

Immunomodulatory Activity of the Aqueous Extract of Seeds of *Abrus precatorius* Linn (Jequirity) in Mice

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ABSTRACT

Background: Various compounds of plant origin have been widely investigated since ancient times for their possible immunomodulatory properties as well as for the treatment of a wide range of diseases. **Objective:** To study the immunomodulatory functions of the aqueous extract of the seeds of *Abrus precatorius* commonly known as *Indian liquorice* (Fabaceae), a medicinal plant native to central India. **Methods:** Swiss albino mice were intraperitoneally treated with three doses (0.75, 1.25 and 2.5 µg/kg b.w.) of extract for 7 days. Relative organ weight, delayed type hypersensitivity (DTH) response, haemagglutination titre (HT) and Phagocytic index (PI) were studied in various groups of animals. **Results:** The results showed no significant difference in relative organ weight of spleen, liver, thymus and kidney in various groups of animals. Treatment of rats with increasing concentrations of the extract decreased the footpad thickness indicating a dose related inhibitory effect of the extract on delayed type hypersensitivity. In the HT test, the plant extract showed a suppressive effect at all doses, and these changes were significant as the dose increased. Phagocytic index was also increased in a dose dependent manner. **Conclusion:** The reduction of antibody titre, delayed type hypersensitivity response and the increase in phagocytic index indicates that *Abrus precatorius* has an inhibitory effect on the immune functions in mice.

Keywords: *Abrus precatorius*, Delayed Type Hypersensitivity, Haemagglutination, Immunomodulation, Phagocytic Index,

INTRODUCTION

Immunology is probably one of the most developing areas of biomedical research which has great promises for the prevention and treatment of a wide range of disorders such as

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inflammatory diseases of the skin, gut, respiratory tract, joints and central organs (1). Immune dysfunction may result in infectious diseases and cancer, and hyper immune reactions may cause autoimmune diseases including allergy and rheumatoid arthritis. Thus the development of the immune modifier which is of herbal origin and stimulates the necessary functions and suppresses the unnecessary ones is highly desirable (2-4). Synthetic drugs are used as immunosuppressive and immunostimulating agents, but there are major limitations to the general use of these agents such as an increased risk of therapeutic and generalized effect throughout life. Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health; a concept of prevention of diseases and strengthening of both physical as well as mental health. Ayurvedic medicine thus constitutes rich sources of active substances for immunomodulation based on herbal preparations. Among the wide range of plants claimed to possess the rasayana effect, the most popularly used are *Withania Somnifera*, *Ocimum sanctum*, *Azadiracta indica*, *Curcuma longa*, and *Tinospora cordifolia*, etc. (5-7).

Abrus precatorius is a leguminous plant of the Fabaceae family that is also called Indian liquorice, Jequirity, Crab eye, Glycyrrhizin glabra, among others. The plant grows widely in fairly dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and the West Indies. The main constituent of the seeds of this plant are isoflavonoids, flavonoids, proteins, alkaloids, carbohydrates and triterpenoids. It has been used for centuries by the Hindus, who employ the seeds as external applications in skin infections, ulcers, and also used to excite artificial inflammation in fistulae. It is asserted that Indian singers chew the leaves of the white seeded variety for the cure of hoarseness. It is also used as anti-spasmodic, anthelmintic, anti-diarrhoeal, inhibitor of intestinal motility, anti-convulsant, anti-bacterial, and insecticidal (8). Jenkins et al. (9) showed that the whole seed of this legume or its extracts could be helpful in reducing the high blood sugars in diabetes because of their low glycaemic index (10,11).

In the present study, we focused on the immunomodulating effect of the aqueous extract of *Abrus precatorius* in mice to analyse its involvement in the cellular, humoral and non specific immunities.

MATERIALS AND METHODS

Animals. Inbred Swiss albino mice of either sex, weighing 25-30 g were maintained in the animal house of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, under standard conditions of temperature ($23 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 5\%$) and light (10:14 h of light and dark, respectively). The animals were housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding and fed with standard animal feed and filtered, acidified water *ad libitum*. Research was conducted in accordance with the guidelines of internationally accepted principles for laboratory animal use and care (CPCSEA), as approved by the Institutional Animal Ethics Committee.

