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ANTI VENOM AND IMMUNOMODULATORY FUNCTIONS OF *CORALLOCARPUS EPIGAEUS* L.

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ABSTRACT

Venom neutralizing activity of crude root extract of *Corallocarpus epigaeus* was determined by *in vivo* procedure recommended by WHO. Neutralization assays for antivenom is necessary to replace the *in vitro* neutralization assay. Albino mice were used to estimate venom neutralizing activity of tuber extract. *Corallocarpus epigaues* tuber extract preparations exhibit immunomodulatory properties. This extract strongly induces the proliferation of PBMC. The stimulatory potency of tuber extract for PBMC, from two different groups was examined. The present findings indicate that a mitogen associated antigen from *C. epigaues* tuber extract is able to activate PBMC from healthy and allergic individuals, thereby demonstrating sensitization to probably highly conserved plant antigens.

KEYWORDS: ***Corallocarpus epigaues*, Tuber extract, Cobra venom, Albinomice, Neutralization, Mitogens, Lymphocyte proliferation.**

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INTRODUCTION

In rural and tribal medical practice many tuberous plants are used to treat snake bite victims. Favorable response to these extracts was observed in some patients suffering from syphilitic and chronic rheumatism[1]. The immunomodulatory properties of *C. epigaeus* tuber extract preparations have been intensively studied during the last few years. *C. epigaeus* is used in the treatment of chronic rheumatism[2], snake bite[3], asthma[4], dysentery and syphilitic disorders[5]. Petroleum and ether, chloroform, acetone and methanol extracts of *C. epigaeus* leaf, stem and tuber exhibited antibacterial activity against various pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*[6]. The present study is aimed as studying the immunomodulatory properties of purified fractions of *C. epigaeus*. In view of the venom neutralizing functions reported earlier we examined the immunomodulatory properties of *C. epigaeus* root extract using lymphocyte transformation test. Preliminary results revealed that some of the fractions of tuberous root extract stimulate the human T-cell transformation. This has encouraged us to evaluate the effect of tuber extract on the expression of humoral and cell mediated immune responses in albino mice to sheep-red blood cells (SRBC). Thus tuber extract significantly enhances human active T-cell rosette formation *in vitro*. In view of this observation we have planned to demonstrate cytokine production (IL-2) with the influence of this tuber extract (unpublished data).

These medicinal plants are not scientifically explored for its therapeutic efficacy and immunomodulatory functions. *C. epigaues* tuber extracts are a mixture of different components, such as proteins, glycoproteins (e.g. lectins), oligo and polysaccharides and others[7]. Compounds like bioflavonoids present in the extract may be responsible for the anti-inflammatory action because of decreasing capillary permeability[8]. The flavonoids have been reported to produce several anti-inflammatory effects[9]. Snake bite could spell death to countless victims and there are no antidotes available. Polyvalent snake venom serums (PSVS) contain thousands of antibodies, which have been extracted from RBC and also from horse blood[10]. Polyvalent venoms from commercial laboratories have large quantities of poorly catabolized foreign antigens. Most of the antivenom against snakes are extracted from horse blood. During preparation and purification of antivenom factor from horse blood, large quantities of horse serum proteins are also present. These proteins cause serum sickness or systemic type-III hypersensitivity reaction when administered along with anti-snake venoms. Among all these antibodies of the polyvalent snake venom only few mop the snake venom and all remaining horse serum protein cause the victim to suffer from acute allergic reactions. Hence rural and tribal populations are in need of an alternative to the existing antivenom serums. Present finding revealed that tuber extract has an antivenom potency and also shows the proliferative response lymphocytes *in vitro*. These findings raise the question, whether antigens other than aqueous extract factor might have been responsible for the immunomodulating effect or not.

C. epigaeus a creeper, popularly known as 'Nagagadda' is widely distributed in India, Africa and tropical Asia. Tuberous root of *C. epigaeus* is traditionally used as folk medicine for cough, asthma, fever, syphilis is and rheumatoid arthritis because of its therapeutic value[11]. Furthermore, the response to these antigens may differ from one individual to another and may be controlled by genetic factors as shown, for instance, for common bacterial antigens[12]. In the present study we investigated the antivenom factor in the crude tuber extract from the roots of *C. epigaeus* and it was tested for the estimation of antivenom factor involving albino mice *in vivo* neutralization of Indian cobra venom. Present study was also extended to observe proliferative response and was chosen for further analysis in order to evaluate natural immunity to *C. epigaeus* tuber associated antigens. Peripheral Blood

Mononuclear Cells (PBMC) from normal and allergic individuals who never had been treated with any form of preparation was investigated.

