STUDY OF THE IMMUNOMODULATORY ACTIVITY OF TRIKKATTU CHURANUM IN MICE

V.V. Venkatachalam*[1], K.Kannan[1], V.Suresh[3], A.Suresh[3], P.Balaji[2], R. Mohankumar [3]
[1]University Department of Pharmacy, Annamalai University, Chidambaram -608002.
[2]Thanthai Roever College of Pharmacy, Perambalur-621212.
[3]JKK Munirajah Medical Research Foundation College of Pharmacy, Komarapalayam-638183

ABSTRACT

Trikkattu Churanum was administered orally at doses of 100, 200, 400, 800 mg/kg/day to healthy mice's divided into six groups consisting of three animals each. The assessment of immunomodulatory activity was carried out by testing the humoral (antibody titer) and cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs. On oral administration of test drug showed a significant increase in humoral antibody titer and delayed type hypersensitivity (DTH) response. With a dose of 400 mg/kg/day of test drug extract produced increased in humoral antibody (HA) titer was 180.2 ± 0.2^a respectively, in compared to the untreated control group value is 8.5 ± 5.5 and potentiated the cellular immunity by facilitating the footpad thickness response to sheep RBCs in sensitized mice's and the DTH response (Mean ± SD % increase in paw volume) was 0.43 ± 0.077^a respectively, in comparison to the corresponding value of 0.31 ± 0.007 for the untreated control group. These differences in HA titer and DTH response were statistically significant ($P<0.05$). The study demonstrates that Trikkattu Churanum extract has promising immunostimulant properties.

Key words: Trikkattu Churanum, Humoral antibody titer, Hypersensitivity, Immunomodulatory

1. INTRODUCTION

Immunology involves the study of human body's response to foreign substance which gets in to the body, its "defense mechanism against injury". Immunomodulation is procedure which can alter the immune system of an organism by interfering with its function; its results is 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 effectors substances. Natural adjutants, synthetic agents, antibody reagents are used as immunosuppressive & immunostimulative agents.

Literature taken from "Introduction about system of Ayurvedic Formulary of India" published by the ministry of heath and family planning, department of health, government of India. Traditional Indian systems 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 (Atal et al., 1986; Patwardhan et al., 1990; Puri et al., 1994; Balachandran and Panchanathan, 1998; Ziauddin et al., 1996).

Most of the ayurvedic compound formulations based on herbal drugs include invariably the following pungent drugs namely (i) Dry ginger (sunthi) or (ii) Long Pepper (Pippali) or (iii) Black Pepper (Marica) or the combinations of three crude drugs in equal proportion called "Trikkatu".

The dried rhizomes of *Zingiber officinalis* (Rose) of the family Zingiberaceae. It contains 5% of Zingeberene, dried fruiting influorescence of *Piper longum* (Linn) of the family Piperaceae. It contains 5 to 6.4% Piperine and dried fruits of *Piper longum* (Linn) of the family Piperaceae. It contains 4 to 5% Piperine.

The objective of present investigation was to study the immunomodulatory activity of Trikkatu Churanum in animal models.

***Corresponding author**
V.V.Venkatachalam
University Department of Pharmacy, Annamalai University, Chidambaram -608002.

2. MATERIALS AND METHODS

Animals

Swiss Albino mice, (Rajah Muthiah Medical College, Annamalai University by central animal house, Chidambaram. Regd No: 160/1999/cpcsea) weighing between 20 and 25 g of either sex were used to evaluate the immunomodulatory activity of Trikkatu Churanum. Animals were housed under standard conditions of temperature (23±1°C), 12 h light/dark cycle and fed with standard pellet diet (Gold Muhor, Lipton India Ltd.) and tap water.

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 x 10^8 cells for immunization and challenge.

Plant material and extract preparation

Fresh air dried rhizhomes of *Zingiber officinalis* (Rose) of the family Zingiberaceae, the dried fruiting influorescence of *Piper longum* (Linn) of the family Piperaceae and the dried fruits of Piper longum (Linn) of the family Piperaceae were dried at room temperature for 2 weeks. They were authenticated in the botany department of Annamalai University by the Prof.Dr.AL.Chidambaram. M.sc, Ph.d., and all the above were separately powdered and then the three powders were mixed in the ratio of 1:1:1: (i.e.) 50g each; from the 150g of the powder, 50g was separately weighed. Later the 50g was boiled for 30 minutes with distilled water and filtered. [The filtrate was subjected to freeze drying by the freeze drier at the Center for Advanced Studies (CAS) at the marine biology department, of Annamalai University at Parangipettai.] The freeze dried powder was suspended in sodium carboxyl methyl cellulose 1% to prepare suitable dosage forms. The animals were divided into six groups consisting of three animals each. A group of three untreated mice's were taken as control (Group I). Cyclophosphamide was administered at a dose of 50mg/kg/day (Group II) and days 4, 5 and 6. The Trikkatu Churanum extract was fed orally for 14 days at a dose of 100 mg/kg/day (Group III), 200 mg/kg/day (Group IV), 400 mg/ kg/day (Group V) and 800 mg/kg/day (Group VI) for assessment of immunomodulatory effect. The animal experimental protocols were approved by the Institute Animal Ethics Committee.

Delayed type hypersensitivity (DTH) response

Three animals per group (control and treated) were immunized on day 0 by i.p. administration of 1 x 10^8 SRBC and challenged by a subcutaneous administration of 1 x 10^8 SRBC/ml into right hind foot pad on day +14. The test drug was administered orally from day 0 until day +13. DTH response was measured at 24 h after SRBC challenge on day +14 and expressed as mean paw volume (Plethysmograph).

