Research Article

Immunomodulatory Activity of Alcoholic Extract of *Tinospora cordifolia*

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ABSTRACT
The objective of the present study was to investigate the immunomodulatory activity of *Tinospora cordifolia* on cellular and humoral immunity. Oral administration of the ethanolic extract of stem of *Tinospora cordifolia* at the doses of 200mg/kg in mice, dose-dependently potentiated the delayed-type hypersensitivity reaction induced by sheep red blood cells. It significantly enhanced the production of circulating antibody titre in mice in response to sheep red blood cells.

Keywords: Cyclophosphamide, Ethanolic extract, Immunomodulatory activity.

1. INTRODUCTION

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 reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world. Traditional Indian system of medicines 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[1,2].

Tinospora cordifolia, belonging to the genus Tinospora is commonly known as Gudduchi or Giloe. Categorised as 'Rasayanas' it is used for its general adaptogenic and pro-host immunomodulatory activity in fighting infections. It is widely used in the Ayurvedic system of medicine for its general tonic, anti-inflammatory, antiarthritic, antiallergic and aphrodisiac properties. The chemical constituents can be broadly categorized as alkaloids, glycosides, sterols, lactones, fatty acids with the alkaloids being the active constituents of the plants[3].

2. MATERIALS AND METHODS

2.1. Animals

Swiss albino mice weighing between 20-25 g of either sex were used to evaluate the immunomodulatory activity of alcoholic extract of stem of *Tinospora cordifolia*. Animals were housed under standard conditions of temperature (25 °C), 12 h/12 h light/dark cycles and fed with standard pellet diet (Godrej food) and tap water. All experiments were conducted in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines.

2.1 Plant material

The dried stem of *Tinospora cordifolia* was collected from Ralamandal area (Dense forest) Indore (M.P) and identified and authenticated at Government Agriculture College, Indore. A voucher specimen has been kept in the herbarium file of our department for future references.

2.2 Extraction

Tinospora cordifolia stem extracted with 90% ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure at a temperature below 50^0c to yield a syrupy mass

(Yield -7.56%), which was used for the present investigation.

2.3 Preliminary phytochemical investigation

Preliminary phytochemical analysis shows the presence of alkaloids, glycosides, phenolic compounds, sterols, lactones, fatty acids and flavonoids[4].

2.4 Acute toxicity

Acute toxicity study was carried out according to Miller and Tainter methods in albino mice of either sex (wt.20-25gm.) were used[5]. An approximate LD_{50} can be initially determined as a pilot study by a so called 'staircase method' using a small number of animals (2 each dose) and increasing the doses of the drug. Five doses can be chosen for determination of LD_{50} starting from no death to 100% mortality. In our study for estimation of LD_{50} of *Tinospora cordifolia*, 5 doses were given orally to 5 groups of rats, 10 in each group. The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours, the number of deceased rats was counted in each group and percentage of mortality calculated.

The LD_{50} dose of ethanolic extract of bark of *Tinospora cordifolia* in mice was found 2000 mg/kg.1/10 of LD_{50} dose 200mg/kg used as therapeutic dose.

2.5 Drugs

Ethanolic extract of stem of *Tinospora cordifolia* was suspended in 1% sodium carboxy methyl cellulose to prepare suitable dosage forms (200mg/kg p.o.).

The control animals were given an equivalent volume of the sodium carboxy methylcellulose vehicle. Cyclophosphamide (Khandelwal Laboratories, Mumbai) was used as a standard immunosuppressant agent and vitamin-E (Evion-merck) was used as standard drug (150 mg/kg).

Antigen: Fresh blood was collected from sheep's 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 1×108 cells for immunization and challenge.

2.6 Humoral antibody response to SRBC[6]

Mice of either sex were divided into four groups of six each. *Tinospora cordifolia* stem ethanolic extract (200 mg/kg, p.o.) was administered on day 0 and continued till the day of the experiment. Cyclophosphamide (50 mg/kg, p.o.) was administered 2 days before the experiment. On day 7, the mice were immunised with 0.1 ml of $1x10^8$ SRBC, i.p. Blood samples were collected from the orbital plexuses of individual animals on day 14 and the antibody titres were determined. Briefly, an aliquot (25 ml) of two fold diluted sera in saline was challenged with 25 ml of 0.1% v/v SRBC suspension in microtitre plates. The plates were incubated at 37^0C for 1 h and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The mean ranks of different groups were statistically compared (Table-I).

