

PHARMACOLOGICAL STUDY OF *TINOSPORA CORDIFOLIA* AS AN IMMUNOMODULATORVAIBHAV D. AHER*^{1,2}, ARUNKUMAR WAHI³¹College of Pharmacy, IFTM Moradabad, Uttar Pradesh India. ²National Institute of Medical Sciences, Jaipur Rajasthan India. ³College of Pharmacy, MIT Moradabad, Uttar Pradesh India.

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ABSTRACT

Immunomodulators are natural or synthetic agents, which by modifying the immune system affect a therapeutic benefit. They may have ability to augment (immune stimulant and /or immune enhancer), restore (immune restorative), inhibit (immune suppressant) or help to produce (adjuvant) the desired immune response. The present work described that *Tinospora cordifolia* alcoholic extract shows immunomodulator activity. The various parameters determined were Delayed Type Hypersensitivity (DTH), effect on the bone marrow cellularity and α -esterase cells and zinc sulphate turbidity test. Orally administration of *T. cordifolia* alcoholic extract (100 mg/kg, p. o) was found to increase in the there was distinct increase in foot pad thickness after treatment with *T. cordifolia* alcoholic extracts which indicates immunomodulatory effects of *T. cordifolia* as compared to vehicle and cyclophosphamide treated groups. Also significant increase in the WBC counts and bone marrow cells significantly indicating stimulatory effect on haemopoietic system. In zinc sulphate turbidity test *T. cordifolia* treated rats serum showed the more turbidity (cloudy) which indicate the increase in the immunoglobulin level as compared to vehicle, SRBC sensitized and cyclophosphamide treated group. Finally it can be concluded that *Tinospora cordifolia* (stem) mango plant climber shows potent immunomodulatory action.

Keywords: Immunomodulator, *T. cordifolia*, DTH, Bone marrow cellularity and α -Esterase cells, Zinc sulphate turbidity test.

INTRODUCTION

Tinospora cordifolia Miers (Menispermaceae), is an important medicinal plant cultivated throughout the Indian subcontinent. Through centuries, it has been extensively used in various Ayurvedic preparations for the treatment of various ailments^{1, 2}. There are several herbal preparations used in the indigenous system of medicines which can enhance the body's immune status. A variety of plant-derived materials polysaccharides, lectins, peptides, etc. have been reported to stimulate the immune system³. Plants with known immunomodulatory activity are *Viscum album*, *Panax ginseng* and *Asparagus racemosus*. However, use⁴ of plants in immunotherapy is still at an early stage. *Tinospora cordifolia*, used in several indigenous drug preparations for general health and other disease conditions, has been shown to possess antiallergic⁵ antidiabetic⁶, antihepatotoxic⁷ and antipyretic⁸ properties. In the present study, we have investigated the immunomodulatory activity of *T. cordifolia* stem alcoholic extract in male wister rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The stems of *Tinospora cordifolia* (climber from Mango plant) were collected from Ranikhet, Uttarakhand. The plant was identified by Dr G.C. Joshi (Botanist), Ranikhet, Uttarakhand. Voucher specimens were preserved in the Department of Pharmacy, IFTM, Moradabad, Uttar Pradesh India.

Plant material and preparation of extraction

The fresh stem of *T. cordifolia* was shade dried and the coarse powders (350g) were extracted separately in Soxhlets using alcohol for 32 hrs. The extract were then concentrated to dryness under reduced pressure by using rotary evaporator at 42-45°C, yielded 14g of dry extract and preserved in a desiccator for further use.

Animals

Male Wister rats weighing 150-180 g were procured from Laboratory Animals Resources, Division of Animal Genetics, Indian Veterinary Research Institute (IVRI), Izatnagar Reg No. CPC-196 and acclimatized to laboratory condition at Animal House, IFTM, at Moradabad at room temperature 23±5°C with a 12/12h/light /dark cycle and relative humidity (55±10%). The Institutional Animal Ethical Committee reviewed the animal protocol prior to the experiment. All rats were treated in accordance with the guideline for the Care and Use of Laboratory Animals (NIH Publication No.86-23, revised 1985) with the permission of Institute Animal Ethical

Committee (Proposal No.11). The animals were kept in polypropylene cage and maintained on balanced ratio provided by Feed Technology Unit, Division of Animal Nutrition, IVRI, Bareilly Uttar Pradesh.

Animal grouping

For experimental procedure, Male Wister rats were divided in the following four groups containing six rats in each group.

Group I (n=6): Negative control: Rats treated with 2 ml of 1% gum acacia solution in distilled water.

Group II (n=6): Positive control: Sensitized rats (by administering 1x10⁸ SRBCs, i. p.) treated with 1% gum acacia solution orally.

Group III (n=6): Rats treated with cyclophosphamide 100 mg/kg/p. o.

Group IV (n=6): Sensitized rats treated with *Tinospora cordifolia* alcoholic extract 100 mg/kg/p.o.

a) 4 days prior to sensitization (days -3, -2, -1, 0).

b) 7 days after sensitization (days +1, +2, +3, +4, +5, +6, +7).