Plant Material and Extract Preparation. Seeds of *Abrus precatorius* (voucher specimen no. 18367) were collected from the suburbs of Bhopal in the month of August-October and identified at the State Forest Research Institute, Jabalpur, M.P. India, where a voucher specimen has been preserved for future identification. The plant parts were thoroughly washed with tap water and dried on filter paper sheets under shade at room temperature for more than one month. Thoroughly shade dried, coarsely

powdered parts of plants were extracted with water by the method of Suffness and Douros (12). Briefly, 100 g of the powder was extracted with 1 litre of double distilled water at 80°C in a water bath for 72 hours. The cooled extract was filtered with muslin cloth. The extracts were then concentrated on a rotatory evaporator below 40°C, stored in an airtight container in freezer (-20°C) for further experimental studies.

Preparation of the Test Sample. A suspension was made in normal saline and administered to animals in a dose volume of 0.2 ml.

Preparation of Sheep Red Blood Cells (Antigen). Fresh sheep blood was collected from the local slaughterhouse in vials under sterile conditions in sterile Alsevere's solution in a 1:1 proportion of Alsevere's solution (Freshly prepared). Blood was kept in a refrigerator and processed for the preparation of SRBCs batch, by centrifugating at 2000 rpm for 10 minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use (13) and finally adjusting to a concentration of 1×10^8 cells/ml for immunization and challenge.

Preparation of Carbon Ink Suspension. Commercially available Camel brand black ink suspension was purchased from the local market and diluted in a ratio of 1:50 with normal saline and used for carbon clearance test in a dose of 1 ml/200 g body weight.

Experimental Design. Animals were divided into 4 groups consisting of 6 animals each.

Control Group received 0.2 ml of normal saline (i.p.) for 7 consecutive days.

Group-I received 0.75 µg/kg b.w. of *Abrus precatorius* extract i.p. for 7 consecutive days.

Group-II received 1.25 µg/kg b.w. of *Abrus precatorius* extract i.p. for 7 consecutive days.

Group-III received 2.5 µg/kg b.w. of *Abrus precatorius* extract i.p. for 7 consecutive days.

Relative Organ Weight. The animals were sacrificed 24 hrs after last dose. Relative organ weight (Organ weight/100 g of body weight) of kidney, liver, spleen and thymus were determined for each animal.

Humoral Antibody Response to SRBCs. On the 8th and the 13th day of the study, the rats from all the groups were immunized and challenged, respectively, with SRBCs in normal saline (0.1 ml of suspension containing 1×10^8 SRBC), intraperitoneally. Blood was withdrawn from the retro orbital plexus of all antigenically sensitized and challenged mice on day 14 and centrifuged to get serum. Serial two fold dilution of serum was made in normal saline in microtitre plates and Sheep RBC (25µl of 1% SRBC prepared in normal saline) were added to each of these dilutions and the plates were incubated at 37°C for 1 hr and then examined for haemagglutination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titer, as described by Puri et al. (14).

Delayed Type Hypersensitivity (DTH) Response Using SRBC's as an Antigen. On the 8th day of the study, all the groups of mice were primed by subcutaneously injecting 0.1 ml of suspension containing 1×10^8 SRBC into the right hind footpad. The contra lateral paw received an equal volume of 0.1% CMC. On the 13th day, the animals were challenged by subcutaneously injecting 0.1 ml of 1×10^8 SRBCs into the left hind footpad of the mice. The extent of DTH response in mice was determined by measuring the footpad thickness after 24 hrs of challenge using vernier callipers. The difference in the thickness of the right hind paw and the left hind paw was used as a measure of

delayed type hypersensitivity (DTH) reaction and expressed as a mean percent thickness/oedema (15).

Phagocytic Index. Phagocytic activity of the ‘reticuloendothelial’ system in vivo was determined by carbon clearance test, after completion of the extract treatment (16). On the 8th day, immediately after the last dose was administered to all animals, each control as well as the treated group received an intravenous injection of carbon suspension (1:50 dilution of Indian ink, camel) in a dose of 1 ml/200 g body weight. Blood was withdrawn from the retroorbital venous plexus before injection (0 min) and 12 min after injection of the carbon suspension and 50 µl of blood was lysed with 4 ml of 0.1% sodium carbonate solution (Na₂CO₃). The optical density was measured spectrophotometrically at 650 nm.

