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RESEARCH ARTICLE

In vivo immunomodulatory activities of the aqueous extract of bonduc nut *Caesalpinia bonducella* seeds

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Abstract

This study evaluated the *in vivo* immunomodulatory activities of the aqueous extract of *Caesalpinia bonducella* Fleming (Caesalpiniaceae) seeds. *C. bonducella* is a plant widely used in the traditional medicinal systems of India. In the present investigation, the aqueous extract of *C. bonducella* seeds was tested for its effect on cell mediated and humoral components of the immune system in rats. Administration of *C. bonducella* seed extract produced an increase of 93.03 ± 4 mean hemagglutinating antibody (HA) titer and a change of 0.56 ± 0.058 mm in delayed type hypersensitivity (DTH) as compared to control at a dose of 400 mg/kg body weight. Thus, the results of this study indicate that *C. bonducella* extract could be a promising immunostimulatory agent.

Keywords: Caesalpinia bonducella*; cell mediated immunity; humoral immunity*

Introduction

The immune system is known to be involved in the etiology as well as pathophysiological mechanisms of many diseases. The role of the immune system has become increasingly important in the understanding of the mechanisms that are involved in disease prevention. Ayurveda gives emphasis on the concept of strengthening host defenses against different diseases (Thatte & Dahanukar, 1986).

Immunomodulation is a procedure by which the immune system of an organism can be altered by interfering with its functions. An immunostimulative drug primarily implies stimulation of non-specific systems, i.e., granulocytes, macrophages, certain T-lymphocytes and different effector substances. Immunosuppression indicates reduced resistance against infections or stress, which may be due to environmental or chemotherapeutic factors (Wagner & Proksch, 1985).

Immune stimulation and immune suppression both need to be tackled in order to regulate normal immunological functioning. Hence, immunostimulating and immunosuppressing agents have their own significance. The search for better agents exerting these activities is becoming a field of major interest all over the world (Patwardhan et al., 1990). 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 (Diasio & LoBuglio, 1996).

The use of plant products as immunomodulators 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

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Indian medicinal plants and various *rasayanas* have been claimed to possess immunomodulatory activities. 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 (Atal et al., 1986; Patwardhan et al., 1990; Puri et al., 1994; Balchandran & Panchanathan, 1998; Ziauddin et al., 1996).

Caesalpinia bonducella Fleming (Caesalpiniaceae), known as bonduc nut, fevernut, molucca bean and "nata karanja" is a prickly shrub found throughout the hotter parts of India and Sri Lanka. Seeds are gray, hard, and globular in shape with a smooth shiny surface, and consist of a thick, brittle shell with a yellowish-white bitter fatty kernel (Nadkarni, 1954). *C. bonducella* is reported to have multiple therapeutic uses including antipyretic, antidiuretic, anthelmintic, antibacterial (Neogi & Nayak, 1958), antiviral (Dhar et al., 1968), antiasthmatic (Gayaraja et al., 1978), antiamebic and anti-estrogenic (Raghunathan & Mitra, 1982) activities. The aqueous solution of the outer shell of the seeds of *C. bonducella* is traditionally used by the tribal people of Andaman and Nicober Islands for the relief of the symptoms of diabetes mellitus. Blood sugar lowering activity of *C. bonducella* has been primarily evaluated with significant results in rabbit (Rao et al., 1994) and rat models (Biswas et al., 1997; Sharma et al., 1997). However, there is no report available in the literature on the immunomodulatory activity of *C. bonducella* seeds. Therefore, the present investigation evaluated the immunomodulatory activity of the aqueous extract of *C. bonducella* seeds in animal models.

Materials and methods

Plant material

The seeds of *C. bonducella* were collected in March 2006 from Sagar District, Madhya Pradesh, India. Further taxonomic identification was conducted by Pradeep Mehta at the Department of Botany, Dr. H.S. Gour University, Sagar, Madhya Pradesh, India. A voucher specimen has been deposited in the herbarium at the laboratory under the number Bot/H/2692.

Preparation of aqueous extract

The air-dried seeds (50 g) of *C. bonducella* were finely powdered (mesh size 20) and stirred with eight parts of distilled water at about 70°-80°C for 2 h. The liquid extract was filtered through a sieve (mesh size 200). The filtrate was concentrated up to two parts using a rotary vacuum evaporator (Model PBU – 6D, Scientific India, Banglore, Karnataka, India). The concentrated liquid was spray dried to obtain the dry powder of the extract with a yield of 3.5 g, which was measured in mg/mL.

Animals

Swiss albino rats of either sex weighing between 100-125 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. Animal studies were approved by animal ethical committee of the university (Eths. Comm./I.E/98 Reg. No. 379/01/ab/CPCSEA).