2.7 Cellular immune response[7]

To study the cellular immune response the edema was induced in the right paw of mice by injecting SRBC($0.025x10^9$ cells) in the subplanner region on 20^{th} day, the increase in paw volume in 48 h i. e. on 22^{nd} day was assessed by plethysmometer. The mean percentage increase in foot pad volume was considered as delayed type hypersensitivity and as an index of cell mediated immunity. The volume of the left hind paw, injected similarly with phosphate-buffered saline served as a control (Table-II).

2.8 Statistical analysis

All the data are expressed as mean±SEM and analyzed by ANOVA followed by Dunnett's *t* - test (n=6).

3. RESULTS AND DISCUSSIONS

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 *Tinospora cordifolia* on humoral response, its influence was tested on sheep erythrocytespecific haemagglutination antibody titre in mice. Cyclophosphamide at a dose of 50 mg/kg, p.o., showed significant inhibition in antibody titre response, while ethanolic extract of *Tinospora cordifolia* was found to significantly enhance the production of circulating antibody titre. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis.

Cyclophosphamide induced suppression of humoral as well as cell mediated response were significantly attenuated by daily oral treatment with alcoholic extract of *Tinospora cordifolia* Vitamin E treated group exhibited similar attenuation of the suspension in

immune responces. *Tinospora cordifolia* ethanolic extract at the dose of 200mg/kg was found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice.

4. CONCLUSION

Results of the present investigation showed increased antibody titre in response to an overall elevation of humoral immune response. Delayed-type hypersensitivity is antigen specific and causes erythema and induction at the site of antigen infection in immunized animals. The general characteristics of delayed-type hypersensitivity are an influx of immune cells at the site of injection, macrophages and basophils in mice. Macrophages are acting in both nonspecific defenses by phagocytes cellular debris and pathogen and specific defense by stimulates lymphocytes and other immune cells to respond to the pathogen. The ethanolic extract of *Tinospora cordifolia* produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals and suggest its therapeutic usefulness in disorder of immunological origin.

Thus it can be concluded that flavonoids and phenolic compounds present in stem extract contribute to the effect of *Tinospora cordifolia* on the humoral and cell mediated immune response in the animal experiments in the present study. Further studies to elucidate the exact immunostimulatory mechanism of ethanolic extract of stem of *Tinospora cordifolia* are in progress.

Table I: Effect of ethanolic extract of stem of *Tinospora cordifolia* and vitamin E on humoral responses to sheep RBC

S.No.	Groups	Mean antibody titre
1.	Control	6.1±0.64
2.	Immunosuppressant (Cyclophosphamide,50mg/kg)	4.8±0.51*
3.	Treated with ethanolic extract 200mg/kg p.o.	7.0±0.88*
4.	Standard (vitamin E/150mg/kg)	8.11±0.82*

n=6, Data are presented as mean ±SEM (ANOVA followed by Dunnett's test)
*P≤0.01 when compared with control.

Table II: Effect of ethanolic extract of stem of *Tinospora cordifolia* and vitamin E on cell mediated immune responses to sheep RBC

S.No.	Groups	Mean increase in paw volume
1.	Control	28.42±2.24
2.	Immunosuppressant (Cyclophosphamide)	14.64±1.38*
3.	Treated with ethanolic extract 200mg/kg p.o.	21.18±2.94*
4.	Standard (vitamin E,150mg/kg)	17.22±2.12*

n=6, Data are presented as mean ±SEM (ANOVA followed by Dunnett's test)
*P≤0.01 when compared with control.

REFERENCES

1. Patwardhan B, Kalbag D, Patki PS and Nagsampagi BA. Search of immunomodulatory agents: a review. Indian Drugs.1990;28(2):348–358.
2. Balchandran P and Panchanathan S. Immunomodulatory activity of Semecarpus anacardium Linn. Nut milk extract in aflatoxin B1-induced hepatocellular carcinoma in rats. Pharmacy Pharmacology Communication. 1998;4:507–510.
3. Venkatarao E and Saiprakash CV. Chemical and Pharmacological studies in tinospora species. Indian Drugs. 1999;36 (2):81-89.
4. Kokate CK. Practical Pharmacognosy,4th edn., Vallabh Prakashan, New Delhi. 1991;110.
5. Miller and Tainter. Quantal Response Calculation of LD^{50}" in "Screening methods in Pharmacology. Ed;Robert A.Turner, Newyork Academic Press,1944.
6. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V and Tandon JS. Immunostimulant activity of Nyctanthes arbor tristis L. Journal of Ethnopharmacology. 1994;42:31–37.
7. Miller LE and Ludke HR. In Manual of Immunology II edition, London ,Lea and Febiger. 1991;1-57.