Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 12 (7): 214-218
1st October, 2012
DOI: 10.3923/jms.2012.214.218

Immunomodulatory Potential of *Asparagus racemosus* Wild and *Chlorophytum 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 *Chlorophytum 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 Chlorophytum 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. arundinaceumand 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., *Chlorophytum arundinaceum* baker

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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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 (Gabhe *et al*., 2006), *Actinidia macrosperma* (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* (Chakraborthy, 2009a) etc.

Among these plants *Asparagus racemosus* and *Chlorophytum 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 *Chlorophytum 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 *Chlorophytum arundinaceum*.

MATERIALS AND METHODS

Plant material: Dry roots of the plant *Asparagus racemosus* and *Chlorophytum 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 *Chlorophytum 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 1×10^8 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 *Chlorophytum arundinaceum* Baker

Neutrophil adhesion test: Neutrophil adhesion test was done by using Wilkinson method (Wilkonson, 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 day^{-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:

$$\text{Neutrophil adhesion (\%)} = \frac{N_{lu} - N_{lt}}{N_{lu}}$$

where, N_{lu} is the neutrophil index of untreated blood sample, N_{it} 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 (1×10^8 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 1×10^8 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 20.64±16.4. then all five groups were compared by using one way ANOVA with Tukey's Multiple Comparison Test, there is a significant ($F_{4,20}$= 31.18 ($p<0.0001$) difference between these group, Tukey's test also shows significant ($p<0.001$) difference among the group. Results are expressed as Mean±SD from five.

In vivo Chlorophytum arundinaceum extract at 300 mg kg^{-1} dose statistically significantly inhibit the adhesion of neutrophils to nylon fiber. Percentage inhibition was higher for *in vitro* extract of *Asparagus racemosus*.

Among all the samples *in vitro* extract of *Asparagus racemosus* was found to suppress delayed type hypersensitivity reactions induced by SRBC in mice with high intensity 11.04+3.30% compared to *in vitro A. racemosus* and *Chlorophytum arundinaceum* extract. Foot volume was enhanced 19.09+1.22% after ethanolic *in vitro Chlorophytum arundinaceum* extract treatment and suggesting cell mediated immune response enhancement by SRBC. Significant decrease (33.90) in DTH reaction indicates that the extract of *in vitro Chlorophytum arundinaceum* is unable to stimulate the macrophage function to stimulate T cells for the hypersensitivity reaction. all four group were compared by using one way ANOVA with Tukey's Multiple Comparison Test, there is a significant (F3,16 = 31.22 ($p<0.0001$) 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 (Chakraborthy, 2009b) which also contain saponins (Sahgal *et al*., 2009; Maneemegalai 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 vitro* ethanolic extracts of *Asparagus racemosus*.

Table 1: Effect of root extracts of *Asparagus racemosus* and *Chlorophytum arundinaceum* on neutrophil Adhesion test in rats

	TLC (cells mm^{-3}) (A)		Neutrophil (%) (B)		Neutrophil index (A×B)			
Dose (mg kg^{-1})	UB	NFTB	UB	NFTB	UB	NFTB	Neutrophil adhesion (%)	Inhibition (%)
20	9.5±0.36	10.6±2.160	25.06±0.95	9.60±0.92	238.07±64.00	101.76±16.4	57.26±1.02	-----
100	11.01±0.96	12.95±1.86	21.12±1.76	14.25±2.70	232.53±5.080	184.54±9.04	20.64±16.4	63.95***
300	9.75±1.09	10.18±1.07	19.48±2.10	16.06±1.06	189.93±2.120	163.49±9.90	13.92±1.06	75.69***
300	10.01±1.06	11.80±1.50	19.31±2.06	14.7±2.00	193.29±6.400	173.49±5.1	10.26±9.05	82.08**
300	10.36±2.07	8.01±1.30	17.21±1.70	11.12±2.19	178.30±5.120	89.07±7.12	50.04±4.01	12.60*

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 *Chlorophytum arundinaceum* on delayed type hypersensitivity

Effect of extract on hypersensitivity foodpad thickness		
Parameters	Dose (mg kg^{-1} P.O.)	Paw volume at 27 h (%)
Control (pH 7.4)	20	29.12+4.01
Test 1	300	11.04+3.30 (52.02)
Test 2	300	14.60+3.29 (45.60)
Test 3	300	19.09+1.22 (33.90)

P.O.: Paw oedema

This indicates that *in vitro* ethanolic extract of *Chlorophytum arundinaceum* increase 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 ethanolic root extract, hydroalcoholic root extract of *Chlorophytum arundinaeceum* 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 *Chlorophytum arundinaceum* and *in vitro* 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 *Chlorophytum arundinaceum* extract. These results was 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 *Chlorophytum 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.

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