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Research Article

# EVALUATION OF IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *GLYCOSMIS PENTAPHYLLA* IN SWISS ALBINO MICE

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**ABSTRACT**

The Immune System is the most complex biological systems in the body. The present study was aimed to investigate the immunomodulatory activity of the ethanolic extract of leaves of *Glycosmis pentaphylla* in swiss albino mice. The effect of humoral antibody response was evaluated by Haemagglutination antibody titre method. Cellular immunity was evaluated by Delayed type hypersensitivity method and neutrophil activation was evaluated by neutrophil adhesion method.on 7th and 14th day of the study,mice from all the groups were immunized and challenged with sheep red blood cells (SRBCs) i.p. The data was analyzed by One-way ANOVA followed by Tukey-kramar multiple comparison test. On oral administration, Ethanolic extract of leaves of *Glycosmis pentaphylla* for 21 days showed a significant increase in the humoral antibody responses, by increasing the haemagglutination antibody titre at doses of 100 and 200mg/kg/p.o. There was a significant dose dependent increase in percentage neutrophil adhesion at doses of 100 and 200mg/kg/p.o. extracts. The present study reveals that the ethanolic extract of *Glycosmis pentaphylla* holds a promise as an immunomodulatory agent, which acts probably by stimulating both the specific and nonspecific arms of immunity.

**Keywords:** Immunomodulatory activity, *Glycosmis pentaphylla*, Humoral antibody response, Delayed type hypersensitivity, Neutrophil activation.

## INTRODUCTION

The medicinal properties of certain plants have been known for centuries[1]. More than a quarter of the medicines in use today come from plants, i.e. from traditional medicine. Modulation of immune response to alleviate the disease has been interest for many years and the concept of Rasayana in Ayurveda is based on related principles. The function and efficacy of immune system may be influenced by many exogenous factors like food and pharmaceuticals, physical and psychological stress and hormones etc. resulting in either immunostimulation or immunosupression[2]. The healthy state is believed to be based on a sophisticated fine tuning of immonoregulatory mechanisms.

Immunomodulation using plant material can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the condition of impaired immune response.

*Glycosmis Pentaphylla (Retz)* [3][4]belonging to the family Rutaceae is a moderate sized shrub which is commonly called kattukkonci in tamil as panal in Malayalam. It is generally known for its medicinal properties. Root were used for facial inflammation, rheumatism, anemia, jaundice. Leaves were used for fever, eczema, skin diseases, helmenthiasis, wounds, lever complaints, vermifuge. Fruits are used for dysentery, cough and bronchitis.. Flowers were used for astringent, expectorant[5][6].

The present study was aimed to evaluate leaves of *Glycosmis Pentaphylla* for immunomodulatory activity in swiss albino mice.

## MATERIALS AND METHODS

### Plant extract

Fresh leaves were collected and dried at room temperature. The dried, coarsely powdered leaves (500g) were subjected to soxhlet extraction with 95% ethanol[7] to obtain ethanolic extract. The extract obtained was subjected to preliminary phytochemical screening for the identification of various chemical constituents [8][9].

### Animals

Swiss albino mice (20-25g) were procured from King Institute,Guindy and acclimatized to laboratory condition for 10 days after their arrival. They were housed in groups under standard light/dark cycle with food and water provided *ad libitum*. All the experimental procedures and protocols used in the study were approved by (IAEC) and the proposal number being IAEC/141/2011.

### Experimental design

The albino mice were divided into seven groups of six animals each [10][11]. Group I received vehicle for 21 days. Group II received Cyclophosphamide (100mg/kg/p.o) on 9th and 16th day as a single dose. Group III, IV were administered with two different concentrations of extracts for 21days.In addition, Group V & VI received Cyclophosphamide (100mg/kg/p.o) along with extract on 9th and 16th day. Group VII received Levamisole (50mg/kg/p.o) for 21days.

### Preparation of Sheep RBC

Sheep blood was collected in sterile Alsevier's solution in 1:1 proportion of Alsevier's solution (freshly prepared) [12]. Blood was kept in the refrigerator and processed, for the preparation of SRBCs batch, by centrifugating at 2000 rpm for 10 minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use.

