

## EVALUATION OF IMMUNOMODULATORY EFFECT OF HYDROALCOHOLIC ROOT EXTRACT OF *CHLOROPHYTUM ARUNDINACEUM* BAKER IN MICE

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Received: 23 July 2011, Revised and Accepted: 16 Nov 2011

### ABSTRACT

*Chlorophytum arundinaceum* is a plant possessing various medicinal properties. The aim of present study was to investigate the immunomodulatory activity of the hydroalcoholic extract (HRC) obtained from the root of the plant at the dose of 100 mg kg<sup>-1</sup> and 200 mg kg<sup>-1</sup>. To evaluate the immunomodulatory activity of the hydroalcoholic root extract of *Chlorophytum arundinaceum* Baker, to justify the traditional claim endowed upon this herbal drug as a rasayana in Ayurveda. The effect of the hydroalcoholic root extract of *Chlorophytum arundinaceum* Baker (HRC) on the specific immune response was evaluated by the humoral antibody response. The effect of HRC on the phagocytic activity was evaluated by the carbon clearance test and neutrophil activation was evaluated by the neutrophil adhesion test for a nonspecific immune response. The data was analysed by one-way ANOVA followed by Tukey-Kramer multiple comparison tests. On oral administration, HRC showed a significant increase in the humoral antibody responses, by increasing the hemagglutinating antibody titre at doses of 100 and 200 mg/kg/p.o. There was a significant dose dependent increase in the phagocytic index and percentage neutrophil adhesion at doses of 100 and 200 mg/kg/p.o. The present study reveals that the hydroalcoholic root extract of *Chlorophytum arundinaceum* Baker holds a promise as an immunomodulatory agent, which acts probably by stimulating both the specific and nonspecific arms of immunity. The results, thus justifies the traditional use of *C.arundinaceum* as a rasayana drug.

**Keywords:** Immunomodulation, Humoral immunity, Cellular immunity, Phagocytic activity.

### INTRODUCTION

An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune response.

Traditional medicine refers to the ancient medical practice that existed in human societies before the application of modern science to health. The importance of traditional medicine (TM) as a source of primary health care was first officially recognized by the World Health Organization (WHO) in 1976 by globally addressing its Traditional Medicine Programme. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination, in maintenance of health and the treatment of diseases. The WHO estimates that about 80 percent of world population relies on TM for primary health care.

Although modern medicine is widely spread, TM still exists in all countries. It is interesting to note that 25 per cent of modern medicines are derived from plants that were used traditionally. For example, the Chinese herbal remedy *Artemisia annua*, used in China for almost 2000 years, has been found to be effective against resistant malaria, and has created a breakthrough in preventing almost a million deaths annually, most of them of children, from severe malaria.

#### TM systems

The major systems of TM in South- East Asia are Ayurveda and Chinese traditional medicine. Ayurveda originated in India long back in the pre-Vedic period. The *Rigveda* and *Atharva-veda* (5000 BC), the earliest Indian documents have references on health and diseases.

Ayurvedic texts like *Charaka Samhita* and *Sushruta Samhita* were documented about 1000 BC. Ayurveda, developed from the *Vedic* concept of life, became the important source of all systems of medical sciences. In course of time, it became a part of the culture and heritage of the people of the Indian subcontinent. Ayurvedic medicinal preparations consist mainly of plant materials in the form of powders, semi-solid preparations, decoctions, elixirs and distillates. Many of them also contain inorganic chemical substances,

minerals and animal products. Alcoholic extracts and alcoholic solutions of the ingredients, tinctures and elixirs are also frequently used in *Ayurvedic* medicine. Over thousands of years, traditional Chinese medicine has developed a theoretical and practical approach to the treatment and prevention of diseases. The first documented source of Chinese medical theory, the *Huangdi Nei Jing* ("Inner Classic of the Yellow Emperor") was written between 300 and 100 BC. It describes the diagnosis and treatment of a huge range of disorders and gives advice about healthy lifestyles, exercise, and diet, which conforms remarkably well to current recommendations for the prevention of chronic diseases<sup>1</sup>.

#### Concept of 'Vyadhirodhak Chamatav' in Ayurvedic Medicine

There is a difference in the concept of body's resistance to disease in traditional Indian System of Medicine, i.e., Ayurveda and the Modern System of medicine which is of Western origin. According to Ayurvedic theory a harmonious balance between three humors of the body viz., 'Vayu', 'Pith' and 'Kaf' is needed for positive health; imbalance of these may cause disease(s). A significant part of Ayurvedic therapeutics is preventive health so that individuals do not suffer from disease. This is the concept of "Vyadhirodhak chamatav", i.e. capacity of the body to resist disease. Obviously, the immune system, as recognized in modern biology, which provides protection against microbes, should be a part of it. An entire section of *Materia Medica* of Ayurveda termed 'Rasayanas' is devoted to enhancement of body's resistance.