Toxicity study

C. bonducella dried extract was dissolved in water and administered orally to different groups of rats in dose ranging from 100-1000 mg/kg body weight, varied for the LD_{50} study using the method of Miller and Tainter (1944). There was no mortality in any of the groups after 7 days of treatment.

Antigen

Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen-free normal saline and adjusted to a concentration of 0.5×10^9 cells/mL for immunization and challenge.

Effect of Caesalpinia bonducella *seed extract on HA titer and DTH response using SRBCs as an antigen in rats*

The present study was conducted according to the method suggested by Puri et al. (1994). Rats were divided into six groups, each group consisted of six rats. Drugs were administered to various groups, i.e. Group I - control (normal saline), Group II and III - aqueous extract of *C. bonducella* seeds (2 dose levels 200 and 400 mg/kg per ora.

The animals were immunized by injecting 0.1 mL of SRBCs suspension containing of 0.5×10^9 cells intraperitoneally on day 0. Blood samples were collected in micro centrifuge tubes from individual animals by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the hemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. Two-fold serial dilutions of pooled serum samples made in 25 μL volumes of normal saline in microtitration plates were 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 hemagglutination under microscope. The reciprocal of the highest

dilution of the test serum giving agglutination was taken as the antibody titer. On day 7, the thickness of the right hind foot pad was measured using vernier calipers.

The rats were then again challenged by injection of 0.5×10^9 cells SRBCs in the right hind foot pad for measuring delayed type hypersensitivity (DTH). Foot thickness was measured before injection and after +24 h of SRBCs challenge. The differences measured between the pre- and post challenge foot thickness for DTH were expressed in mm. The extract was administered orally on day 0 and continued till day 7 of challenge (Shivaprasad et al., 2006).

Statistical analysis

Data were expressed as the mean standard deviation of the mean values (SD), and statistical analysis was carried out employing one way ANOVA.

Results and discussion

Immunomodulatory agents of plant origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. The animals from all the groups (I, II, and III) were sensitized on day 0. The control group received only vehicle (saline) from day -7 to +7. The humoral antibody titer value was found to be 93.03 ± 4. Administration of aqueous extract of *C. bonducella* seeds produced a dose-dependent increase in the HA titer after incubation with SRBCs (Table 1). Administration of higher dose of aqueous extract of *C. bonducella* seeds, i.e., 400 mg/kg produced significant increase in HA titer as evident from hemagglutination after incubation of serum with SRBCs. DTH was determined 24 h after the challenge. A higher dose of aqueous extract of *C. bonducella* seeds (400 mg/kg) showed statistically significant increase in mean paw edema.

Antibody production to T-dependent antigen SRBC requires co-operation of T- and B-lymphocytes and macrophages (Benacerraf, 1978). The high values of haemagglutinating antibody titer obtained in case of aqueous seed extract of *C. bonducella* have indicated that immunostimulation was achieved through humoral immunity. CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity, and delayed-type hypersensitivity reactions (Miller et al., 1991). Therefore, increase in DTH reaction in rats in response to T cell-dependent antigen revealed the stimulatory effect of aqueous seed extracts of *C. bonducella* on T cells (Table 1).

Some of the constituents from *C. bonducella* include triterpenoids, saponins, and phenolics that may be responsible for immunostimulatory effects. Saponins in particular are described as immunostimulatory agents (Liu et al., 1995). Recently, phenolic glucosides were found to be responsible for the enhancement of HA titer and DTH response (Lakshmi et al., 2003).

The results obtained in the present study showed that *C. bonducella* seed extract displayed a dose-dependent immunostimulatory effect in relation to antigenic stimulation. Injecting rats i.p. with 0.5×10^9 SRBCs (suspended in normal saline) sensitized to rats for elicitation of DTH and also induced antibody formation, therefore, this system has major advantages, i.e. it enables two components of immune response to be measured in the same species under ideal condition and is relatively simple and inexpensive to perform (Doherty, 1981). *C. bonducella* seed extract produced dose dependent increase in both the parameters, i.e. antibody production and delayed type hypersensitivity. It is thus concluded that the aqueous extract of *C. bonducella* seeds has promising immunostimulant properties.

Table 1. Effect of *Caesalpinia bonducella* seed extract on HA titer and DTH response using SRBCs as an antigen in rats.

Group	Treatment	Dose (mg/kg)	HA titer	DTH response (mm)
I	Control (normal saline)	-	22.04 ± 1.2	0.18 ± 0.019
II	Aqueous extract of *C. bonducella*	200	38.14 ± 2.6*	0.33 ± 0.056*
III	Aqueous extract of *C. bonducella*	400	93.03 ± 4*	0.56 ± 0.058*

Values are mean ± SD, n = 6, P < 0.001 significant*.

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***Declaration of interest*:** The authors report no conflicts of interest.

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