SCREENING OF AN IMMUNOMODULATORY POTENTIAL OF CHAVANAPRASHA: AN AYURVEDIC FORMULATION

M. Sajjad Khan[1*], Nitin Nema[1], M.D. Kharya[1], Salma Khanam[2]

[1] Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar-470 003, Madhya Pradesh, India
[2]Al-Ameen College of Pharmacy, Bangalore-560 028, Karnataka, India

Address for correspondence
M. Sajjad Khan
Near Moti Masjid Mominpura,
Tulsinagar ward, Bhagwan Ganj, Sagar (M.P) 470003
Phone: +91-9977317284 (p) 07582-247386 Fax: 07582-204306,
E-mail: sahil2sahil@gmail.com

Summary

Chavanprasha is a comprehensive herbal tonic with multiple health benefits, prepared according to an ancient Ayurvedic formula. In the present study, The Chavanaprasha has been investigated for its effect on cell mediated and humoral components of the immune system in mice. Administration of Chavanaprasha formulation produced an increase in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) in mice. It was concluded that the Chavanaprasha is a promising ayurvedic formulation with immunostimulant properties.

Key words: Cell mediated immunity, Humoral immunity, Chavanaprasha.

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, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections or stress and may be due to environmental or chemotherapeutic factors.
Immunostimulation and immunosuppression both need to be tackled in order to regulate normal immunological functioning. Hence both immunostimulating and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming a field of major interest all over the world[1].

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitations to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system[2].

The use of plant products as immunomodulator is still in a developing stage. There are several herbs used in the indigenous system of medicines that can modulate the body's immune system. A variety of plant derived materials such as polysaccharides; lectins, peptides, flavonoids and tannins have been reported to modulate the immune system.
Traditional Indian systems of medicines such as 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. Medicines of the *rasayana* group are believed to promote health, immunity, and longevity. According to Ayurveda, they strengthen all tissues of the body, prevent aging, promote intellect and prevent disease[3-6].

The most widely used rasayana today is Chavanprasha, a reputed herbal formulation that has been used in India since ancient times. It is a complex mixture of many herbal ingredients. According to the Charaka Samhita, Chavanprasha is the foremost of all rasayanas, especially good for alleviating cough and asthma; it also nourishes weakness, injury, old age and regeneration of tissues. "Through the use of this rasayana" a person acquires intelligence, memory, freedom from disease, longevity, strength of the senses, great pleasure in the companionship with women, great increase in the strength of the digestive fire, improvement of the complexion, and the restoration of wind to its normal course[7]. There is no enough scientific data to support the therapeutic benefits of this popular herbal formulation. Hence there is a need to study the desired therapeutic activities of chyavanprasha formulations containing various ingredients from different plants[8].

Since, there is paucity of scientific data on the *in vivo* activities of Chavanaprasha. Hence we took the objective of investigating the immunomodulatory activity of the reputed polyherbal formulation, Chavanaprasha in animal models.

Materials and Methods

Materials
Marketed formulation of Chavanaprasha (Zandu) was purchased from local ayurvedic bhavan, Bangalore, India.

Animals
Swiss albino mice of either sex weighing between 20- 25 g were used for the study. Animals were housed under standard conditions of temperature (25 °C), 12 h/12 h light/dark cycles and fed with standard pellet diet and tap water.

Toxicity study
Chavanaprasha formulation was administered orally to different groups of mice in dose ranging from 100-1000 mg/kg for the LD_{50} study using the method of Miller and Tainter[9]. There was no lethality in any of the groups after 7 days of treatment.

Antigen
Fresh blood was colleted from sheep sacrificed in the local slaughterhouse. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5 x 10^9 cells/ml for immunization and challenge.

Effects of Chavanaprasha on HA titre and DTH response using SRBCs as an antigen in mice
Mice were divided into six groups, each group containing six mice. Drugs were administered in various groups, i.e. Group I–Control (Normal saline), Group II–VI Chavanaprasha (5 dose levels 100–500 mg/kg p.o.)[4]

The animals were immunized by injecting 0.1 ml of SRBCs suspension containing of 0.5 x 10^9 cells intraperitoneally on day 0. Blood samples were collected in micro centrifuge tubes from individual animal by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25 µl volumes of normal saline in microtitration plates was added 25 µl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37 °C for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre. On day 7, the thickness of the right hind foot pad was measured using vernier caliper. The mice were then challenged by injection of 0.5 x 10^9 cells SRBCs in right hind foot pad. Foot thickness was measured again +24 h after this challenge. The difference between the pre and post challenge foot thickness expressed in mm was taken as a measure of DTH. The extract was administered orally on day 0 and continued till day 7 of challenge.