Various Indian plants which have the potential of immunomodulating activity are identified from various sources in the literature. Some of them are as *Asparagus racemosus*, *Azadiracta indica*, *Curcuma longa*, *Ocimum santum*, *Panax ginseng*, *Picrorhiza kurroa*, *Tinospora cordifolia*<sup>2</sup>, *Withania somnifer*<sup>3</sup> etc., considerable work is to be done on the remainder plants.

In the present study, the roots of the plant *Chlorophytum arundinaceum* which is used as rejuvenating tonic in Ayurveda, was used to evaluate immunomodulator activity in mice.

### MATERIALS AND METHODS

#### Collection and Extraction

The dried root of *Chlorophytum arundinaceum* Baker is collected from the hills of Tirupathi, Andhra Pradesh and authenticated by

Professor Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai. Voucher number PARC/2010/636 and submitted to SRM College of Pharmacy. The dried roots (250 g) were crushed, finely powdered, and sifted from #40 sieve. The powder was defatted with petroleum ether (60-80°C, 500 ml x 4 times) and then extracted with 50% alcohol (1:1 ratio of alcohol and water) by reflux distillation and evaporated under reduced pressure. The product was stored in a refrigerator at 4°C in a glass container throughout the study.

#### Preliminary Phytochemical Screening

The hydroalcoholic root extract of *Chlorophytum arundinaeum* was subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents <sup>4</sup>.

#### Animals

The experiment was carried out on Swiss albino mice (20-25g) are procured from the “Kings Institute, Guindy, Chennai” and will be adapted to the laboratory conditions of “Animal House, SRM College of Pharmacy They will be fed with standard laboratory diet and water *ad libitum* and will be maintained under 12hr light/ dark cycle at 25 ± 2°C and 50 ± 10% humidity. The study was approved by Institutional Animal Ethical Committee, CPCSEA and the proposal number is IAEC/122/2010.

#### Chemicals

Cyclophosphamide (SIGMA Ltd., India), SRBC from Animal house of SRM UNIVERSITY, were used in pharmacological studies.

#### Grouping of Animals

The animals were divided into two main sets. First set having 3 groups and second having 2 groups, each group having 6 animals.

#### First set

Group I: Vehicle control (Treated with 0.1ml of SCMC with Cyclophosphamide).

Group II: Treated with HRC 100mg kg<sup>-1</sup> p.o., from day 1 to 7 along with Cyclophosphamide 50 mg kg<sup>-1</sup> for last three days.

Group III: Treated with HRC 200 mg kg<sup>-1</sup>p.o., from day 1 to 7 along with Cyclophosphamide 50 mg kg<sup>-1</sup> for last three days.

#### Second set

Group I: Treated with HRC 100mg kg<sup>-1</sup> p.o., for Seven days.

Group II: Treated with HRC 200 mg kg<sup>-1</sup> p.o., for seven days.

#### Pharmacological Evaluation

##### Humoral mediated Immune response

##### Haemagglutination Antibody titre

Mice were pre treated with extract for 7 days and each mice will be immunized with 0.1ml Of 20% SRBC by intraperitoneal route. The day of immunization was referred as day 0. Blood was withdrawn from all the animals on the seventh day from retro orbital plexus, under mild anesthesia, centrifuge to obtain the serum. The antibody titre was determined by using microtitre plates. Each well of microtitre plate was filled initially with 25µl of normal saline and 25µl of serum was mixed with 25µl of normal saline in the first well of microtitre plate. Subsequently the 25µl diluted serum was removed from the first well and added to the next well to get two fold dilutions of this diluted serum were carried out till the last well of second row(twenty-first well),so that the antibody concentration of any of the dilutions is half of the previous dilution. Twenty-five microlitres of 1%SRBC was added to each well and the microtitre plates were incubated at 37°C for one hour and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titre obtained on seventh day after immunization challenge with SRBCs were considered as the humoral immune response.

#### Cell Mediated Immune Response

##### Foot Pad Thickness

The thickness of right hind footpad was measured by plethysmometer on 7<sup>th</sup> day. The mice were then challenged by injecting 0.5ml of 1%SRBC in right hind footpad and after 24hr of this challenged the foot thickness was measured again. The pre and post challenged difference was expressed in mm and taken as a measure of DTH.

