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STUDY OF THE IMMUNOMODULATORY ACTIVITY OF TRIKKATTU CHURANUM IN MICE

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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 increase in humoral antibody (HA) titer as 180.2 ± 0.2^a respectively, as 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

INTRODUCTION

Immunology involves the study of human body's response to foreign substance which gets into the body and, 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 and it is named as an immunostimulative drug, which primarily implies stimulation of non-specific system, i. e.,

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Granulocytes, macrophages, complement, certain T-lymphocytes and different effectors substances. Natural adjutants, synthetic agents and antibody reagents are used as immunosuppressive and immunostimulative agents.

Literature taken from "Introduction about system of Ayurvedic Formulary of India" published by the Ministry of Health 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[1,2].

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 contains 5% of zingeberene, dried fruiting influorescence of *Piper longum* (Linn) of the family Piperaceae contains 5 to 6.4% piperine and dried fruits of *piper longum* (Linn) of the family piperaceae contains 4 to 5% piperine.

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

EXPERIMENTAL

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. A. L. Chidambaram. All the above were separately powdered and then the three powders were mixed in the ratio of 1 : 1 : 1 : (i. e.) 50 g each; from the 150 g of the powder, 50 g was separately weighed. Later the 50 g 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 mices were taken as control (Group I). Cyclophosphamide was administered at a dose of 50 mg/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

Mices 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 mices. 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.

RESULTS AND DISCUSSION

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.

Table 1. Humoral antibody titer and DTH response

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 ± 0.13^a	3.12 ± 1.5^a
III	Test extract I	100	0.35 ± 0.0212^b	45.2 ± 22.0^c
IV	Test extract II	200	0.34 ± 0.0141^c	86.2 ± 25.1^b
V	Test extract III	400	0.43 ± 0.077^a	325.3 ± 115.2^a
VI	Test extract IV	800	0.39 ± 0.05b	302.1 ± 180.8^b

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 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.

The statistical significance for the various treatments was :

For cyclophosphamide $p < 0.05$

Test extract I $p < 0.02$

Test extract I I $p < 0.01$

Test extract I II $p < 0.05$

Test extract I V $p < 0.02$

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

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] $p < 0.01$

Results 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 found again for the test extract III was 325.3 ± 115.2 and it produced large clumps (due to antigen and very high antibody

reaction). Cyclophosphamide treatment increases DTH response and decreases 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

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