The results were expressed as phagocytic index:

$$K = (\text{Ln OD } t_{12 \text{ min}}) - (\text{Ln OD } t_{0 \text{ min}}) / (t_{12 \text{ min}} - t_{0 \text{ min}})$$

Where, OD_{12 min} and OD_{t 0 min} are the optical densities at 12 min and 0 min respectively

Statistical Evaluation. All the results were expressed as the Mean ± Standard Error of the Mean (SEM) and data were analyzed using Student’s t- test.

RESULTS

The results obtained for relative organ weight in the control and treated groups are shown in Table 1.

Table 1. Effect of the Aqueous extracts of *Abrus precatorius* on the relative organ weight of mice.

Treatment Groups	Dose (µg/kg)	Relative Organ weight (mean ± SE) in grams			
		Spleen	Liver	Thymus	Kidney
Extract	0.75	0.43 ± 0.2	4.5 ± 0.4	0.25 ± 0.3	1.4 ± 0.3
Extract	1.25	0.42 ± 0.17	4.7 ± 0.3	0.26 ± 0.7	1.6 ± 0.1*
Extract	2.5	0.45 ± 0.21	4.9 ± 0.5	0.26 ± 0.2	1.5 ± 0.2
Control (NS)#	0.2 ml	0.39 ± 0.7	4.8 ± 0.4	0.23 ± 0.5	1.3 ± 0.2

Values are presented as mean ± SE, n=6; *p<0.005 considered to be significant difference when compared with control animals; #NS: Normal Saline

None of the doses of *Abrus precatorius* extract showed toxicity or mortality in the extract treated animals. No significant relative organ weight gain differences were recorded in various groups of animals when compared with the control group. In the humoral antibody titre (HA response), doses of 0.75, 1.25 and 2.5 µg/kg showed the titre value of 387.02 ± 19.76, 154.67 ± 73.17, and 95.33 ± 13.49, respectively, compared to 565.102 ± 78.29 in the non treated mice, indicating a significant inhibitory effect in a dose dependent manner.

Daily administration of aqueous extract of *Abrus precatorius* for 7 consecutive days produced a significant ($p < 0.05$) inhibition in humoral antibody titre at 0.75, 1.25 μg , but no significant effect was observed at the higher dose of 2.5 $\mu\text{g}/\text{kg}$ b.w. (Figure 1).

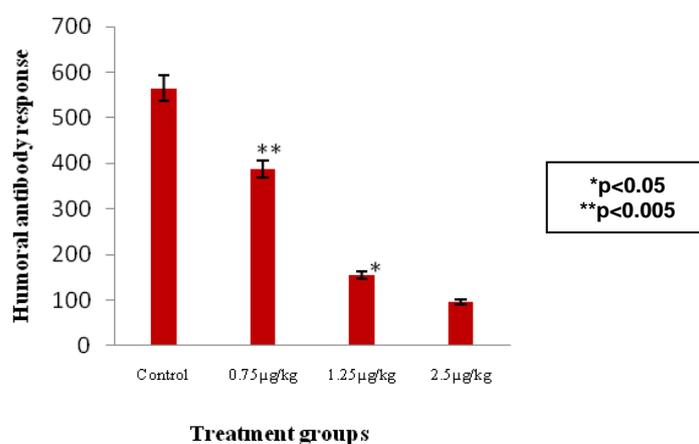


Figure 1. Effect of aqueous extract of *Abrus precatorius* on humoral antibody Response on Swiss albino mice. Values are expressed as mean \pm SEM (n=6).

Daily administration of aqueous extract of *Abrus precatorius* for 7 consecutive days, produced a significant ($p < 0.05$) suppressive change in DTH response of mice to sheep RBC at 0.75, 1.25 μg and 2.5 μg in a dose dependent manner when compared with control animals (Figure 2).

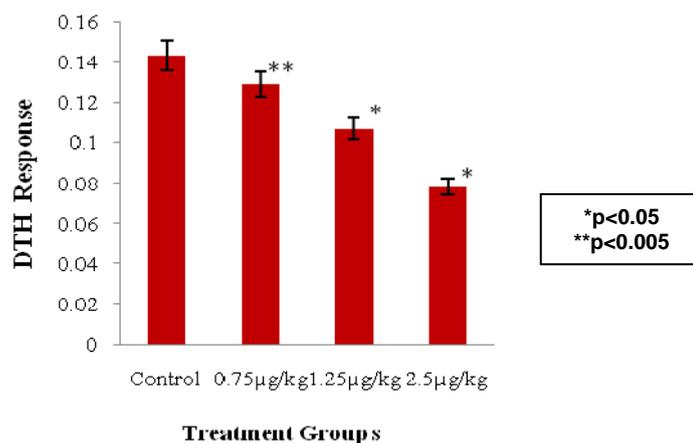


Figure 2. Effect of aqueous extract of *Abrus precatorius* on DTH Response in Swiss albino mice. Values are expressed as mean \pm SEM (n=6).

