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Research Article

Immunomodulatory Activity of the Methanol Extract of *Amorphophallus campanulatus* (Araceae) Tuber

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Abstract

Purpose: *Traditionally, Amorphophallus campanulatus tuber is used for the treatment of enlarged spleen, rheumatism and tumour. Therefore, this study was designed to evaluate the immunomodulatory activity of the plant material.*
Method*: The effect of the methanol extract (ME) of Amorphophallus campanulatus tuber on immunological function in mice was studied using charcoal clearance, spleen index and delayed-type hypersensitivity (DTH) response models. The extract was administered orally at doses of 250 and 500 mg/kg.*
Results: *The extract exhibited immunomodulatory activity by causing a significant decrease in charcoal clearance, spleen index and delayed-type hypersensitivity (DTH) response.*
Conclusion: *Amorphophallus campanulatus caused decreases in the charcoal clearance rate and cellular immunity by facilitating the footpad thickness response to sheep red blood cell (RBC) in sensitized mice.*

Keywords*: Amorphophallus campanulatus; immunomodulatory activity, Charcoal clearance, Spleen index, Delayed-type hypersensitivity response.*

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INTRODUCTION

The majority of people in developing countries still rely on herbal medicines to meet their health needs, especially in cases where synthetic medicines cannot provide relief from hard-to-cure illnesses. *Amorphophallus campanulatus* (Roxb.) Bl. (Family: Araceae), locally known in India as *Suran*, is a perennial herb with rounded tuberous root stock (corm) that is widely distributed in India, Bangladesh, and Africa. The tuberous roots of the plant are used traditionally for the treatment of piles, abdominal pain, tumours, enlargement of the spleen, asthma and rheumatism[1]. The roots of the plant also possess tonic, stomachic and appetizer properties[2]. The tuber has been reported to have antiprotease activity[3], antibacterial, antifungal and cytotoxic as well as analgesic[4] activities. Some of its traditional uses, including treatment of tumours, enlarged spleen and rheumatism suggest that the tuberous roots of the plant might possess immunomodulatory activity. However, the roots of the plant have not yet been explored for their immunomodulatory activity; hence, the present study was carried out to determine the immunomodulatory activity of the methanol extract of the tuberous roots of *Amorphophallus campanulatus.*

EXPERIMENTAL

Plant material

The fresh tubers of *Amorphophallus campanulatus* were collected from the local market of Tambram, Chennai, India. Authentication of the plant was by ***Dr*** P ***Jayaraman,*** a taxonomist of the Plant Anatomy Research Centre, Tambaram, Chennai, India. A voucher specimen (no. PARC-2007-87) was deposited at the herbarium of the Plant Anatomy Research Centre, Tambaram, Chennai, India.

Extraction of plant materials

The Tuber of *Amorphophallus Campanulatus* was cut into small pieces and dried in an oven at 40 – 50 ℃. After coarse grinding, it was subjected to maceration in methanol for 5 days, the extract obtained by vacuum evaporation and the yield determined. phytochemical screening was carried out using conventional methods [5].

Animals

Wistar albino mice of either sex (18 - 22 g, 6 - 8 weeks old) were used for the study. The Animal Ethical Committee approved the experimental protocols based on the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals, New Delhi, India) and the Institutional Animal Ethical Committee (approval ref no. IAEC/26/2007),. The animals were housed in polypropylene cages at room temperature with a 12-h day/light cycle for 2 weeks with food and water *ad libitum* prior to the animal studies.

Immunomodulatory Tests

The mice were divided into four groups of six mice each. The mice were immunized with 0.2 ml of 2 %v/v (1×10^6/ml) sheep RBC (SRBC) for the assessment of delayed-type hypersensitivity (DTH) response and charcoal clearance test.. Group I was the control group and was treated with vehicle (water : Tween 80, 80:20); Group II received 250 mg/kg of the extract; Group III received 500 mg/kg of the extract; and Group IV received the reference standard (SD), i.e., cyclosporine (5 mg/kg)

Charcoal clearance test

The mice were randomized into the treatment groups as outlined above. The animals were administered the extract or standard drug for 15 days. After the last dose, charcoal particle clearance test was performed. Briefly, each mouse was injected with 1:50 diluted Indian

ink in a dose of 1 mL/100 g body weight through its tail vein, and 20µl whole blood was sampled from the *medial canthus* of each mouse at the 2nd and 10th minute. Two millilitres of 1 % Na_2CO_3 was added to the sampled blood and absorbance was determined at 680 nm [6,7]. Charcoal clearance index (K) was calculated as in Eq 1.