Humoral antibody (HA) titer

Mice's of group III, IV, V and VI were pretreated with test drug for 14 days and each mice was immunized with 1 x 10^8 SRBC by i.p. route, including control and cyclophosphamide treated mice's. The day of immunization was referred to as day 0. Blood samples were collected in micro centrifuge tube from individual animals by retro orbital puncture on day 14.The titre was determined by titrating serum dilutions with SRBC (1 x 10^8 SRBC). The microtitre plates were incubated at room temperature for two hours and examined visually for agglutination. The highest number dilution of serum showing haemagglutination has been expressed as HA titer.

Statistical analysis

Data were expressed as the mean standard deviation of the means (S.D) and statically analysis was carried out employing student's *t*-test values <0.05 were considered significant.

3. RESULTS

Delayed type hypersensitivity response

The results indicates the animals treated with lower doses test extract I (100 mg/kg) and test extract II (200 mg/kg) did not show significant increase in paw edema. The optimum dose of the test extract III (400 mg/kg) showed significant increase in paw edema. At still higher dose of test extract IV (800 mg/kg) there was again a decrease in paw edema. Cyclophosphamide treatment appears to be more potent than test extract I in producing paw edema.

The statistical significance for the various treatments was:

For cyclophosphamide	$p<0.05$
Test extract I	$p<0.02$
Test extract II	$p<0.01$
Test extract III	$p<0.05$
Test extract IV	$p<0.02$

3.2. Humoral antibody titer and DTH response

The humoral antibody titer value was found to be 21.5 ± 9.2 for control. Administration of test extract I

produced a dose dependent (100 – 800 mg/kg per day for 14 days) increase in humoral antibody as evident by haemagglutination at that dilution. In a cyclophosphamide treatment reduced the HA titer.

Effect of test extract and cyclophosphamide on DTH response and HA titer using SRBCs as an antigen in mice- 14 days pretreatment.

Group	Treatment	Dose mg/kg P.O for 14 days	DTH response (mm) mean paw edema ± S.D	HA titer (mean ± S.D)
I	Control	-	0.31 ± 0.007	21.5 ± 9.2
II	Cyclophosphamide	50	0.51 ± 0.13a	3.12 ± 1.5a
III	Test extract I	100	0.35 ± 0.0212b	45.2 ± 22.0c
IV	Test extract II	200	0.34 ± 0.0141c	86.2 ± 25.1b
V	Test extract III	400	0.43 ± 0.077a	325.3 ±115.2a
VI	Test extract IV	800	0.39 ± 0.05b	302.1 ± 180.8b

Control: 1% sodium carboxyl methyl cellulose; n=3 per group results were expresses as mean ± S.D Significant differences compared from control by student's *t*- test [a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$

4. DISCSUSSION

Result obtained in the present study showed that when the test extract III was administered, DTH response was the best. The test extract III was administered the paw edema was 0.43 ± 0.077 which is only next to the Cyclophosphamide treatment. The statistical significance was $p<0.05$. For humoral antibody titre the best response was found again for the test extract III was 325.3 ± 115.2 and it was produced large clumps (due to antigen & very high antibody reaction). Cyclophosphamide treatment there was increases in DTH response and decrease in HA titer. The results obtained in the present studies showed that the test extract displays a dose dependent immunostimulating effect in relation to antigenic stimulation up to 400 mg. The test extracts produced dose dependent increase in both the parameters up to 400 mg, i. e, (antibody production and delayed type hypersensitivity). However for 800 mg there is a slight decrease in both the parameters. It is thus concluded that trikkatu churanum extract has promising immunostimulant properties.

REFERENCES

Atal CK, Sharma ML, Kaul A, Khajuria A, Immunomodulatory agents of plant origin I: Preliminary screening, Journal of Ethnopharmacology, 18, 1986, 133-141.

Fulzele SV, Satturwar PM, Joshi SB, Dorle AK, Study of the Immunomodulatory activity of Haridradi Ghrita in Rats, Indian Journal of Pharmacology, 35, 2003, 51-54.

Gokhare AB, Damne AS, Sanad MN, Investigation in to the Immunomodulatory activity of Argyreia speciosa, Journal of Ethnopharmacology, 84,2003,109-114.

La Gangre PH, Makaness GB, Miller TE, Potentiation of cell mediated immunity by selective suppression of antibody formation with Cyclophosphamide, Journal of Experimental Medicines, 139, 1974, 1529 – 1539.

Leemol Davis, Girija Kuttan, Immunomodulatory activity of Withania somnifera, Journal of Ethnopharmacology, 71, 2000,193-200.

Manjrekar PN, Joly CI, Narayanan S, Comparative studies of the immunomodulatory activity of Tinospora cardifolia and Tinospora sinesis, Fitoterapia, 78, 2001, 85-87.

Mohan PV, Devi KS, Chemo protective effect of Sobatum against Cyclophosphamide toxicity in mice, Journal Experimental Clinical Cancer Research, 17, 1998,159-164.

Neelam Makare, Subhash Bodhankar, Vinod Rangari, Immunomodulatory activity of alcoholic extracts Mangifera indica. L in mice", Journal of Ethnopharmacology, 78, 2001, 133-137.

Puri A, Saxena RP, Saxena KC, srivastava V, Tandon, JS, Immunostimulant activity of Nyctanthes arbortristis. L, Journal of Ethnopharmacology, 42, 1994, 31-37.

Ramesh Agarwal, Sham Diwanar, Pralhadpatki, Studies on Immunomodulatory activity of Withania somnifera extracts in experimental immune inflammation, Journal of Ethnopharmacology, 67, 1999, 27-35.

Sunila ES, Kuttan G, Immunomodulatory activity and Anti-tumor activity of Piper longum Linn and piperine, Journal of Ethnopharmacology, 90, 2004, 339-346.