Immunomodulatory Potential of *Asparagus racemosus* Wild and *Chlorophytm arundinaceum* Baker in Wistar rats

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The aim of present study was to evaluate immunomodulatory potential of ethanolic extract of *Asparagus racemosus* Wild. and *Chlorophytm arundinaceum* Baker. in comparison to established immunosuppressant *Cedrus deodara* wood oil (100 mg kg\(^{-1}\) b.wt.) in mice. The assessment of immunomodulatory activity on specific and nonspecific immunity was studied by Delayed Type Hypersensitivity (DTH) and neutrophil adhesion test respectively. Through Neutrophil adhesion test maximum sensitivity was observed in *in vivo* extract of *C. arundinaceum* while percent inhibition was maximum in *in vitro* extract of *A. racemosus*. *In vivo Chlorophytm arundinaceum* extract at 300 mg kg\(^{-1}\) dose statistically significantly inhibit the adhesion of neutrophils to nylon fiber. However, *in vivo A. racemosus* at the dose of 300 mg kg\(^{-1}\) was found to significantly decreased rat paw edema as compared to control, *in vivo C. arundinaceum* and *in vitro A. racemosus* extract. The present study has shown the immunomodulatory activity of both plants by potentiating humoral as well as cellular immunity.

**Key words:** Immunomodulatory, neutrophil adhesion, delayed type hypersensitivity, *Asparagus racemosus* wild, *Chlorophytm arundinaceum* baker
INTRODUCTION

Plant secondary metabolite Saponins posses' immunomodulatory activity due to their haemolytic and adjuvant activity. There are many plants which have immunomodulatory potential because of these saponins viz. Curcuma longa (Al-Sultan, 2003), Asparagus racemosus (Arora et al., 2005), Ficus benghalensis roots (Gabi et al., 2006), Actinidia macropermona (Lu et al., 2007), Ricinus communis Linn, Acacia catechu (Hossain et al., 2008), Hibiscus rosa sinensis Linn (Kolewe et al., 2008), Momordica charantia (Juvekar et al., 2009), Aesculus indica (Chakraborty, 2009a) etc.

Among these plants Asparagus racemosus and Chlorophyllum arundinaceum which are belong to family Asparagaceae also contain steroidal saponins (Hayes et al., 2006; Tandon and Shukla, 1992, 1993, 1995, 1996, 1997; Tandon et al., 1992). In Asparagus racemosus Immunomodulatory potential is identified by Gautam et al. (2009), Romagnani (2000) and Thatte et al. (1987) and in Chlorophyllum arundinaceum by Kumar et al. (2011). The objective of present study was to compare the immunomodulatory activity of the in vivo and in vitro ethanol root extract of Asparagus racemosus and in vivo ethanol root extract of Chlorophyllum arundinaceum.

MATERIALS AND METHODS

Plant material: Dry roots of the plant Asparagus racemosus and Chlorophyllum arundinaceum were obtained, identified and authenticated from standard resources.

Preparation of the extract: The experimental material (50 g) was dried and ground in small particles and placed in a porous cellulose thimble. This material was soxhlet extracted successively with solvents. Asparagus racemosus and Chlorophyllum arundinaceum root powder were defatted with petroleum ether and then extracted with ethanol in a soxhlet apparatus. Extraction was done with each solvent until the supernatant in the soxhlet became transparent (approximately 30 h). Ethanol extract was then filtered, concentrated by distilling off the solvents and evaporated to dryness using water bath to get crude ethanol extract.

Antigen: Sheep's erythrocytes (SRBCs) collected in Alsever's solution, were washed three times with pyrogen-free sterile 0.9% normal saline and adjusted to a concentration of 1×10^7 cells mL⁻¹ for sensitization and challenge.

Animals: Healthy Wistar adult male rats (150-200 g) were used for the study. They were divided into 5 groups of 5 animals each. The dosages of drugs administered to the different groups were as follows:

Group 1: Control (normal saline 20 mL kg⁻¹)
Group 2: Standard: Cedrus deodara wood oil (100 mg kg⁻¹)
Group 3: Test 1 (300 mg kg⁻¹ b.wt.)
Group 4: Test 2 (300 mg kg⁻¹ b.wt.)
Group 5: Test 3 (300 mg kg⁻¹ b.wt.)