### Neutrophil adhesion test

The Group I, III and IV animals will be used for this test. The control Group I will be receive vehicle, while animals of group II and III will be given cyclophosphamide (100mg/kg/p.o))and extract (100mg/kg/p.o) [13]. On 7th day before immunizing the blood will be collected by puncturing retro-orbital plexus under mild ether anaesthesia. Blood will be collected in vials pre-treated by disodium EDTA and analyzed for total leukocyte count (TLC) and Differential leukocyte count (DLC) estimation.

The present neutrophil adhesion will be calculated as follows;

$$\text{Neutrophil adhesion} = \frac{\text{NIu} - \text{NIt} \times 100}{\text{NIu}}$$

Where,

$NI_u$: Neutrophil Index before incubation with nylon fiber.

$NI_t$: Neutrophil index after incubation with nylon fiber.

Neutrophil index = TLC ×% Neutrophil.

### Delayed type hypersensitivity (DTH) response

Delayed type hypersensitivity response of the animal will be measured on 21st day. On 14th day of the study, all the groups I to VIII were immunized with SRBCs (0.1ml of 20% SRBC i.p.) in normal saline[14][15]. On day 21st all animals from all the groups were challenged with 0.03 ml of 20% SRBCs in subplantar region of right

hind paw. Foot pad oedema in mice was used for detection of cellular immune response. On 21st day, injection of 0.1ml of 20% SRBCs in the subplantar region of right hind paw in the volume of 0.03 ml and normal saline in left hind paw in same volume. Foot pad reaction was assessed after 24hr. on 22nd day, in terms of increase in the thickness of footpad as a result of hypersensitivity reaction due to oedema; the thickness of the right hind foot pad was measured using plethysmometer[16] 17r. The footpad reaction was expressed as the difference in the thickness (m.m.) between the right foot pad injected with SRBC and the left footpad injected with normal saline.

**Haemagglutination(HA) antibody titre**

On 7th and 14th day of the study, mice from all the groups (i.e. group I to VIII) will be immunized and challenged respectively, with SRBCs in normal saline (0.1ml of 20% SRBCs) intraperitoneally[18] [19]. Blood will be withdrawn on 14th and 21st day from retro-orbital plexus under mild ether anaesthesia from all antigenically sensitized and challenged mice respectively. Blood will be centrifuged to obtain serum, normal saline will be used as a diluent and SRBCs count will be adjusted to (0.1% of SRBCs). Each well of a microtitre plate will be filled initially with 20 µl of saline and 20 µl of serum will be mixed in the first well of micro titre plate. Subsequently the 20 µl diluted serum will be removed from first well and added to the next well to get two fold dilutions of the antibodies present in the serum. Further twofold dilutions of this diluted serum will similarly carried out till the last well of the second row (24th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 20 µl SRBC (0.1% of SRBCs) will be added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for haemagglutination. The highest dilution giving haemagglutination will be taken as the antibody titre[20]. The antibody titres were expressed in the graded manner, the minimum dilution (1/2) being ranked as 1, and mean ranks of different groups will be compared for statistical significance. Antibody titre obtained on 14th day after immunization (on 7th day) and on 21st day after challenge (on 14th day) with SRBCs will be considered as primary and secondary humoral immune response respectively.

**RESULTS**

Preliminary phytochemical investigations of ethanolic extract of *Glycosmis pentaphylla* showed the presence of Saponins,Glycosides,Alkaloids,Carbohydrates,Phenols,Tanins,Steroid.

Neutrophil adhesion index of the ethanolic extract of *Glycosmis pentaphylla* increased the adhesion of neutrophils to nylon fibres,which correlated to the process of migration of neutrophils in blood vessels. The neutrophil adhesion was significantly increased in the extract 200mg/kg when compared with control(Table 1).

The Cell-mediated immune response of ethanolic extract of *Glycosmis pentaphylla* 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 (Fig.no.1)

The HA titre value was used to assess humoral immune response. Administration of ethanolic extract of leaves of *Glycosmis pentaphylla* produced a dose depent increase in the HA titre after 1hr incubation with SRBCs(Table 3).administration of higher dose of extract i.e.200mg/kg produced significant increase in HA titre as evident from haemagglutination after incubation of serum with SRBCs.(Fig,no.2)

