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**Research Article**

**Study of Immunomodulatory activity of *Tinospora cordifolia* extract**

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**ABSTRACT**

Polymorphonuclear leucocytes (PMN) cells are an important component of the host defence system or immunity. Literature has defined the importance of many plants like *Tinospora cordifolia* for their immunomodulatory action. We tested some of the extracts of *T. cordifolia* for the stimulation of the immune defense system using an *in vitro* phagocytosis method. Human PMN cells were used in the assay along with *Candida* culture as an antigen. Various *T. cordifolia* extracts from external vendors and inhouse extracts were tested. The results were analysed by student's t test. The tested extracts were able to stimulate the PMN cells for phagocytosis of added *Candida* cells. The % phagocytosis of the test samples as compared to the control proves potent immunostimulatory activity of the *T. cordifolia* extract. The assay results substantiate the use of this plant as an immunomodulatory agent.

**KEYWORDS:** PMN cells, immunomodulatory action, *Tinospora cordifolia*, phagocytosis

**INTRODUCTION**

Host Immune response plays a major role in any type of infection. Neutrophils or Polymorphonuclear leucocytes (PMN) cells are important components in the surveillance and protection systems for a broad spectrum of host defenses<sup>1</sup>. They play the main role as an effector or killer cells for many types of antigenic challenges especially for infections. The primary function of these blood cells in host resistance is the migration towards the challenge, which is called chemotaxis and the intracellular killing of microorganisms by the formation of oxygen radicals<sup>1,4,6</sup>. Chronic and recurrent infections are associated with diminished immunity. Also, the protective blood cells from patients suffering from recurrent infections fail to show or exert their normal microbiocidal functions<sup>1,3</sup>. Many traditional plants have been used since ancient times because of their various pharmacological properties<sup>1, 4</sup>. One such important property is immunomodulation, which means modification of the immune response or the functioning of the immune system by the action of an immunomodulator. Immunomodulators are those

extrinsic or intrinsic substances which regulate or alter the scope, type, duration or competency of the immune response.

Some of the plant species are studied for their chemotactic and phagocytosis enhancing properties. One such studied plant is *Tinospora cordifolia*, commonly known in India as Guduchi. *T. cordifolia* is reported to benefit the immune system in a variety of ways. The alcoholic and aqueous extracts of *T. cordifolia* have been tested successfully for immunomodulatory activity. It has been observed that it stimulates the macrophages, leucocytosis and enhances neutrophil activity in various animal studies conducted.<sup>2,5,6</sup>

*Tinospora cordifolia* extract has been reported against bacterial growth and improved phagocytic and intracellular bacterial capacities of neutrophils in mice. Its natural compounds have been reported to improve the phagocytic activity of macrophages, enhancement in nitric acid production by stimulation of splenocyte, and production of reactive oxygen species (ROS) in human neutrophil cells<sup>7</sup>. In another scientific investigation using human white blood cells, it is shown that *Tinospora* increased the killing

ability of macrophages, the immune cells responsible for fighting invaders<sup>8,9,10</sup>.

This study included the evaluation of *T. cordifolia* extracts for the stimulation of the immune defense system using an *in vitro* phagocytosis method. The assay demonstrated the immunomodulatory activity of *Tinospora cordifolia* and can be used as a preliminary method for screening of immunomodulation activity.

#### **MATERIALS AND METHOD:**

##### **Samples:**

*Tinospora cordifolia* extracts were procured from two different extract manufacturers in India (Kisalaya Herbals Limited, Madhya Pradesh and Sanat Products Limited). Hydro-alcoholic extracts were used for the study. Also some inhouse samples were included in the study.

##### **Isolation of Polymorphonuclear leucocytes (PMN) cells:**

Blood (20 ml) from healthy human volunteers was collected aseptically. Appropriate consent of the human volunteers and approval from the company – Piramal Enterprises limited was obtained for the experiment. Add 4.0 ml of 4.5 % dextran in saline along with 500 units of heparin. Shake it gently and allow standing for 60 minutes. Aspirate the leucocytes rich plasma. Centrifuge at 400x g for 10 minutes at 4°C. Discard supernatant and resuspend cells in 0.9% Sodium chloride solution to a final concentration of  $5 \times 10^7$  cell/ml. Layer 5.0 ml of cell suspension over the percoll gradient and centrifuge at 700 x g for 30 minutes at 25°C in a swinging bucket rotar. Use a sterile pipette and collect the pellet of enriched PMN cells. Wash PMN cells in 0.9% Sodium chloride solution and use for assay.

##### **Preparation of *Candida albicans* cells:**

Dilute a suspension of *Candida* cells with saline solution. Make final concentration to  $25 \times 10^7$  cell/ml. Heat in a waterbath at 100°C for 30 minutes.

##### **Assay procedure:**

Take 0.2 ml of PMN cells ( $2 \times 10^7$  cells/ml) in 1 ml of HBSS containing 5 % fetal calf serum and 0.05 ml of test sample. Incubate at 37°C for 7 minutes. Add 0.1 ml of *Candida* cell suspension and incubate at 37°C for 60 minutes. Add EDTA to stop the reaction. Count the number of *Candida* cells phagocytosed by PMN cells after staining with 5 % fuchsin in phenol solution.

##### **Calculation:**

% phagocytosis = No. of PMN cells containing ingested *Candida* cells/ 100 PMN cells

##### **Statistical analysis:**

Student's *t*-test was used to determine the significance of difference in the mean values.

#### **RESULTS AND DISCUSSION:**

Plants can provide a supportive therapy to conventional methods for an impaired immune system. In one of the recent study, *Barleria prionitis* was studied for immunomodulatory activities and the plant extract fraction IFBp was found to be a potent immunostimulant, stimulating both the specific and non-specific immune mechanisms<sup>11</sup>. In another study *Stachys mialhesi* plant extract was evaluated for its immunostimulatory effect using an *in vivo model* and the plant was found effective<sup>12</sup>.