##### Neutrophil Adhesion Test

On the 7<sup>th</sup> day of drug treatment, blood was collected by puncturing the retro-orbital plexus into heparinised vials and analysed for total leukocyte counts (TLC) and differential leukocyte counts (DLC) by fixing blood smears and staining with Field stain I & II-Lieshman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibres for 15 min at 37°C.

The incubated blood samples were again analysed for TLC and DLC. The product of TLC and % neutrophil adhesion gives Neutrophil Index (NI) of blood sample. Percent Neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion (\%)} = \frac{NI_u - NI_t}{NI_u} \times 100$$

NI<sub>u</sub> – Neutrophil Index of untreated blood, NI<sub>t</sub> – Neutrophil Index of fibrin treated blood.

##### Carbon Clearance Test

Carbon ink suspension was be injected via tail vein to each mice 48hours of the five-days drug treatment. Blood samples (25µl) were then withdrawn from the retro-orbital plexus under mild anaesthesia at 0 and 15minutes after injection of colloidal carbon ink and lysed in 0.1% sodium carbonate solution (3ml). The optical density was measured by spectrophotometrically at 660nm. The phagocytic index was calculated by using the following formula

$$K = \frac{(\ln OD_1 - \ln OD_2)}{(t_2 - t_1)}$$

##### Statistical Analysis

All the data were analysed by using one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparisons test. All the values were expressed as Mean ±S.E.M. All statistical analyses were performed using Graph Pad software (San Diego, CA).

#### RESULTS

##### Phytochemical screening

Preliminary phytochemical screening of the hydroalcoholic root extract of *Chlorophytum arundinaeum* (HRC) revealed the presence of alkaloids, flavonoids, phenol compounds, glycosides, saponins.

##### Humoral antibody response

The HA titre value was used to assess humoral immune response. Administration of hydroalcoholic root extract of *Chlorophytum arundinaeum* (HRC) produced a dose dependent increase in the HA titre after 1 h incubation with SRBCs (Table 1). Administration of higher dose i.e.200mg/kg produced significant increase in HA titre as evident from haemagglutination after incubation of serum with SRBCs. Effect HRC on Humoral immune response is showed in Table 1

##### Delayed type hypersensitivity

The Cell-mediated immune response of HRC was assessed by DTH reaction, i.e., foot pad thickness as shown in the **Table 2**, the HRC produced a significant, dose related increase in DTH reactivity in mice. Increase in response to cell dependent antigen revealed the stimulatory effect of HRC on T-cells.

**Table 1: Effect of HRC on Humoral immune response**

Group	Treatment	Dose	Ha titre value
I	Vehicle control	1 ml of 1% SCMC	147.9 + 1.188
II	Cyclophosphamide (Cyp)	50 mg/kg	9.6 + 1.054***
III	HRC + Cyclophosphamide	100 mg/kg	192 + 1.342***a
IV	HRC + Cyclophosphamide	200 mg/kg	254.8 + 0.909***a

Values are expressed in Mean + S.E.M. (n=6). Significantly different from control\*\*\*p < 0.05; Significantly different from control Cyclophosphamide group<sup>a</sup> p < 0.05.

**Table 2: Effect of HRC on Delayed type hypersensitivity**

Group	Treatment	Dose (mg/kg)	DTH response (mm) mean paw oedema + S.E.M
I	Control	10mg/ml	0.55 + 0.0176
II	Cyclophosphamide	50 mg/kg	0.90 + 0.0154**a
III	HRC + Cyclophosphamide	100 mg/kg	1.25 + 0.0202***a,b
IV	HRC + Cyclophosphamide	200 mg/kg	1.99 + 0.1012***a,b

Values are expressed in Mean±S.E.M, n=6,\*\*p<0.05,\*\*\*p<0.05, a:when compared with control ; b:when compared with

**Table 4: Effect of the HRC on Neutrophil Adhesion Test**

Group	Treatment	Dose	TLC (10 <sup>3</sup> /mm <sup>3</sup> ) [A]		% Neutrophil [B]		Neutrophil index (A x B)		Neutrophil adhesion(%)
			UB	FTB	UB	FTB	UB	FTB	
I	CONTROL	SCMC 0.1ml	6.3± 0.33	6.0 ±0.57	46.67± 0.66	41.67± 0.88	295.3± 13.78	236.78 ±18.41	19.93± 4.33
II	HRC	100 mg/kg	7.0 ±0.57	6.3 ±0.88	52.33 ±1.20	42.33± 1.20	366.0 ±29.96	269.0± 42.12	27.60± 5.23
III	HRC	200 mg/kg	7.3 ±0.33	5.6 ±0.33	54.33 ±1.20	44.33± 1.20	399± 25.24	274.0 ±36.36	31.87± 5.30*

Values are expressed in Mean ± S.E.M, n=6,\*p<0.05, HRC treated groups are compared with group of control.