Test 1: In vivo ethanol root extract of Asparagus racemosus Wild
Test 2: In vitro ethanol root extract of Asparagus racemosus Wild
Test 3: In vivo ethanol root extract of Chlorophyllum arundinaceum Baker

Neutrophil adhesion test: Neutrophil adhesion test was done by using Wilkinson method (Wilkinson, 1978). The rats of control group I were treated orally with normal saline, group 2 with Cedrus deodara wood oil and test groups 3, 4 and 5 with extracts at the doses of 300 mg kg⁻¹ for 8 days. On the 8th day of treatment, blood samples from all the groups were collected by puncturing the retro-orbital plexus into heparinized vials under mild ether anesthesia. Blood samples were analyzed for Total Leukocyte Count (TLC) using Erma PC -607 cell counter. Differential Leukocyte Count (DLC) was obtained by fixing blood smears and staining with leucofine. After determine percent neutrophil count, blood samples were incubated with nylon fibre (80 mg mL⁻¹ of blood sample) for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The percentage of neutrophils in the treated and untreated blood was determined and the difference was taken as index of neutrophil adhesion. Percentage of neutrophil adhesion was calculated as follows:

Percent neutrophil adhesion was calculated from the following formula:

Neutrophil adhesion (%) = \( \frac{N_u - N_a}{N_u} \times 100 \)

where, \( N_u \) is the neutrophil index of untreated blood sample, \( N_a \) is the neutrophil index of treated blood sample.

Delayed type hypersensitivity (DTH): Delayed type of hypersensitivity response to SRBC was calculated as a measure of paw oedema thickness (mm) (Hajra et al., 2012). Each animal treated with ethanol extract at the concentrations of 20, 300, 300 and 300 mg kg⁻¹ weight and compared with control. After administration of extracts all
groups except control were sensitized with 10% SRBC (1×10^6 cells) at day 0 and day 7 subcutaneously whereas control group was administered with equal volume of PBS (pH 7.4). On 10th day all groups were challenged with 1×10^6 SRBC cells intradermally into the left footpad of each mouse, while PBS (pH 7.4) was injected into right hind paw. An increased in paw oedema thickness was calculated after 27 h by volume differential meter. The difference between pre and post challenge foot pad thickness expressed in mm was taken as a measure of DTH reaction (Hajra et al., 2012) was calculated as the mean percentage increase in paw oedema thickness (plethysmometrically) over the control values. The data obtained was subjected to statistical analysis.

**Statistical analysis:** The collected data of all five groups was analyzed using one way ANOVA with Tukey's Multiple Comparison Test. Data were expressed as Mean±the correspondent standard error of mean (SD) and n = 5.

**RESULTS**

Effect of ethanol extract on neutrophil activation by the neutrophil adhesion test is shown in Table 1. In vivo, in vitro ethanol root extract of Asparagus racemosus and in vivo ethanol extract of Chlorophytum arundinaceum with doses 300 mg kg^-1 b.wt. showed percentage of neutrophil adhesion as 13.92±1.06, 10.26±9.05 and 50.04±4.01. Whereas, in case of control group it was 57.26±1.02 and in standard sample 20.6±16.4. then all five groups were compared by using one way ANOVA with Tukey’s Multiple Comparison Test, there is a significant (F_{2,5} = 31.18 (p<0.0001) difference between these group, Tukey’s test also shows significant (p<0.001) difference among the group. Values are expressed as Mean±SD, n = 5 per group. In brackets, inhibition percentage is reported (Table 2). Asparagus racemosus in vitro extract at 300 mg kg^-1 showed highly significant activity (p<0.001) for DTH response indicates that it significantly inhibit the edema formation induced by SRBC.

**DISCUSSION**

Phytochemical studies of both plants showed presence of saponins which are known as immunostimulatory agents. This was most likely reason of immunomodulatory potential of both plants. Similar trends were observed in Swietenia mahagoni seeds methanolic extracts and Cassia auriculata leaves petroleum ether extract (Chakraborty, 2009b) which contain saponins (Sahgal et al., 2009; Manimekala and Naveen, 2010) shown a significant stimulation of the cell mediated immunity and humoral immunity in Wistar albino rats.

Neutrophil granulocytes form an essential part of the innate immune system, are normally found in the blood stream. In acute phase of inflammation, being part of first line of defense they migrate towards inflammatory site through chemotaxis. In vitro ethanol extract of Chlorophytum arundinaceum showed a significant increase in the neutrophil adhesion to nylon fibres compared to in vitro and in vivo ethanolic extracts of Asparagus racemosus.

