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Evaluation of Immunomodulatory Activity of Saya Churnam (A Poly Herbal Formulation) on Albino rats.

Key words:
Saya churnam, Immunomodulation, Antibody titer, Delayed type hyper sensitivity, Ig E.

Corresponding Author:
Dr. G. Pramod Reddy, Department of Pharmacology, Siddha Central Research Institute, Anna hospital Campus, Arumbakkam, Chennai, T.N., India.
gpramod01@gmail.com

Abstract:
The immunomodulatory activity of Saya churnam (a poly herbal formulation) on Albino rats was evaluated by using Cyclophosphamide as an immunosuppressant. The Poly herbal formulation was administered orally at the dosage levels of 200mg/kg/day and 400mg/kg/day body weight of rat. The assessment of immunomodulatory activity on specific and non- specific immunity were studied by Hemagglutination antibody (HA) titer, delayed type hypersensitivity (DTH), Identification of Ig E, hematological , biochemical analysis. Induction of immune suppression in rats was achieved by using Cyclophosphamide (CP) (100mg/kg/day, p.o). Oral administration of Saya Churnam (SC) showed a significant increase in the production of circulating antibody titer in response to sheep red blood cells (SRBCs). A significant ($p< 0.001$) increase in the both primary and secondary HA titer was observed when compared to control group, where as in Cyclophosphamide treated group Saya Churnam showed significant ($p<0.01$) increase in HA titer. Saya churnam showed significant ($p<0.01$) Delayed type hypersensitivity (DTH) reaction by facilitating the foot pad thickness response to SRBCs in sensitized rats. Also biochemical and hematological analysis showed a significant ($p<0.001$) increase in Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and WBCs. The study demonstrates that Saya churnam triggers both specific and non-specific responses to a greater extent. The study showed significantly increased results in Heme agglutination (HA) titer, DTH response, Ig E test, biochemical, hematological analysis.

INTRODUCTION

Indian traditional systems of medicines like Siddha and Ayurveda which generally involve herbal formulations have suggested the body's natural resistance to disease (Sagrawat and Khan, 2007). Recent studies with plants have revealed many compounds with potent antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential (Wanger, 1990). Immune activation is an effective as well as protective approach against emerging infectious diseases, and this immune system known to be involved in etiology and pathophysiological mechanisms of several diseases. It is believed that the immunomodulatory drugs promote positive health and maintain organic resistance against infections by establishing body's equilibrium and conditioning the body tissues (Dasgupta et

al., 1998). The restorative and rejuvenating power of these herbal remedies might be due to their action on the immune system and thereby responsible for the protection of the organism from extraneous substance and maintaining homeostasis.

Plant and plant products are being used as a source of medicine since long. Non – toxic herbal preparations are used to improve the general health by stimulating body's immunity. In this poly herbal formulation *(Terminalia chebula, Piper longum, Piper cubeb, Alpinia galangal and Myristica*

MATERIALS AND METHODS

Site of the experiment: The experiment was conducted at the Department of Pharmacology, Siddha Central Research Institute, Chennai, in February, 2011.

Plant material: Fruits and rhizome of the plants were procured from local market and it was authentified by Sasikala Ethirajulu, Assistant Director, Department of Pharmacognosy, Siddha Central Research Institute, Chennai.

Preparation of poly herbal formulation:

inflammatory, anti-ulcer, and other activities.

The Siddha system of medicines not only provides hat alternative, but also scores over the side effects and cost factors of allopathic medicine. Herbal preparation can be more effective and safer (Vinothapooshan et al., 2011) than conventional medicines. Non-toxic herbal drug preparations that are used to improve general health by stimulating body's immunity (Kumar et al., 1999).

Herbal medicine has become an integral part of standard health care (Agarwal et al., 2010). This formulation of Saya churnam was obtained from the ancient Siddha text (Agasthiar, 1995). In Siddha normally this Saya churnam is prescribed for Tuberculosis, Jaundice, Anemia, Diabetes mellitus, Arthritis. On the basis of literature there are no reports on Saya Churnam for immunomodulatory system.

The present study was undertaken to investigate and to validate the Immunomodulatory activity of Saya churnam.

of Alpinia galangal was collected. It was cleaned without any dust particles or other unwanted materials. Each herb is finely powdered and it was mixed in different ratio. The collected fruits and rhizome are fried little and powdered as mentioned in the ancient text. Then the poly herbal powder was used for the further studies. Now this formulation is used for the evaluation study.

Drug and chemicals: All the drugs and chemicals are analytical grade while the other drugs procured were Cyclophosphamide (Biochem pharmaceutical, Mumbai), ELISA commercial kit from Biotran Diagnostics, U.S.A.

