

IMMUNOMODULATORY ACTIVITY OF AQUEOUS EXTRACT OF *AMORPHOPHALLUS CAMPANULATUS* TUBER

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Abstract: Traditionally *Amorphophallus campanulatus* (AC) tuber is used for tumor, rheumatoid arthritis, carminative and liver tonic. It is also used in the treatment of piles and given as the restorative in dyspepsia debility, Anti-inflammatory, antihaemorrhoidal, haemostatic, expectorant, digrstaive and anthelmintic. In present study effect of aqueous extract of *Amorphophallus campanulatus* tuber on immunological function in mice was studied. The Aqueous extract (AE) of *Amorphophallus campanulatus* tuber, given orally at the doses of 250 and 500 mg/kg showed a significant results of test parameters viz., charcoal clearance, spleen index and delayed type hypersensitivity (DTH) response. In mice immunized with sheep RBC, aqueous extract of AC decreases the charcoal clearance rate and the cellular immunity by facilitating the footpad thickness response to sheep RBC in sensitized mice.

Key word - *Amorphophallus campanulatus*, Immunomodulatory activity

INTRODUCTION

Modulation of immune responses to alleviate diseases has been of interest for many years and the concept of 'Rasayana' in Ayurveda is based on related principles. Immunostimulation in a drug-induced immunosuppression model and immuno-suppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation [1]. Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes, hence are called immunomodulatory agents [2]. The most important breakthrough is the development of adjuvants to be used in vaccination programs or immunosuppressants which can be safely used in organ transplant cases. These basic areas of immunomodulators are currently receiving adequate attention. A number of plant products are being investigated for immune response modifying activity [3].

The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs in cases where synthetic medicine could not relieve patients who suffer from hard-to-cure illnesses. *Amorphophallus campanulatus*, belonging to Family Araceae, locally known as Suran, is a perennial herb with rounded tuberous corm that is widely distributed in India, Bangladesh, and Africa [4]. The tuberous roots of the plant are used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma and rheumatism [5]. The tuberous roots of the plant also have tonic, stomachic and appetizer properties [6]. Some of its traditional uses, such as in the treatment of tumors and enlargement of spleen and rheumatism, have indicated that it might also possess immunomodulatory activities, which have not been explored till to day. Therefore, the present study was designed to determine immunomodulatory activity of the aqueous extract of tuberous roots of *Amorphophallus campanulatus*.

MATERIALS AND METHODS

Collection and authentication of plant material:

The fresh tubers of *Amorphophallus campanulatus* were collected in the months of Dec - Jan from the local market of Tambaram town, Chennai. Authentication of plant was done by Dr. Jairaman, Professor of Botany, Tambaram.

Extraction of plant material: Tuber part of *Amorphophallus campanulatus* was cut into small pieces and dried in oven at 40 to 50 °C and after grinding coarsely was subjected to maceration in aqueous media for 24 hours. The percentage yield was found to be 23.3%.

Animals and treatments: Wistar albino mice of either sex weighing between 18-22 g were used for the present investigation. Animal ethical committee approved experimental protocol under guidelines of CPCSEA (iaec/26/2007), New Delhi. The animals were housed in polypropylene cages at room temperature with a 12 hours day-night cycle for 2 weeks with food and water ad libitum. The aqueous extract was used for pharmacological screening.

Albino mice were divided into four groups and each group was immunized with 2% 0.2 ml (1×10^6 /ml) (v/v) SRBC. In each immunized group six mice were taken for assessing delayed type hypersensitivity response and charcoal clearance test. The groups were treated as follows: Group 1: vehicle treated control group, group 2: aqueous extract of *Amorphophallus campanulatus* treated group (250 mg/kg), group 3: aqueous extract of *Amorphophallus campanulatus* (500 mg/kg), group 4: STD (cyclosporine 5 mg/kg) treated group.

Charcoal clearance test: Albino mice (6-8 weeks old) weighing 18-22 g were randomized into four groups and fed with extract of *Amorphophallus campanulatus* for 15 days at the same dosages as the above. After the last dosage was given, charcoal particle clearance test was performed as per the method given by Sheng et al. [7] and Farong et al. [8].

Each mouse was injected with diluted Indian ink through its tail vein, 20 μ l whole blood was sampled from the medial canthus of each mouse at the 2nd and 10th min respectively, to it 2 ml of 1% Na₂CO₃ was added to it and the absorbance at 680 nm was determined. The spleen indices were determined

from the weights of organs of mice surviving up to 15 days. On the 15th day of treatment, 6 mice from each of the four groups were sacrificed and the spleen was recovered and weighed.

