyzed using SRBC as antigen (pretreatment for 7 days) and presented in table 1. The antibody titer has been measured using haemagglutination (HA) test and it has been in increasing (8.5, 16.3, 32.4, 64.5, 256.6 and 512.4) proportionate to the concentration of extract dose up to 1200 mg/kg. The DTH results for the same extract have also been identified. This has also been in increasing (0.28, 2.85, 3.15, 4.86, 5.12 and 4.76 mm) proportionate to the concentration of extract dose up to 1200 mg/kg.

The results of the batches of 15 days pretreatment in plotted in Table 2. The antibody titer has been measured using haemagglutination (HA) test and it has been in increasing (16.4, 32.5, 64.6, 128.5, 512.4 and 1024.5) proportionate to the concentration of extract dose up to 1200 mg/kg. The DTH results for the same extract have also been identified. This has also been in increasing (0.30, 3.15, 4.24, 5.12, 5.65 and 4.54 mm) proportionate to the concentration of extract dose up to 1200 mg/kg. Results of the batches of 7 days pretreatment have almost been comparable to 15 days pretreatment. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduced resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Both, the immunostimulation and immuno-suppression need to be tackled in order to regulate the normal immunological functioning. Hence both immunostimulating and immunosuppressing agents have their own standing and a search for better agents exerting these activities is becoming the field of major interest all over the world (Patwardhan *et al*., 1990).

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitations to the general use of these agents such as an increased risk of infection and a generalized effect throughout the immune system (Diasio and LoBuglio, 1996). Traditional Indian systems of medicine like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'rasayanas' have been claimed to possess immunomodulatory activity (Atal *et al.,* 1986; Puri *et al*., 1994; Ziauddin *et al*., 1996; Balchandran and Panchanathan, 1998). The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by

phagocytic cells. To evaluate the effect of *C. zeylanica* on humoral response, its influence was tested on sheep erythrocyte specific haemagglutination antibody titre in mice (Kuby, 1997; Encyclopedia of immunology, 1998). EFCZ and NFCZ treatment improved the heamagglutination antibody titer reflecting an overall elevation of humoral immune response.

The DTH response, which directly correlates with cell-mediated immunity (CMI), was found to be the highest at the maximum dose tested in the methanolic extract (200 mg/kg). The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T- lymphocytes. When challenged by the antigen, they are converted to lymphoblasts and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction (Mitra *et al.,* 1999). The infiltrating cells are probably immobilised to promote defensive (inflammatory) reaction (Dash *et al.,* 2006). Treatment of EFCZ and NFCZ enhanced DTH reaction, which is reflected from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site. This study may be supporting a possible role of EFCZ and NFCZ in assisting cell-mediated immune response. The increase in thymus weight was accompanied by increase in its cell counts. This may be partly due to stimulatory effect of plant extract on the lymphocytes and bone marrow haematopoietic cells, which ultimately home in the thymus. However, this homing may be temporary and in due course of time normalcy may ensue (Bin-Hafiz *et al.,* 2003; Asha *et al.,* 2005).

Conclusion

It can therefore be concluded that the extract of *Borreria articularis* is a potent immunostimulant and can be used as a complimentary therapeutic agent.

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