K = (lgA2 - lgA10)/(t2 - t1) …….. (1)

where lgA2 and lgA10 are the optical densities at 2 min and 10 min, respectively.

Spleen index

Spleen index was determined from the weight of the spleen of the mice surviving up to 15 days. On the 15th day of treatment, 6 mice from each of the four groups were sacrificed and the spleens were recovered and weighed. The results are expressed as the organ index using the formula: *weight of spleen (mg)/body wt (g)* [8].

Delayed-type hypersensitivity response test

The mice were treated with the extract at a dose of either 250 or 500 mg/kg/day for 15 days. On the tenth day, 0.2 ml of 2 %v/v (1×10^6/ml) SRBC was injected intraperitonially into each mouse. Four days later, another dose of 20 µl, 2 %v/v (1×10^6/ml) of SRBC was injected subcutaneously into the left plantar tissue of each mouse paw. Forty-eight hours after injection, the thickness of the left paw of each treated mouse was measured with a plethysmometer [9]. Swelling was calculated as in Eq 2 while inhibition was derived as in Eq 3,

Swelling (%) = (V-Vi/Vi) ×100 …….. (2)

where Vi is the mean initial volume of mercury rise in the plethysmometer and V is the mean final volume of mercury rise in the plethysmometer.

Inhibition (%) = {(1 – DG)/CG} x 100 ……. (3)
where Dg is the swelling of the drug-treated group and CG is the swelling of the control group.

Statistical analysis

All the results were expressed as mean ± standard deviation (SD).and the data were analyzed using one-way analysis of variance (ANOVA) with Dunnett's post test. *P*-values (*$p < 0.05$, **$p < 0.01$) were in comparing the test data with control to determine statistically significant differences.

RESULTS

The extract yield was 10 %w/w while phytochemical screening indicated the presence of steroids and flavonoids.

The effect of the extract of *Amorphophallus campanulatus* on phagocytic activity, spleen index and DTH response is shown in Table 1. The phagocytic activity of the reticulo-endothelial system is generally measured by the rate of removal of carbon particles from the blood stream. All the extract-treated groups exhibited significantly low clearance index compared with the control group. 500mg/kg dose producing a lower value than 250 mg/kg ($p<0.05$). This indicates depression of the reticuloendothelial system.

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

Group	Clearance index	Spleen index	DTH response
Control	0.034 ± 0.002	4.46 ±0.51	19.78 ± 3.92
Cyclosporin (5 mg/kg)	0.023 ± 0.002**	3.16 ±0.22**	13.41 ± 0.74**
ME (250 mg/kg)	0.029 ± 0.001*	3.96 ±0.10^{ns}	15.5 ± 1.97*
ME (500 mg/kg)	0.024 ± 0.00**	3.46 ±0.26**	12.23 ± 1.91**

*Values are mean ± SD (n = 6); *p < 0.05; ** p < 0.01 compared to control; ME = extract*

Spleen index was lower at the extract dose of 500 mg/kg than at 250 mg/kg with the index significantly higher for control. DTH response was also lower in the extract-treated group than in the control group but the difference was only significant ($p<0.05$) at the extract dose of 500 mg/kg.

DISCUSSION

The study was carried out in order to evaluate the possible immunomodulatory activity of methanol extract of *Amorphophallus campanulatus* tuber. Active phagocytosis is the major defense mechanism against infection. The clearance rate of granular foreign bodies from circulation reflects the phagocytic function of mononuclear macrophages. Pretreatment of mice with the *Amorphophallus campanulatus* extract resulted in a significant decrease in clearance index compared to the control group., The higher dose of the extract (500 mg/kg) caused a significantly greater decrease in clearance index than the group treated with 250 mg/kg.

Spleen serves as a reservoir for blood and filters or purifies the blood and lymphatic fluid that flows through it. When the spleen is damaged or removed, the individual is more susceptible to infections, and hence spleen index is a suitable parameter for monitoring immune system function. The present study showed a decrease in spleen index in groups treated with the extract, compared to the control group.

The DTH response, which is a direct correlation of cell-mediated immunity (CMI), was decreased to a greater extent by both doses of the extract compared to the control group. However, the higher dose caused a more significant reduction in DTH response than the lower dose.

CONCLUSION

The methanol extract of *Amorphophallus campanulatus* tuber significantly and dose-dependently suppressed the immune system in mice. Further studies are required to confirm this preliminary finding, which may provide the rationale for the ethnopharmacological uses of this plant.

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