Animals: Healthy Wistar albino rats both male and female (180-230 gm) were used for the study. All the animals were housed which were under standard conditions of temperature (25 ± 2˚C), 12h light/ dark cycles and fed with standard pellet food and water. The animals were divided into four groups consisting of six animals each. A group of

six un- treated rats were taken as control (group I). Group II animals received standard drug Cyclophosphamide on day 9th and 16th orally in a dosage range of 100mg/kg/day. The Saya churnam was fed orally for 21 days at a dosage of 200 mg/kg/day (group III), 400) mg/kg/day, (group IV) mg/kg/day for assessment of immunomodulatory activity.

Test compound formulations: The different ratio of all the fruit and rhizome powder was measured and prepared in honey with water in the ratio of 7:3 prior to oral administration of animals. Freshly prepared drug solution was used. The vehicle alone served as control.

EXPERIMENTAL PROCEDURE

Antigenic material: Preparation of sheep RBCs (SRBCs): Sheep blood was collected in sterile Alsever's solution in 2:1 proportion of Alsever's

visually for agglutination has been expressed as HA titer.

Delayed Type Hypersensitivity (DTH) Response: Each group of animals (group I-IV) was immunized with SRBCs on day 7th and 14th. On day 21st Rats are administered 0.1ml of 1% SRBCs in the left hind foot pad by subcutaneous (Fulzele et al., 2003) injection, in the right hind foot pad administered 0.1 ml of 0.9% of normal saline was injected and the increased level of paw volume was measured by plethysmometer (Gayathri et al., 2005) in three different time (0 hour, 1 hour, 3 hour) intervals. The thickness between left and right hind paw volume was measured.

Identification of Ig E: On 21st day serum was obtained from all the group of animals. 25µl of serum was added to all the wells, add 100µl of Ig E biotin reagent then allowed to incubation of an hour. Discard the contents and add 300 µl of wash

SRBCs batch, by centrifugating at 2000rpm for ten minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use (Kirtikar et al., 1961).

Heamagglutinating Antibody (HA) Titer: The rats of all groups are pre-treated with drug for 21 days. And each rat immunized with $0.1X10^9$ SRBC/rat by i.p. route, including control rats. The immunization (Agarwal et al., 1999) was given on day 7^{th} and $14^{th.}$ The animals were treated with Saya churnam for 21 days. The titer was determined by titrating serum dilutions with SRBCs. The micro titer plates were incubated at room temperature for one hour and examined allowed it for incubation of half an hour. Discard the contents and added 300 µl of wash buffer and discarded it was carried out 4 times after that.1 ml of working substrate solution was added allowed for 15 minutes incubation then added 0.05ml of stop solution read the absorbance at 450 nm.

Liver function and blood parameters test: Activities of Serum Glutamate Oxalate Transaminase (Sharififa et al., 2009) (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and Hematological parameters (RBC, WBC, Hb, PLT) were estimated by using kits (Span Diagnostics, India). For this purpose four groups of animals (one control+three

Table 1: Effect of Saya Churnam on primary and secondary antibody titer.

Treatment	Primary antibody titer	Secondary antibody titer
Control	6.88±0.83	7.66 ± 0.98
CP 100mg/kg	5.23±0.63**	4.86 ± 1.04**
SCLD 200mg/kg	5.72 ± 1.04***	6.12 ± 0.89***
SCHD 400mg/kg	7.76 ± 1.32***	8.56 ± 0.98***

The values are expressed as (Mean ± S.D), n=6,**p<0.01 and *** p<0.001

Treatments) as described above were used and treated with drug for 21 days.

Statistical analysis: The statistical analysis was performed by using student t Test followed by one way analysis (ANOVA).

RESULTS

Heamagglutination antibody (HA) Titer: Effect of Saya churnam low dose (SCLD) and Saya churnam high dose (SCHD) on primary and secondary antibody response on HA titer is shown in (Table 1). Primary antibody response on day 14^{th} in saya churnam (200mg, 400mg/kg/p.o) treated group with normal immune status showed significant increase (p<0.01) in HA titer when compared with the control group. A significant decrease in the antibody titer was observed in the Cyclophosphamide–treated group when compared with the control group. In immunosuppressed groups, where the immunity was suppressed by dose treated groups with normal immune status group showed a significant rise (p<0.01) in the antibody titer when compared with the control group. In the immunosuppressed groups where the immunity was suppressed by administration of Cyclophosphamide on day sixteenth Saya churnam both low dose and high dose showed a significant rise (p<0.01) in HA titer when compared with the Cyclophosphamide group.

Delayed type hypersensitivity: Effect of Saya churnam on cell mediated immune response by DTH induced foot pad oedema is shown in (Table 2). In the all groups of rats with normal immune status, of Saya churnam low dose (200mg/kg/p.o) and Saya churnam high dose(400mg/kg/p.o) showed significant (**p<0.01,*P<0.05) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with control group. The drug treated group of rats

produced a significant ($p<0.01$) rise in the antibody titer when compared with the Cyclophosphamide – treated group. Secondary antibody titer on twenty first day in Saya churnam both low dose and high difference of paw thickness. Heightened delayed type hypersensitivity reaction suggests activation of cellular immune system.