<table>
<thead>
<tr>
<th>Dose (mg kg^-1)</th>
<th>TLC (cells mm^-2) (A)</th>
<th>Neutrophil (%) (B)</th>
<th>Neutrophil index (A×B)</th>
<th>Neutrophil adhesion (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UB</td>
<td>NFTB</td>
<td>UB</td>
<td>NFTB</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9.5±0.36</td>
<td>13.92±1.06</td>
<td>10.26±9.05</td>
<td>50.04±4.01</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>11.01±0.96</td>
<td>12.93±1.86</td>
<td>12.71±2.70</td>
<td>42.5±2.70</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>9.75±1.09</td>
<td>10.18±1.07</td>
<td>19.48±2.10</td>
<td>66.0±4.01</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>10.01±1.06</td>
<td>11.89±1.50</td>
<td>19.31±2.06</td>
<td>14.7±2.00</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>10.16±2.07</td>
<td>8.01±1.30</td>
<td>17.2±1.70</td>
<td>11.12±2.19</td>
<td></td>
</tr>
</tbody>
</table>

UB: Untreated blood, NFTB: Nylon fiber treated blood, *p<0.01, **p<0.001, ***p<0.0001
Table 2: Effect of root extracts of *Asparagus racemosus* and *Chlorophyllum arundinaceum* on delayed type hypersensitivity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (mg kg⁻¹ P.O.)</th>
<th>Paw volume at 27h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (pH 7.4)</td>
<td>20</td>
<td>29.12±4.01</td>
</tr>
<tr>
<td>Test 1</td>
<td>300</td>
<td>11.04±3.30 (52.62)</td>
</tr>
<tr>
<td>Test 2</td>
<td>300</td>
<td>14.60±3.29 (45.60)</td>
</tr>
<tr>
<td>Test 3</td>
<td>300</td>
<td>19.09±1.22 (33.90)</td>
</tr>
</tbody>
</table>

P.O.: Paw oedema

This indicates that *in vitro* ethanol extract of *Chlorophyllum arundinaceum* increased the neutrophil chemotaxis towards inflammation site. This may be due to the up-regulation of the β2 integrins that are present on the surface of the neutrophils through which they adhere firmly to the nylon fibres (Hajra et al., 2012). Increase in β2 integrins stimulates the process of migration of cells in the blood vessels. Likewise, ethanol extract, hydroalcoholic root extract of *Chlorophyllum arundinaceum* stimulate both cellular and humoral immune response in Swiss albino mice (Kumar et al., 2011).

Type IV hypersensitivity is often called delayed type hypersensitivity correlated with CD8+ cytotoxic T cells and CD4+ helper T cells, induces cell mediated response. The histology of DTH can be different for different species, but the general characteristics are that when antigens are challenged T-cells, sensitized T-lymphocytes to convert lymphoblasts and secrete cytokines including Interleukin-2, Interferon, macrophage migration inhibition factor and tumor necrosis factor. This will attract more scavenger cells such as macrophages and basophiles within 24-72 h in rats. This process is same in human and mice.

Administration of ethanol *in vitro* extract of *Chlorophyllum arundinaceum* and *in vitro* root ethanol extract of *Asparagus racemosus* enhanced DTH reaction, which is measured from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site. Decrease in paw volume in the mouse treated with *in vitro* extract of *Asparagus racemosus* suggest that it was effective to suppress delayed type hypersensitivity reactions induced by SRBC in mice with high intensity compared to *in vitro* *A. racemosus* and *Chlorophyllum arundinaceum* extract. These results were also supported by previous results of Gautam et al. (2009) in which *Asparagus racemosus* root aqueous extract up-regulate CD3 and CD4/CD8 positive percentages in peripheral blood.

These results may be helpful in conventional chemotherapy in development of new drugs.

**CONCLUSION**

In the present study, ethanol *in vitro* extract of *Asparagus racemosus* and *Chlorophyllum arundinaceum* showed immunomodulatory activity which strengthens the ancient texts of ayurveda claiming immunomodulatory potential of these plants. Further experimentation is required to check effectiveness of various fractions of the extract and comparison of immunomodulatory potential of *in vitro* and *in vitro* extracts.

**REFERENCES**