MATERIALS AND METHODS

The method used in this study is based on *in vivo* neutralization of common Indian cobra venom as antigen by using horse antivenoms[13]. However, it has been observed that the anti-sera against snake venoms and the antibody contents are estimated by the intensity and number of precipitin bands not correlating with the neutralizing capacities[14]. Venom was collected from common Indian cobra with the help of tribals in Eastern Ghats, Andhra Pradesh. Fresh tubers of *C. epigaeus* were collected from Erramalai hills of Andhra Pradesh (Fig. 1). The tuber has a repute remedy for snake bite when it administered internally and by applying to the bitten part[15]. Fresh tubers (1000g) were thoroughly rinsed in triple distilled water, sliced into small pieces, shades dried and were pulverized. Crude extract was prepared by mixing the pulverizing powder with phosphate buffer saline (PBS, pH 7.2) and was used for neutralization assay. Groups of six mice weighing 25 ± 2g were injected intraperitoneally with cobra venom at doses ranging from 0.16 - 0.61 µg/g mouse. The venom was prepared in normal saline and the volume of injection was kept constant at 0.1 ml/25 g mouse. Control mice were injected with normal saline only. The percentage of mortality of mice was recorded 24 hrs after the injection.

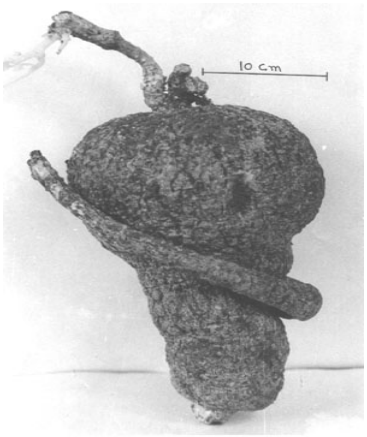

Figure 1
Tuberous root of C. epigaeus (Cucurbitaceae)

Neutralizing activity of crude root extract was determined *in vivo* procedure recommended by WHO[16]. Cobra venom at different concentrations in normal saline 0.2 - 0.8 mg/ml were incubated with equal volume of crude root extract for 1 hr at 37ºC before injection. The volume of injection was kept constant at 0.1 ml/25g mouse. The LD_{50} of the cobra venom was estimated by the method of Litchfield and Wilcoxon[17].

$$\text{Neutralizing activity} = (LD^{1}_{50} - LD_{50}) \frac{\text{Weight of the mouse}}{\text{Volume of crude root extract}}$$

Where LD_{50} - Medium lethal dose of cobra venom alone.
LD^{1}_{50} - Medium lethal doses of the venom and crude root extract.

Proliferative response was analysed by isolating PBMC from two groups. PBMC were isolated by Ficoll density centrifugation as described earlier[18]. Cells were adjusted to 2 x 10^6 cells/ml suspended in RPMI 1640 (Gibco) supplemented with penicillin and Streptomycin at 0.1 mg/ml (Sigma, USA). Different concentrations of the tuber extract were added ranging from 10 to 10,000 µg/ml (final concentration corresponding to the weight of the tuber) with PBMC and were incubated for seven days in 96 wells flat bottom microtiter plates (Tarson, India) in a humidified 5% CO_2 atmosphere at 37°C. Sixteen hours before harvesting 10 µl of ^{3}H-thymidine (BARC, Bombay, India) was added to determine the proliferative response. Samples were counted in a liquid scintillation counter. Stimulation indices (Si) were expressed as Si = Cpm with extract / without extract. All experiments were performed in triplicates.

RESULTS

Neutralizing activity of tuberous root extract

The average neutralizing activity was 311.3 µg/ml. It is clear for various reasons that *in vivo* neutralization assay for antivenom potency is necessary to replace the *in vitro* neutralization assays. Various immunoassays were based on indirect haemagglutination, radioimmuno assay, ELISA (Enzyme linked immunosorbent assay) and gel diffusion[19].

Evaluation of immunomodulatory potency of purified and crude tuber extracts in cultures of healthy PBMC and allergic individuals

Differences in the immunomodulatory effects of *C. epigaeus* tuber extracts were analyzed in atopic individuals who had repeatedly shown pronounced cellular immune reactions. Purified tuberous root extract of *C. epigaeus* induced the highest proliferative response of PBMC in allergic patients. Stimulation indices up to 55 were obtained with purified tuber extract. The other extracts only gave a weak response with indices ranging from 1-20 (Fig. 2).