Delayed type hypersensitivity response: This was done as per the method of Johrapurkar et al. [9]. Albino mice were treated with 250 mg/kg and 500 mg/kg dose of the extract of *Amorphophallus campanulatus* respectively. During the 15 day process of continuous oral feeding, a dosage of 2 % 0.2 ml (1×10^6 /ml) (V/V) SRBC was injected IP to each mouse on the 10th day. Four days later, another dosage of 20 μ l (1×10^6 /ml), 20 mol/L (V/V) of SRBC was injected subcutaneously at the measured site of each mouse. Forty-eight hours after injection, thickness of the left plantar of each treated mouse was measured with the help of Plethysmometer with respect to % increase in volume.

Statistical analysis: All the values are expressed in terms of Mean \pm S.D. of (n=6). The experimental results were compared with control. ** p < 0.01 significant and ^{ns}p>0.05, Non significant. One-way ANOVA followed by Dunnett's test.

RESULTS

Table 1 and figures 1 to 3 Show the changes in charcoal clearance index, spleen index and DTH response while comparing with the control group. Study shows a decrease in the charcoal clearance index with 250 mg/kg AE of *Amorphophallus campanulatus* but the higher dose (500 mg/kg) of extract reduces the clearance index significantly as compared to control. Spleen index also decreased in case of AE of *Amorphophallus campanulatus* treated group and even the dose of 500 mg/kg showed a significant reduction in the spleen index as compared to control. In case of DTH response there is decrease with both the doses of AE of *Amorphophallus campanulatus*. However, it is significant with 500 mg/kg dose when compared with control group. The tuber extract demonstrated the ability to influence the immunologic capacity of mice. Administration of 250 mg/kg and 500 mg/kg dose of *Amorphophallus campanulatus* extract for 15 days resulted in significantly reduction of antigen phagocytosis, spleen index, DTH response in the treated groups as compared with the control group, as indicated by decrease in the clearance index of carbon (for details see table 1 and Figs. 1-3).

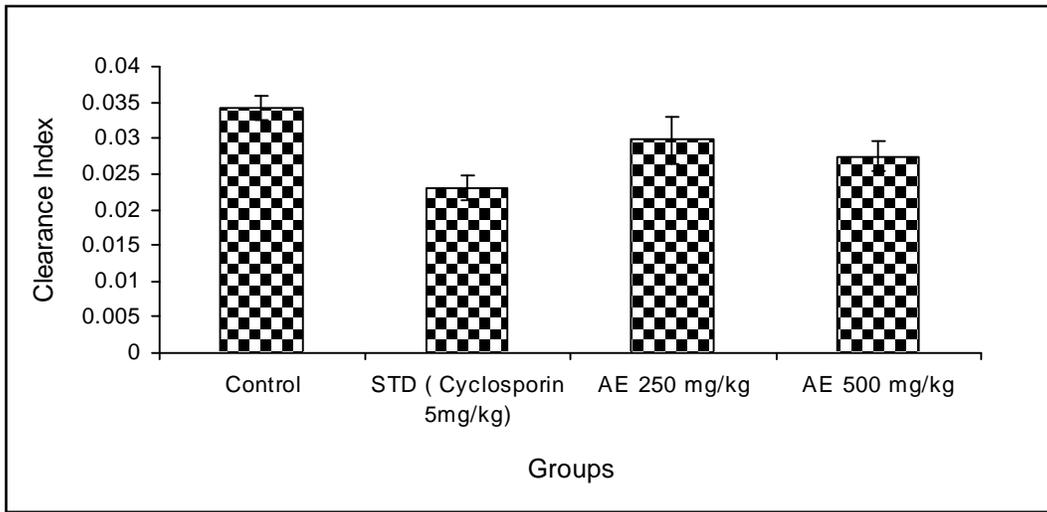


Fig.: Effect of *Amorphophallus campanulatus* extract on charcoal clearance index to antigenic challenge by sheep RBCs in mice.

