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RESEARCH ARTICLE

IMMUNOMODULATORY ACTIVITY OF *TINOSPORA CORDIFOLIA* ON CYCLOPHOSPHAMIDE INDUCED ALBINO WISTAR RATS

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ABSTRACT

In the present investigation immunomodulatory activity was studied for the plant Tinospora cordifolia leaf extract using rat model. *Tinospora cordifolia* is an Indian medicinal plant 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 *Tinospora cordifolia,* against cyclophosphamide (CP) - induced suppression of humoral immunity in mice was reported (Bin Hafeez *et al.,* 1995; Haque *et al.,* 2001). It was also found that *Tinospora cordifolia* had antimutagenic effects against benzo-a-pyrene and CP-induced mutagenicity.

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MATERIALS AND METHODS

Male Albino Wistar Rats were divided into seven groups (I - VII) each comprising of 7 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.

Humoral Antibody (HA) response

Humoral Antibody (HA) response was identified using the method described by Puri *et al.* (1994)was adopted. rat were divided into seven groups, each group containing six rat. Drugs were administered in various groups, i.e. Group I – Control (Sodium carboxy methyl cellulose (SCMC) 1%), Group II – VI test extract 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 1X 10^8 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 essential oil and Cyclophosphamide was administered orally on day 0 and continued till day 7th and 14th 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

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 effect or molecules by activated cells (para immunity). 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, the same experiment but down with 15 days post treatment represented in Table 2 respectively.

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

Tinospora cordifolia Groups/dose mg/kg	Heamagglutination titre	DTH response mean paw edema in mm
I-Control	8.2 ±0.65	0.29±0.03
II-75	16.4 ±1.40	2.93±0.34
III-150	64.3±1.86	5.17±0.47
IV-300	128.5±2.59	5.90±0.46
V-600	256.6 ±2.86	6.10±0.36
VI-1200	512.4±3.51	6.8±0.92
VII- Induced control – 50	4.00±0.65	0.55±0.09

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

Table 2. Effect of Tinospora cordifolia and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in rat 15 day post treatment

Tinospora cordifolia Groups/dose mg/kg	Haemagglutination titre	DTH response mean paw edema in mm
I-Control	16.2±1.77	0.30±0.03
II-75	32.3±2.23	3.00±0.40
III-150	64.5±3.05	4.10±0.38
IV-300	128.6±3.39	4.76±0.47
V-600	512.4±3.62	4.17±0.69
VI-1200	1024.5±4.20	3.65±0.52

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

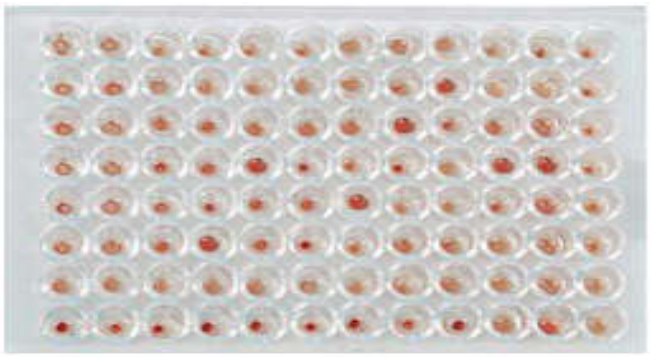

Plate 1. Immunomodulatory activity of cyclophosamide induced Tinospora cordifolia in 7 days pretreatment

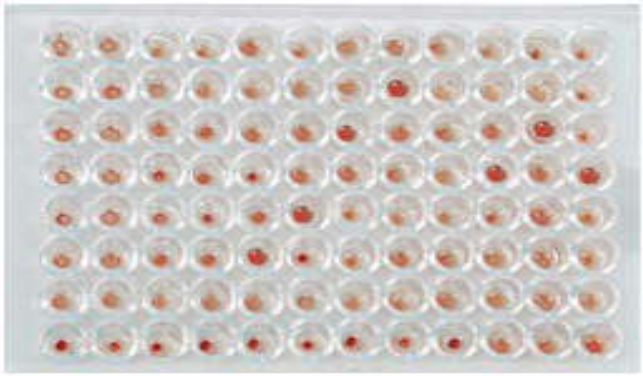

Plate 2. Immunomodulatory activity of cyclophosamide induced *Tinospora cordifolia* in 15 days post treatment

The result for *Tinospora cordifolia* 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 (8.2, 16.4, 64.3, 128.5, 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 (0.29, 2.93, 5.17, 5.90, 6.10 and 6.8 mm) proportionate to the concentration of extract dose up to 1200 mg/kg. The results of the batches of 15 days post treatment in plotted in Table 2. The antibody titer has been measured using haemagglutination (HA) test and it has been in increasing (16.2, 32.3, 64.5, 128.6, 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, 3.0, 4.10, 4.76, 4.17 and 3.65 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 post treatment.

Conclusion

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

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