**Table 1: Effect of the ethanolic root extract of *Glycosmis pentaphylla* on neutrophil adhesion test**

| Group | Treatment | Dose | TLC($10^3$/$mm^3$ [A] | | % Neutrophil [B] | | Neutrophil index[A×B] | | Neutrophil adhesion% |
|---|---|---|---|---|---|---|---|---|---|
| | | | UB | FTB | UB | FTB | UB | FTB | |
| I | Control | 0.1ml | 6.3 ±0.1 | 6.2 ±0.08 | 46.0 ±0.85 | 41.0 ±0.36 | 292.75 ±6.3 | 255.55 ±3.9 | 12.5±1.4 |
| II | Extract | 100 mg/kg | 7.1 ±0.08 | 6.8 ±0.10 | 49.16 ±0.7 | 44.16 ±1.49 | 349.01 ±6.2 | 295.86 ±7.4 | ***15.11 ±2.3 |
| III | Extract | 200 mg/kg | 7.2 ±0.13 | 6.8 ±0.16 | 50.0 ±0.85 | 39.5 ±3.12 | 359.81 ±7.81 | 270.50 ±18.5 | ***25.02 ± 4.3 |

Values are expressed in Mean ±S.E.M.(n=7),***p<0.0001,**p<0.01.All groups are compared with control group.

**Table 2: Effect of ethanolic extract of *Glycosmis pentaphylla* on delayed type hypersensitivity**

| Group | Treatment | Dose | Mean difference of Paw Oedema in (mm). |
|---|---|---|---|
| I | Vehicle control | 1ml of 1% | 0.437±0.034 |
| II | Cyclophosphamide | 100mg/kg | 0.365±0.027 |
| III | Extract | 100mg/kg | ***0.590±0.016 |
| IV | Extract | 200mg/kg | ***0.612±0.022 |
| V | Extract+Cyclophosphamide | 100mg/kg | 0.408±0.027 |
| VI | Extract+Cyclophosphamide | 200mg/kg | 0.463±0.022 |
| VII | Standard | 50mg/kg | **0.585±0.013 |

Values are expressed in Mean ±S.E.M.(n=7) , ***p<0.0001,**p<0.01 are considered as statistical significant. All groups are compared with control group.

**Table 3: Effect of ethanolic extract of leaves of *Glycosmis pentaphylla* on Humoral immune response**

| Group | Treatment | Dose | HA Titre value |
|---|---|---|---|
| I | Vehicle control | 1ml of 1% | 6.0±0.720 |
| II | Cyclophosphamide | 100mg/kg | 4.7±0.654 |
| III | Extract | 100mg/kg | *8.9±0.180 |
| IV | Extract | 200mg/kg | ***11.81±0.531 |
| V | Extract+Cyclophosphamide | 100mg/kg | ***6.56±0.554 |
| VI | Extract+Cyclophosphamide | 200mg/kg | ***7.1±0.907 |
| VII | Standard | 50mg/kg | ***7.533±0.580 |

Values are expressed in Mean ±S.E.M.(n=7),***p<0.0001,*p<0.01.were considered as statistical significant. All groups are compared with control group