Preparation of sheep red blood cells (SRBC)

From healthy Sheep blood was collected from local butcher house and mixed with sterile Alsever's solution (1:1). It was thoroughly mixed and centrifuged at 3000 rpm for 5 min. Supernatant was discarded, SRBC pellets were washed with sterilized phosphate buffer saline (pH 7.2) 2-3 times. Then the SRBC pellets were prepared in phosphate buffer saline (pH 7.2) and total SRBC was counted using Neubauer chamber, finally 1x10⁸ SRBCs (0.5ml) were injected intraperitoneally for sensitization and challenging the rats⁹.

Determination of delayed type hypersensitivity

The effect of the *T. cordifolia* alcoholic extract on the antigen specific cellular immune response in experimental animals was measured by determining the degree of DTH response using the foot paw swelling test.¹⁰ The rats were divided in different group as described in animal grouping. Seven days later (day+7), the same animals were injected subcutaneously with 0.2 ml of SRBC suspended in 50µl of phosphate buffered saline pH 7.2 (PBS) into the right hind foot pad for elicitation of the DTH reaction. The left hind foot pad was injected with 50µl of PBS as control. The difference between the means of right and left hind footpad thickness gave a degree of foot pad swelling which was used for group comparisons. The control

group was administered with 0.1 ml of PBS. The footpad thicknesses were measured after 8, 24, 48 and 72 hrs of sensitization by using vernier caliper.

Effect on the bone marrow cellularity and α -esterase cells of rat

Bone marrow cellularity

Bone marrow cellularity was determined by the method of BALB/c mice (6 Nos/ group) were divided into two groups as described above. The animals were sacrificed 24 h after the last dose and bone marrow cells from femur was collected into the medium containing 2% fetal calf serum (FCS). The bone marrow cell number was determined using a hemocytometer and expressed as total live cells/femur.¹¹

α -esterase positive cells

The number of α -esterase positive cells was determined by the azodye coupling method¹². A smear of bone marrow cells from the above preparation was made on clean glass slides, stained with α -naphthyl acetate and pararosaniline hydrochloride and counter stained with haematoxylin. The numbers of α -esterase positive cells were expressed out of 4000 cells.

Determination of humoral immunity by zinc sulphate turbidity test

The rats were divided in different group as described in animal grouping, six hours after the last dose blood was collected and the serum was used for estimation of immunoglobulin levels using method devised by Mullen.¹³

Zinc sulphate solution preparation

The triple distilled water was boiled for 15 min. to remove dissolved CO₂ and was used to prepare zinc sulphate solution (208 mg/liter ZnSO₄.7H₂O). The ZnSO₄ solution was kept in an aspirate bottle to protect uptake of carbon dioxide. This was achieved by insertion of soda lime tube into the stopper. A tubing to deliver 6 ml per vial was connected to the aspirator bottle.

Test procedure

A control vial containing 6 ml distilled water and test vial containing 6 ml zinc sulphate solution were taken and added to 0.1 ml serum sample. The solutions were gently shaken to ensure complete mixing and reading was taken spectrophotometrically at 580nm.

Statistical analysis

The results were expressed as mean \pm S.D. and statistical evaluation of the data was done using students t-test and P<0.05 was considered as significant.

RESULTS

Table 1 showed DTH reaction, in vehicle treated rats there was no change in foot pad thickness after 8, 24, 48 and 72 hrs. But *T. cordifolia* alcoholic extract shows the significantly increase (p<0.05) in the foot pad thickness as compared to SRBC sensitized and cyclophosphamide treated rats.

Table 1: The effect of *T.cordifolia* alcoholic extract on DTH

Treatment group	Dose	Mean foot pad thickness (mm)			
		8hrs	24hrs	48hrs	72hrs
Control group	2 ml of 1% gum acacia solution	0.061 \pm 0.02	0.061 \pm 0.03	0.061 \pm 0.03	0.061 \pm 0.01
SRBC sensitized	0.2ml/animal, s. c. SRBC +2 ml of gum acacia solution orally	0.062 \pm 0.02	0.064 \pm 0.02	0.065 \pm 0.01	0.066 \pm 0.01
Cyclophosphamide	0.2ml/animal, s.c. SRBC 100 mg/kg. orally	0.060 \pm 0.04	0.063 \pm 0.03	0.065 \pm 0.01	0.064 \pm 0.11
<i>T. cordifolia</i>	100 mg/kg p.o.	0.061 \pm 0.006*	0.064 \pm 0.06*	0.088 \pm 0.02*	0.092 \pm 0.02

*P<0.05 when compared to SRBC treated group

Table 2: Effect on the bone marrow cellularity and α -esterase cells of rat

Treatment group	Dose	Bone marrow cellularity (10 ⁶ cells/femur)	α -Esterase activity (no. of α -esterase positive cells/4000)
Vehicle treated	2 ml of 1% gum acacia solution	15x10 ⁶ \pm 1.1	1160 \pm 4.13
SRBC sensitized	0.2ml/animal, i p.+2 ml of gum acacia solution orally	12x10 ⁶ \pm 1.2	988 \pm 5.22
Cyclophos-phamide	100 mg/kg. orally	8x10 ⁶ \pm 1.2	634 \pm 2.11
<i>T. cordifolia</i>	100 mg/kg p.o.	28x10 ⁶ \pm 1.3*	1928 \pm 5.13*

*P<0.05 when compared with vehicle treated, SRBC sensitized and cyclophosphamide treated groups.