## DISCUSSION

Immunomodulatory agents of the plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. The results of scientific evidences obtained in this study support the traditional claim of *Chlorophytum arundinaeum* for medicinal purposes. In the present study, the immunomodulatory activities of hydroalcoholic root extract of *Chlorophytum arundinaeum* (HRC), an important plant of indigenous system of Indian medicine were explored.

Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Herbal agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy<sup>5</sup>. The results obtained in the present study indicate that *Chlorophytum arundinaeum* is a potent immunostimulant, stimulating both specific and nonspecific immune mechanisms.

During the preliminary phytochemical studies and from previous literatures the root extract of the *Chlorophytum arundinaeum* consists of saponins, phenolic glycosides. Saponins, in particular, are described as immunostimulatory agents<sup>6</sup>.

Many of the disorders today are based on the imbalances of immunological processes like DTH (cell mediated) reactions and humoral responses<sup>7</sup>. DTH is a part of the process of graft rejection,

Cyclophosphamide.DTH : Delayed type hypersensitivity,\*\*\*highly significant,\*\* moderately significant.

## Carbon clearance test

The phagocytic activity of the reticuloendothelial system is generally measured by the rate of removal of carbon particles from the blood stream. There was a significant increase in phagocytic activity (p<0.05) in the HRC 200mg/kg treated groups when compared with the control, indicating that HRC possesses a macrophage stimulatory activity. Effect of HRC on Phagocytic index was shown in Table 3.

**Table 3: Effect of HRC on Phagocytic index**

Group	Treatment	Dose	Phagocytic index
I	Vehicle control	1 ml of 5% gum acacia	0.011+0.0020
II	HRC	100 mg/kg	0.0188+0.0015
III	HRC	200 mg/kg	0.0333+0.0029***

Values are expressed as Mean ± S.E.M, n=6, \*\*\*p<0.05,HRC treated groups are compared with control.

## Neutrophil index

HRC increased the adhesion of neutrophils to nylon fibres, which correlated to the process of margination of neutrophils in blood vessels. The neutrophil adhesion was significantly increased in the HRC 200mg/kg when compared with control. Effect of the hydroalcoholic root extract of *Chlorophytum arundinaeum*(HRC) on Neutrophil Adhesion Test was shown in Table 4.

tumour immunity and most important immunity to many intracellular infectious micro-organisms, especially those causing chronic diseases viz., tuberculosis. Further, DTH requires the specific recognitions of a given antigen by activated T-lymphocytes which subsequently proliferate and release cytokines. These in turn, increase vascular permeability, induce vasodilation, macrophage accumulation and activations, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. In the present, DTH reaction is measured by foot-pad thickness, after 24hr of antigenic challenge and subsequent immunization with SRBC, the animal showed significant increase in volume of paw edema due to production of antibodies in response to the antigen. Animals treated with cyclophosphamide showed potentiation of DTH response as Cyclophosphamide damaged the short-lived suppressor T-cells in immune regulatory systems<sup>8</sup>. Significant dose dependent increase in the DTH response indicates that *Chlorophytum arundinaeum* possesses stimulatory effect on lymphocytes and on other necessary cell types required for the expression of the reaction<sup>9</sup>.

The index of humoral immune response is the increase in antibody titre value due to increase in immune response<sup>10</sup>. Cyclophosphamide is an immune suppressor, which decreases the antibody titre level; therefore it serves as an ideal standard to compare with the test drug in order to establish whether the immune response is substantial. The humoral immunity involves interaction of B-cells with the antigen and their subsequent

proliferations and differentiations into antibody secreting plasma cells<sup>11</sup>. Further, antibody functions as the effector of the humoral response by binding to antigen by neutralizing it or facilitating its eliminations by cross-linking to form clusters that are more readily ingested by phagocytic cells. In the present study, to evaluate the effect of hydroalcoholic root extract of *Chlorophytum arundinaeum* (HRC) on humoral response, its influence was tested on SRBC specific haemagglutination antibody titre in mice. Cyclophosphamide (50mg/kg, p.o.) showed significant inhibition in antibody titre response, whereas HRC showed an enhanced production of circulating antibody titre. This augmentation of the humoral response to SRBC antigen by increase in haemagglutination antibody titre indicated the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis<sup>12</sup>. Might be due to the presence of phenolic glycosides present in the extract responsible for enhancement of HA titre and DTH response. Recently phenolic glycosides isolated from *Curculigo orchoides* were found to be responsible for the enhancement of HA titre and DTH response<sup>13</sup>.