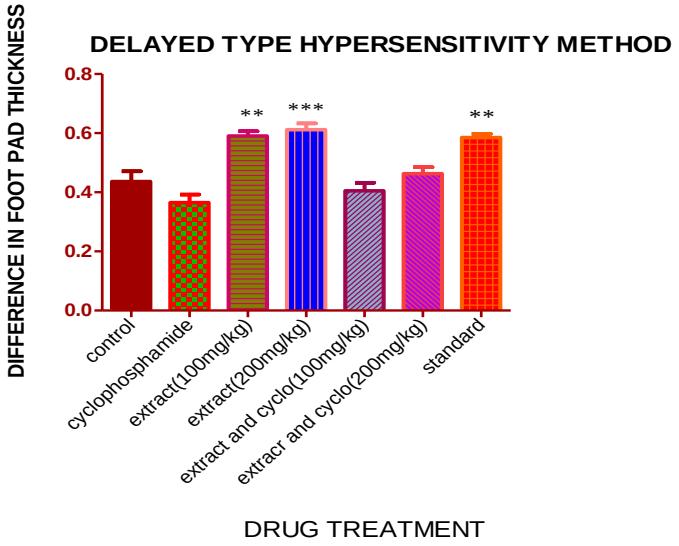


**Fig. 1: Graphical Representation of Delayed Type Hypersensitivity Method.**

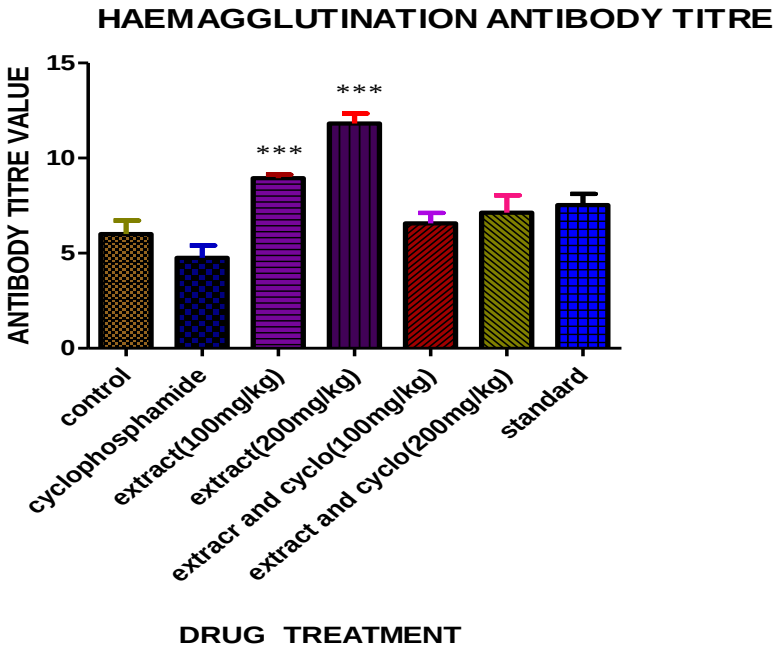


**Fig. 2: Graphical Representation of Haemagglutination Antibody Titre Value.**

## DISCUSSION

Immunomodulatory agents obtained from plant and animal origin generally enhances the immune responsiveness of an organism against a pathogen by activating the immune system. *Glycosmis pentaphylla* is known for several medicinal uses and has been investigated for different pharmacological properties. Modulation of the immune response through stimulation or suppression may help in maintaining a disease free state. Herbal agents that activate host defence mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy.

During the preliminary phytochemical studies and from previous literatures the leaves extract of the *Glycosmis pentaphylla* consist of saponins, phenolic glycosides. saponins, in particular, are described as immunostimulatory agents.

Neutrophils represent a multifunctional cell type in innate immunity. In the present study neutrophil adhesion test, TLC and DLC were analyzed. In this test, a significant increase in total and differential count was observed when leaves extract of *Glycosmis pentaphylla* at a dose of 200mg/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 likes CSF and IL-1 which in turn stimulates neutrophil and increases neutrophil index. This gives host defence the ability to counter the infectious diseases. Our results demonstrated that rise in neutrophil index as shown by enhanced adhesion of neutrophil to nylon fiber further suggests that *Glycosmis pentaphylla* may be useful in promoting the protection of body by phagocytosis, even in diseased conditions where Immunity is depressed.

DTH reaction is measured by foot-pad thickness, after 24hr of antigenic challenge and subsequent immunization with SRBC, the animal showed significant increase in paw oedema 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. Significant dose dependent increase in the DTH response indicates that *Glycosmis pentaphylla* possess stimulatory effect on lymphocytes and on other necessary cell types required for the expression of the reaction.

In the present study,the humoral response, its influence was tested on SRBC specific haemagglutination antibody titre in mice. Cyclophosphamide (100mg/kg,p.o)showed significant inhibition in antibody titre response, where as ethenolic extract of leaves showed an enhanced production of circulating antibody titre. This augmentation of the humoral response to SRBC antigenby increase in haemagglutination antibody titre indicated the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody stnthesis. It might be due to the presence of phenolic glycosides present in the extract responsible for the enhancement of HA titre and DTH response.

From the above investigations the ethanolic extract of *Glycosmis pentaphylla* was found to have a significant immunostimulatory activity on both the specific and non-specificimmune mechanisms. The significant increase in the immunostimulatory activity of extract could be attributed to the presence of carbazole alkaloid glybomines, Quinazoline, Furoquinoline, Quinolone, Acridone, Tannins, Flavanoids, Saponin, Carbohydrate, Protein, Phenols, Steroids.

**CONCLUSION**

The results of the present study suggests that the both low dose(100mg/kg) aswell as high dose(200mg/kg) of ethnolic extracts of leaves of *Glycosmis pentaphylla* may stimulates immune system by acting through cellular and humoral immunity in experimental models of immunity in animal. Further studies using in vivo and in vitro models of immunomodulation are recomended to confirm the Immunomodulatory activity of *Glycosmis pentaphylla* leaves and its mechanism of action.

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