Table 3: Zinc sulphate turbidity test

Treatment group	Dose	Serum immunoglobulin level (ZST units)
Vehicle	2 ml of 1% gum acacia solution	21.398 \pm 0.8543
SRBC sensitize	0.2ml/animal, i p.+2 ml of gum acacia solution orally	18.215 \pm 0.4852
Cyclophosphamide	100 mg/kg. orally	20.191 \pm 0.1184
<i>T. cordifolia</i>	100 mg/kg p.o.	28.562 \pm 0.3641*

*P<0.05 when compared with control, SRBC sensitized and cyclophosphamide treated groups.

Effect on the Bone Marrow Cellularity and α -Esterase cells of rat

T. cordifolia alcoholic extracts are found to increase the bone marrow cellularity (28x10⁶cells/femur) and (23x10⁶cells/femur) as compared to the vehicle treated group (15x10⁶ cells/femur) as well as SRBC sensitized animals (12x10⁶ cells/femur) and cyclophosphamide treated group. Thus show immunosuppression action (8x10⁶ cells/femur) as shown in Table: 2.

Table 2 showed the number of α -esterase positive cells (1928/4000 cells) in *T. cordifolia* and *T. chebula* (1512/4000 cells) alcoholic

extract treated groups were also increased significantly (p<0.05) as compared to that of vehicle treated group (1160/4000 cells), SRBC sensitized rat (988/4000 cells) and cyclophosphamide treated group (834/4000 cells).

Determination of humoral immunity by zinc sulphate turbidity test

Table 3 shows *T. cordifolia* alcoholic extract treated group showed a significant increase (28.562 \pm 0.3641) in the serum immunoglobulin levels whereas SRBC sensitized rats did not show any significant

increase in the serum immunoglobulin levels (18.215±0.4852) as compared to vehicle treated (21.398±0.8543), cyclophosphamide treated (20.191±0.1184)) rats respectively.

DISCUSSION

Earlier workers reported that the drug having immunomodulatory effects show cutaneous reaction which is attributed to liberation of lymphokines, skin reactive factor and monocytes, chemotactic factor from sensitized T- cells.¹⁴ Thickening and reddening of skin in the immunized animals are attributed to vasodilation that causes increase capillary permeability of local influx of mononuclear cells at the site of inoculation^{14,15}

In our studies we found that (Table:1) there was distinct increase in foot pad thickness after treatment with *T. cordifolia* alcoholic extract which indicates immunomodulatory effect of *T. cordifolia*,

The earlier workers have shown that the drugs are having immunomodulatory activity shows increase in the WBC counts and bone marrow cells significantly indicating stimulatory effect on haemopoietic system. Besides, immunomodulatory drugs increases α -esterase positive bone marrow cells.^{16, 17, 18, 19}

Our observation shows (Table:1) that there was enhancement in the bone marrow cellularity as well as α -esterase activity in the rats groups treated with alcoholic extracts of *T. cordifolia* which evidently show that these drugs have immunomodulatory activity. However, as reported in the preceding conclusions in this study activity of *T. cordifolia* was higher than control group. The estimation of serum immunoglobulin levels was used to evaluate the increase in serum immunoglobulin concentration after administration of drug. Immunoglobulins are antibodies that react specifically with SRBC antigen and formation of cloudy serum is concentratic immunomodulatory property.²⁰

In the present study (Table: 3) the *T. cordifolia* treated rats serum showed the more turbidity (cloudy) which indicate the increase in the immunoglobulin level after *T. cordifolia* alcoholic extract treatment as compared to vehicle, SRBC sensitized and cyclophosphamide treated group. The turbidity was expressed as ZST units which in terms indicate the amount of immunoglobulins present in sample. This indicates the immunomodulatory property of *T. cordifolia*.

However, the control and cyclophosphamide treated rats serum showed the less turbidity in serum solution as compared to *T. cordifolia* treated rats group which shows that *T. cordifolia* has strong immunostimulant action as compared to control and cyclophosphamide groups.

CONCLUSION

In conclusion, it is revealed that the alcoholic extracts of *T. cordifolia* obtained from the dried ripe fruits possess good immunomodulatory activity. Although the ongoing research work is still under progress in order to explore the cellular changes and other pharmacological and biotechnological investigations in male wister rat.

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