The results showed a significant inhibition in delayed type hypersensitivity due to exposure of the mice to various doses of the extract.

In order to assess the effect of the extract on phagocytic index, the animals received an intravenous injection of carbon suspension before collection of blood sample.

Result obtained showed a significant increase in the phagocytic index in all the groups treated with *Abrus precatorius* as compared to the control group (Figure 3).

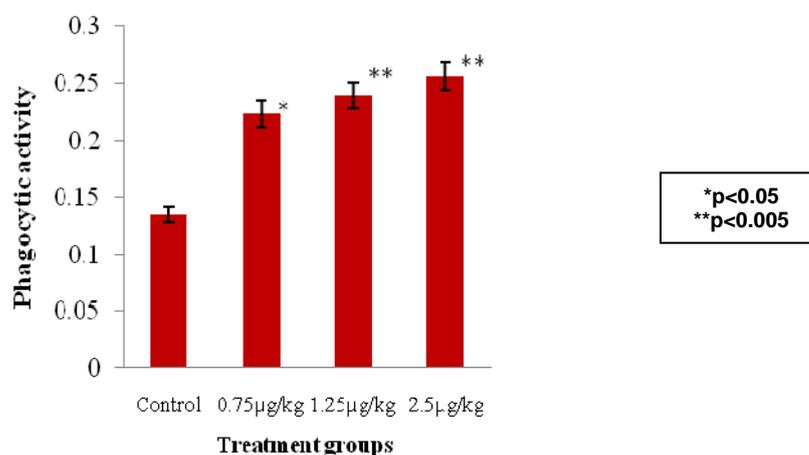


Figure 3. Effect of aqueous extract of *Abrus precatorius* on phagocytic activity in Swiss albino mice. Values are expressed as mean \pm SEM (n=6).

DISCUSSION

Stimulation or suppression of the immune response through may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (17). Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation (18). The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such products, if well tolerated by the patient may be developed into alternative co adjuvants in the treatment of disorders caused by an exaggerated unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc. (19).

In the present study, *Abrus precatorius* showed an overall inhibitory effect on immune response in mice and no significant increase in relative organ weight was observed. This may be due to the inhibitory effect of the extract on the immune system. The suppressive effects were observed on both humoral and cellular immunity. The inhibitory effect on humoral immune response to SRBC as evidenced by a decreased antibody titre in mice, could be due to the suppression of antigen processing and presentation, or might be influenced by the relative amounts of different cytokines produced upon T cell and B cell stimulation (20). SRBC's induced delayed type hypersensitivity was used to assess the effect of the extract on cell mediated immunity. Result of our study showed that injection of the extract at all the doses reduced the immune reaction to SRBC as a T cell dependent antigen, indicating that the extract could affect cellular component of the local immune reaction including lymphocytes

and /or monocytes recruited at the site of interaction (21). Cell mediated immunity (CMI) responses are critical to defence against infectious organisms, infection of foreign graft, tumor immunity and delayed type hypersensitivity reactions (22). The phagocytic index was measured by carbon clearance method. The inhibitory effect of the reticulo endothelial system and the activity of macrophages were evident from the carbon clearance assay (23). In this in vivo assay macrophages are known to secrete a number of cytokines, which in turn stimulate other immunocytes. This may enhance the defence against the infectious stress. This seems to be the general way in which particulate matter is cleared from the blood (24), which indicates significant enhancement in the phagocytic function of the macrophages and thus, non specific immunity. Phagocytosis by macrophages is important against smaller parasites and its effectiveness is markedly increased by opsonisation of the parasites with the antibody and complement c3b.

Further, it is concluded that aqueous extract of *Abrus precatorius* has the capacity to inhibit specific immune response i.e. delayed type hypersensitivity and antibody titre and is also effective against non-specific immunity as evidenced by the phagocytic activity.

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