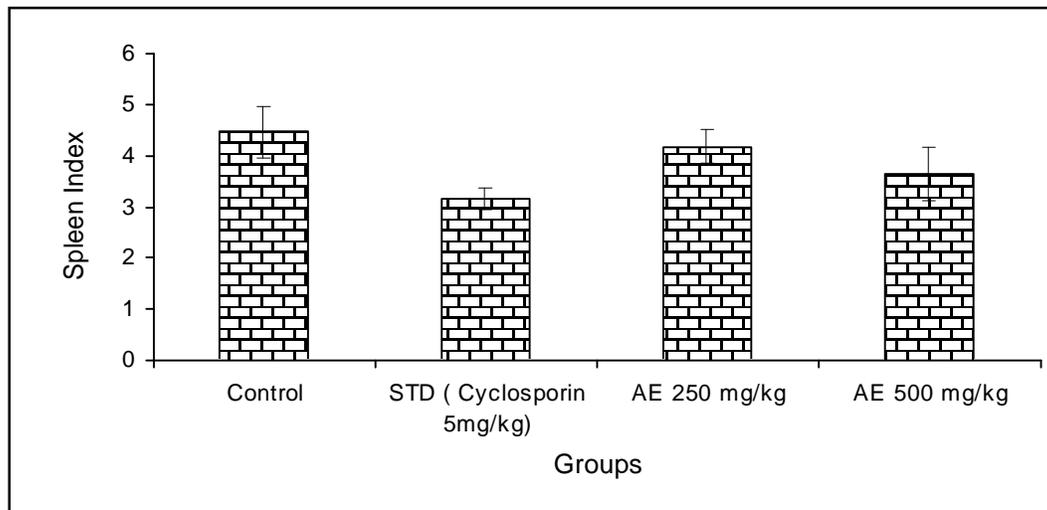


Fig.2: Effect of *Amorphophallus campanulatus* extract on spleen index to antigenic challenge by sheep RBCs in mice.

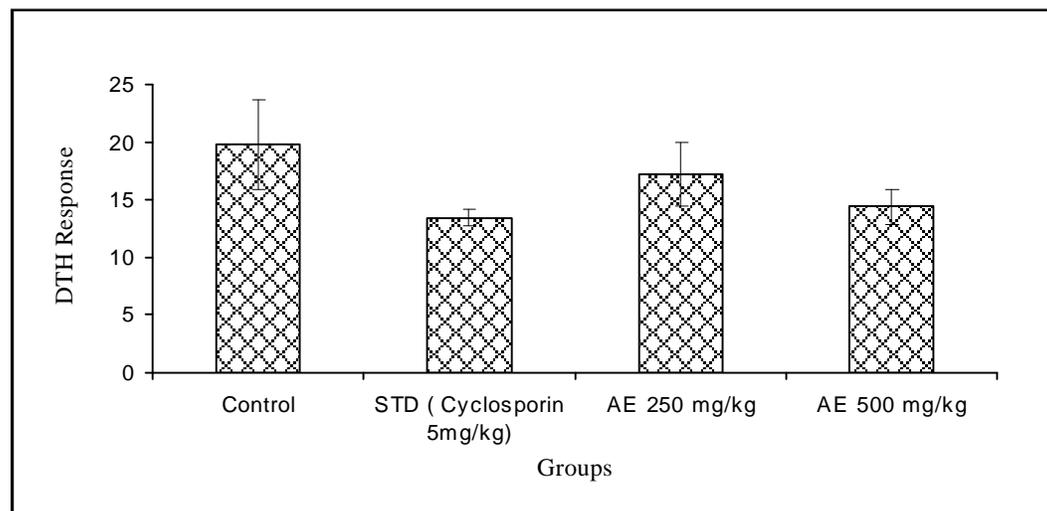


Fig. 3: Effect of *Amorphophallus campanulatus* extract on DTH response to antigenic challenge by sheep RBCs in mice.

Table 1: Effect of *Amorphophallus campanulatus* extract on charcoal clearance index, spleen index and DTH response to antigenic challenge by sheep RBCs in mice.

Sr. no	Groups	Clearance index	Spleen index	DTH response
1	Control	0.0342 ± 0.0016	4.458± 0.505	19.78± 3.92
2	STD (cyclosporin)	0.0230 ± 0.0017**	3.155± 0.22**	13.41 ± 0.74**
5	AE 250 mg/kg	0.0297 ± 0.0033 ^{ns}	4.178± 0.33 ^{ns}	17.25 ± 2.75 ^{ns}
6	AE 500 mg/kg	0.0275 ± 0.0021**	3.647± 0.51**	14.42± 1.49**

DISCUSSION

The study was undertaken in order to evaluate the immunomodulatory activity of aqueous extract of *Amorphophallus campanulatus* tuber. Active phagocytosis is the major defense mechanism against infection. The clearance rate of granular foreign bodies from the circulation reflects the phagocytic function of mononuclear macrophages. When mice were treated with the *Amorphophallus campanulatus* extracts, decrease in clearance index was observed as compared to control group. Studies showed that clearance index decreased significantly in group treated with dose of AE 500 mg/kg as compared to AE 250 mg/kg treated group.

Spleen filters or purifies the blood and lymph fluid that flows through it. When the spleen is damaged or removed, the individual is more susceptible to infections, so for determining the immune system spleen index is determined. Present study showed that there was decrease in spleen index in groups treated with aqueous extract of *Amorphophallus campanulatus*, it was observed that decrease was significant in group treated with AE 500 mg/kg as compared to AE 250 mg/kg.

The DTH response, which is a direct correlate of cell mediated immunity (CMI), in present study it was found to be decreased by the extract of *Amorphophallus campanulatus* as compared to control group. The results are significant with both the doses of extract.

In conclusion, the results obtained in the present study have shown the immunomodulatory activity of aqueous extract *Amorphophallus campanulatus in vivo*. Further study is warranted for understanding the exact mechanisms responsible for immunomodulatory activity, which is in progress.

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