Statistical analysis
Data were expressed as the mean standard deviation of the means (S.D.) and statistical analysis was carried out employing Student's *t*-test.

Results and Discussion

All the animals of groups I–VI were sensitized on day 0. The control group received only vehicle (Saline) from day -7 to +7. The humoral antibody titre value was found to be 24.7 ± 9.9. Administration of Chavanaprasha formulation produced a dose dependent increase in the HA titre after incubation with SRBCs (Table 1). Administration of higher doses, i.e. 300, 400 and 500 mg/kg produced significant increase in HA titre as evident from haemagglutination after incubation of serum with SRBCs. DTH was determined 24 h after the challenge. Higher doses of Chavanaprasha (400 and 500 mg/kg) showed statistically significant increase in mean paw edema. Effect of Chavanaprasha o HA titre and DTH at different doses is given in Table-1. The results obtained in the present studies showed that Chavanaprasha displays a dose dependent immunostimulatory effects in relation to antigenic stimulation. Injecting mice i.p. with 10^9 SRBCs suspended in saline sensitizes them for elicitation of DTH and also induces antibody formation, therefore this system has major advantages, i.e. it enables two component of immune response to be measured in the same species under ideal condition and is relatively simple and inexpensive to perform[10]. Chavanaprasha produced dose dependent increase in both the parameters, i.e. antibody production and delayed type hypersensitivity. It is thus concluded that the Chavanaprasha formulation has promising immunostimulant properties.

Table 1: Effects of Chavanaprasha on HA titre and DTH response using SRBCs as an antigen in mice

Group	Treatment	Dose (mg/kg)	HA titre	DTH response (mm)
I	Control (Normal Saline)	-	27.4 ± 9.9	0.35 ± 0.08
II	Chavanaprasha formulation	100	22.7 ± 11.23	0.38 ± 0.19
III		200	35.1 ± 12.38	0.40 ± 0.15
IV		300	122.5 ± 5.12*	0.48 ± 0.05
V		400	317.4 ± 6.80*	0.48 ± 0.15*
VI		500	419.5 ± 7.33*	0.52 ± 0.20*

Values are mean ± S.D., n = 6, P < 0.05 significant*

Acknowledgement

The authors are grateful to Prof. Salma Khanam (Head of Department - Pharmacognosy), Prof. B. G. Shivananda (Principal) Al-Ameen College of Pharmacy, Bangalore for their constant encouragement.

References

1. Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA (1990): Search of immunomodulatory agents: a review. *Indian Drugs* 1990; 28(2), 348–358.
2. Diasio RB, LoBuglio AF. *Immunomodulators: Immunosuppressive Agents and Immunostimulants*. In: Goodman and Gilman's (Eds.), *The Pharmacological Basis of Therapeutics,* McGraw-Hill, New York; 1996; 9th edition, 1291–1307.
3. Atal CK, Sharma ML, Kaul A, Khajuria A. Immunomodulating agents of plant origin. I: Preliminary screening, *J. Ethnopharmacol* 1986; 18**,** 133–141.
4. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbortristis* L. *J. Ethnopharmacol,* 1994; 42**,** 31–37.
5. Balchandran P, Panchanathan S. Immunomodulatory activity of *Semecarpus anacardium* Linn. Nut milk extract in aflatoxin B1-induced hepatocellular carcinoma in rats. *Pharm. Pharmacol. Commun,*1998; 4**,** 507–510.
6. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of Ashwagandha. *J. Ethnopharmacol.* 1996; 50**,** P. 69–76.
7. Indian Ayurvedic Pharmacopoeia, Department of Ayurveda Publication, India. 1996; 101-07
8. Subramoniam P, Pushpangadan S. *Ind. J. Pharmacol*. 1999; 31, 166–175.
9. Ghosh MN. *Fundamentals of Experimental Pharmacology,* Scientific Book Agencies, Calcutta, India: 1971; 84-88.
10. Doherty NS. Selective effects of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cells in mice. *Agents and Action.* 1981; 11 (3), P. 237-242.