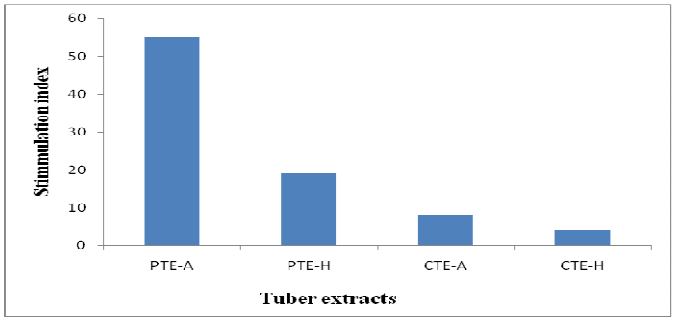

Figure 2

Stimulation of PBMC by Corallocarpus epigaeus tuber extracts purified and crude preparations (PTE, Purified tuber extract and CTE, Crude tuber extact) stimulation index was calculated as Cpm with extract/Cpm without extract. Purified tuber extract (PTE) induced the highest response of PBMC from allergic individuals and the crude extract produced slight stimulation

PTE-A - Purified Tuber extract on Allergic lymphocyte
PTE-H - Purified tuber extract Healthy lymphocytes
CTE-A - Crude Tuber extract on Allergic lymphocytes
CTE-H - Crude Tuber extract on Healthy lympyhocytes

In order to obtain more information about the immunomodulatory functions of PTE a large group of individuals (n=74) were examined (Fig. 3). Weak reactivity with stimulation indices up to 6 was observed in most instances in individuals of group I. Therefore, a Stimulation Indices (SI) of 6 is still normal, it was in controls (Group I) and 80% of the 24 individuals with allergic manifestations (Group II) had PBMC, which proliferate strongly in the presence of purified tuber extract.

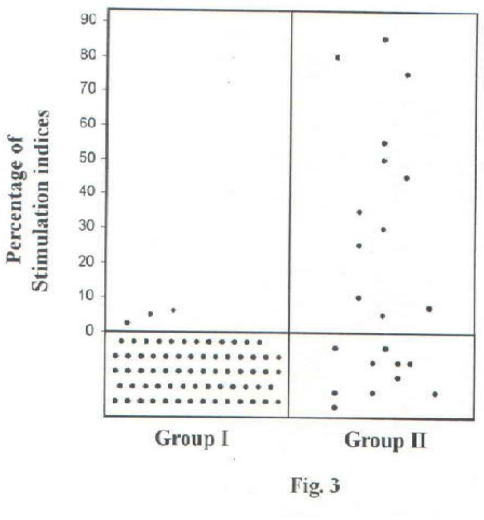

Figure 3

Reactivity of PBMC from normal controls (group I) patients with allergic manifestations (group II) to the purified C. epigaeus tuber extract stimulation indices were given at the optimal and purified tuber extract concentrations. The highest indices were detected in group II individuals. In normal controls indices the index berely exceeded 6, so, this value was taken as the upper limit of the normal range.

In present study we tried to evaluate the natural reactivity to tuber antigens in healthy controls and allergic individuals. After testing at two extracts of tuber it became evident that the purified tuber extract was only one, which caused a strong proliferative response in 7 day cultures of PBMC.

DISCUSSION

In vivo neutralization of common Indian cobra venom by using tuberous root extract was deceptively simple, economical and is very useful for assessment of antivenom potency in crude tuber extract, especially when a large number of samples are involved. Lyophilized and freeze dried tuber extract had saved many snake bite victims. The factor that PBMC from patients with non allergic responses to exogenous antigens particularly, to recognize purified tuber extracts related may indicate that, challenge with these conserved plant protein antigens permitted the demonstration of heightened natural immunity. This concept is further substantiated by other reports showing immunostimulatory activities of other plant extracts such as those from *Echinacea purpurea*[20] and *Thuja occidentalis*[21] in humans. The data obtained by measuring cytokines in the supernatants of mitogen stimulated PBMC suggest that a subpopulation of T-helper cells (T_H) namely T_H2 cells may have been preparatively activated[22]. T_H2 cells have been shown to be involved in the maintenance of natural immunity[23]. Further investigations and immunomodulatory functions of *C. epigaeus* crystallysed tuberous extract fractions are in progress.

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