The literature shows extensive work on immunostimulation property of *Tinospora cordifolia*. Experimental and clinical data is available which says that the immunostimulation property of *Tinospora cordifolia* can be attributed to its ability to stimulate macrophage activation which in turns leads to granulocyte-monocyte colony stimulation factor. In a study carried out by U.Sharma et al, where they have studied the immunomodulatory activity of different fractions and extracts of stem of *Tinospora cordifolia* using the PMN-phagocytic function studies, it has been shown that the immunostimulatory property of *Tinospora cordifolia* can be attributed to the synergistic effect of group of compounds<sup>13</sup>.

In our present study, immunomodulatory activity of different fractions and extracts of stem of *Tinospora cordifolia* were evaluated using the polymorphonuclear neutrophil (PMN) phagocytic function studies. The present study substantiates the same property of *Tinospora cordifolia* through an *in vitro* slide method of phagocytosis. The method is simple to perform and gives an *in situ* view of the internal mechanism of phagocytosis. The method can be employed on routine basis at primary level for screening of plants for immunomodulatory activity.

The plant extract samples from external vendors and extracts prepared in-house were analyzed for the said property. All samples were tested at two different concentrations of 0.5 and 1.0 mg/ml. Six samples of aqueous extracts obtained from different vendors showed the immunomodulatory activity of the plant. Five samples were able to stimulate the cells for phagocytosis in a dose dependent manner and one sample failed to stimulate the PMN cells. The % phagocytosis of approximately 30 % was observed for the active extracts at a 1.0 mg/ml concentration (table 1). Thus it can be said that the plant is able to stimulate the cells for phagocytosis. The inhouse samples of both aqueous and hydro-alcoholic extracts

showed a significant value of % phagocytosis with an average value of 35 to 40% (table 2). The results obtained over here once again prove the immunostimulation property of *Tinospora cordifolia* as the plant extracts were able to stimulate the cells for phagocytosis in dose dependent manner.

Some scientists have investigated the immunostimulation property of *Tinospora* using different study models. The therapeutic potential of *Tinospora cordifolia*, was studied to assess the immunomodulatory activity using in-vitro as well as ex vivo models by R.Upadhyaya et al and significant effect on cytokine profile was obtained<sup>14</sup>. In another study carried out by More P. et al, *Tinospora* was shown to have a significant effect on macrophage activation thus proving its immunomodulation property<sup>15</sup>.

This property of the plant can be explored to use in many disease conditions as an adjuvant therapy. Like many infectious agents are becoming resistant to currently used antibiotics, in such cases this plant based formulation can be used to enhance the immune response of the patient and bring the cells at the site of infection and increase its killing capacity.

In other disease conditions like recurrent UTI infections, this immunostimulatory activity of the *T. cordifolia* will be useful to flush off the adhering infectious organisms from the mucosal surfaces by enhancing the phagocytosing activity of the cells of the immune system. Further studies are carried out to develop this plant extract into an oral formulation.

### CONCLUSION

The evaluated extracts of *Tinospora cordifolia* showed immunostimulatory activity as evident from the results of phagocytosis assay. The activity was dose dependent when tested at different concentrations. These results demonstrate the immunomodulatory potential of *Tinospora cordifolia* extract. The immune system study shows that phagocytosis is an important attribute of the human immune system. The higher phagocytosis percentage value in turn exhibits improved immune mechanism. Thus this study further upholds the immunomodulatory potential of *Tinospora cordifolia* plant extract.

**Table 1**  
Immuno- modulatory activity of *T.cordifolia* extracts (sample from external vendor)

Concentration (mg/ml)	% Phagocytosis (n=4)
Control	7.19 ± 0.94
1. <i>T.cordifolia</i> (KG/282)	
0.5 mg/ml	27.93 ± 2.099*
1.0mg/ml	36.52 ± 1.769*
2. <i>T.cordifolia</i> (113062)	
0.5 mg/ml	19.00 ± 5.00
1.0mg/ml	32.50 ± 0.5*
3. <i>T.cordifolia</i> -13101445	
0.5 mg/ml	4.62 ± 0.66
1.0mg/ml	8.71 ± 0.34
4. <i>T.cordifolia</i> -TE/03/635	
0.5 mg/ml	19.62 ± 0.81*
1.0mg/ml	27.83 ± 0.36*
5. <i>T.cordifolia</i> -SDA/104	
0.5 mg/ml	21.77 ± 1.88*
1.0mg/ml	30.81 ± 0.38*
6. <i>T.cordifolia</i> -KA/16304	
0.5 mg/ml	27.22 ± 0.42*
1.0mg/ml	30.55 ± 4.1*

\*p<0.05 is significant when compared to control by student's 't' test

**Table 2**  
Immuno- modulatory activity of *T.cordifolia* extracts (in house samples)

Concentration(mg/ml)	% Phagocytosis (n=4)
Control	11.25 ± 0.24
1. <i>T.cordifolia</i> -aqs-5	
0.5 mg/ml	34.94 ± 2.42*
1.0mg/ml	41.92 ± 0.51*
2. <i>T.cordifolia</i> -aqs-7	
0.5 mg/ml	31.01 ± 0.47*
1.0mg/ml	36.11 ± 0.75*
3. <i>T.cordifolia</i> -50% (hydro-alcoholic)	
0.5 mg/ml	30.53 ± 1.15*
1.0 mg/ml	34.25 ± 1.17*

\*p<0.05 is significant when compared to control by student's 't' test

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