Neutrophils represent a multi-functional cell type in innate immunity that contributes to bacterial clearance by recognition, phagocytosis and killing whereas leucocytes are responsible for the production of antibodies leading to enhancement of immunity<sup>14</sup>. Further, Neutrophil granules contain a variety of toxic substances that kill or inhibit growth of bacteria, fungi and mediators of this cell will be increased only when the immune system is stimulated. Therefore, in neutrophil adhesion test, the TLC and DLC were analyzed. In this test, a significant increase in total and differential count was observed when root extract of *C.arundinaeum* at a dose of 200 mg/kg was administered orally. Macrophages are polymorphonuclear lymphocytes which play an important role in modulation of the immune system. These cells then secrete number of cytokines like CSF and IL-1 which in turn stimulates neutrophils and increases neutrophil index<sup>15</sup>. This gives host defense the ability to counter the infectious diseases. Our results demonstrated that the rise in neutrophil index as shown by enhanced adhesion of neutrophil to nylon fiber further suggests that *C.arundinaeum* may be useful in promoting the protection of body by phagocytosis, even in diseased conditions where immunity is depressed.

Phagocytic index generally increases whenever there is an increase in immune response and its effects are associated with varied pathologic conditions in humans<sup>16</sup>. Besides, phagocytosis represents an important innate defense mechanism against ingested particulates including whole pathogenic microorganisms<sup>17</sup>. The specialized cells that are capable of phagocytosis include blood monocytes, neutrophils and tissue macrophages. In a view of the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to B-cells, hydroalcoholic root extract of *Chlorophytum arundinaeum* (HRC) was evaluated for its effect on macrophage phagocytic activity. When the carbon particles are injected intravenously, the rate of clearance of carbon from blood by macrophage is governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. In this study, the increase in phagocytic index as seen from the carbon clearance test after treatment with HRC advocate effect of extract on macrophages and therefore on the subsequent stimulation of the immune system.

The plant has rich source of saponins, glycoside compounds which in other plants have been reported to have immunomodulatory activities, for example, *Curculigo orchoides* Gaertn which contains similar constituents shows immunomodulating activity. Some of the chemical constituents isolated from *Chlorophytum arundinaeum* include tetrahydrofurans, Bibenzyl Xyloside, stigmaterols, antibacterial compounds<sup>18</sup>. Further, our preliminary phytochemical studies show the presence of alkaloids, carbohydrates, tannins, phenolic compounds and saponins glycosides. The results of this study were consistent with the work of other investigators in determining the effectiveness of selected natural products against allergen induced inflammation. For example Mungantiwar, et al,<sup>19</sup> demonstrated the immunomodulatory effects of alkaloidal fraction of *Boerhaavia diffusa* in mice. Furthermore, it has been re-ported that, diosgenyl saponins isolated from *Paris polyphylla* possesses immunostimulating properties<sup>20</sup>.

In conclusion, the present evidence suggests that hydroalcoholic root extract of *Chlorophytum arundinaeum* Baker (HRC) may stimulate both cellular and humoral immune response. The extract not only potentiate non specific immune responses, but also humoral as well as cell-mediated immunity effectively. Thus, the immunostimulatory effect produced by hydroalcoholic root extract of *Chlorophytum arundinaeum* in Cyclophosphamide induced immunosuppression may be due to cell mediated and humoral antibody mediated activation of T and B cells. It can be therefore be concluded that HRC is a potential immunostimulant against cytotoxic drugs and can be used as a complimentary therapeutic agent. Therefore, the plant holds promise for being used as an immunostimulating agent might be due to the presence of saponins, and in-depth study on various fractions of the extract effective as immunomodulating entities from the plant is warranted to determine the most potent immunostimulating fraction from *Chlorophytum arundinaeum*. Thus, the study validates the traditional use of herb as a 'Rasayana' in Ayurvedic system of medicine.

#### ACKNOWLEDGMENT

The author thanks to the Department of Pharmacology, SRM University, Tamil Nadu for providing facilities and grants necessary to carry out the work.

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