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Full Length Research Paper

Enhanced Phagocytosis and Antibody Production by *Tinospora cordifolia* - A new dimension in Immunomodulation

M. S. Ranjith[1]*, A.J.A. Ranjitsingh[2], S. Gokul Shankar[3], G. S. Vijayalaksmi[5], K. Deepa[5] and Harcharan Singh Sidhu[1]

[1]Microbiology Unit, AIMST University, Kedah, Malaysia.
[2]Department of Advanced Zoology and Biotechnology, Sri Paramakalyani College, Alwarkurichi, India.
[3]R and D Center, Cholayil Private Limited, Chennai, India.
[4]Sri Paramakalyani Center for Environmental Sciences, M.S. University, Tirunelveli, India.
[5]Anatomy Unit, AIMST University, Kedah, Malaysia.

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Tinospora cordifolia **(guduchi) is a widely used shrub in ayurvedic systems of medicine known to possess immunomodulatory properties. In the present study the aqueous extract of *T. cordifolia* was found to enhance phagocytosis *in vitro*. The aqueous and ethanolic extracts also induced an increase in antibody production *in vivo*.**

Key words: Immunomodulation, phagocytosis assay, *Tinospora cordifolia*.

INTRODUCTION

One of the most important components of immune system in the initial stages of the defense is phagocytosis and it is an innate response. Phagocytic cells include neutrophils, eosinophils, macrophages and monocytes that recognize foreign substances and invading microorganisms. These cells engulf and destroy the foreign substances with their intracellular killing mechanisms. When the innate immune response fails the next level of defense is provided by B cells (antibody mediated immune response) and T cells (cell mediated immune response). Any means by which these defense systems can be catalyzed/enhanced will prove to boost the overall immune response and well being of the host (Gottlieb et al*.,* 1987).

Since ancient times, people have used plants for their healing, preventive, curative, rejuvenative and immunomodulating properties (Swerdlow, 2000). In the recent years, this ancient knowledge has gained global acceptance. Use of plant compounds to enhance the phagocytic ability of macrophages and increase the antibody production by B cells have been well documented by several workers (Chopra et al*.,* 1956; Kirtikar and Basu 1975; Nadkarni et al*.,* 1976; Chopra et al*.,* 1982; Zhao et al*.,* 1991). In the system of alternative medicine several plants are reported to have immune boosting property. Among them, *Tinospora cordifolia* locally known as guduchi is of great interest for several researchers (Rege et al*.,* 1993; Thatte et al*.,* 1994; Gunabakshi et al*.,* 1998). The present study was aimed at evaluating the effect of the above plant in phagocyte mediated immunity and antibody production through *in vitro* and *in vivo* studies.

MATERIALS AND METHODS

Collection of plant

T. cordifolia was locally collected and got authenticated at the R and D Center, Cholayil Private Limited, Chennai, India.

Extraction procedure

Non-destructive cold process extraction was employed using stem powder of *T. cordifolia with* ethanol, ethyl acetate and chloroform. For aqueous extraction, heat distillation process was used. The

*Corresponding author. E-mail: msranjith@yahoo.com. Phone: +6 016 4083740.

crushed semi-dried stem powder of *T. cordifolia* was soaked separately in ethanol, ethyl acetate and chloroform in a ratio of 1:5 solute versus solvent in a conical flask. The entire set up was kept at room temperature for 24 h with intermittent shaking. After 24 h, the mixture was filtered through Whattman No.1 filter paper and the filtrate was dried to evaporate the solvent. The extract settled at the bottom was used for the experiment at varying concentration. The concentrated extracts were dissolved in dimethyl sulfoxide (DMSO) and used for further tests. In the case of aqueous extraction the stem powder was boiled in water at 1:5 ratio at 100°C for 30 min (Sonia et al*.,* 2000). After 30 min the mixture was filtered and the filtrate was stored in a refrigerator until use.

Phagocytosis assay

Preparation of macrophage suspension

Peritoneal exudate was collected from the peritoneal cavity of anaesthetized rats (Vanfurth et al*.,* 1978). The fluid was transferred to a sterile test tube and centrifuged at 500 rpm for 10 min. After removing the supernatant, the pellet was resuspended in 1 ml of cold distilled water along with 1 ml of minimum essential medium (MEM; Hi-media) to destroy the neutrophils. This suspension was centrifuged again for 10 min, the supernatant was removed and the pellet was resuspended in 2 ml of MEM. Total and differential counts of the peritoneal exudate cells were carried out. Recovery of macrophages was approximately 85 - 90%. Viability of cells was determined using a dye exclusion technique and was found to be more than 96%. The final concentration was adjusted to 10^5 cells per ml (Cline, 1973).

Preparation of yeast cell suspension

Candida albicans used in the present study is a clinical isolate from patient with acute vaginal candidiasis (Obtained from Department of Microbiology, The New College, Chennai). This strain was grown in the medium for growth of yeast phase (MGYP). This medium is composed of 0.3% malt extract, 1% glucose, 0.3% yeast extract and 0.5% peptone adjusted to pH 6.4 - 6.8. After incubation in this medium for 24 h at room temperature (28°C - 32°C), the organisms entered the yeast phase. The cells were washed twice in 0.9% saline. The yeast characteristics of *C. albicans* and their viability were checked and verified with a haemocytometer. For this purpose, 0.5 ml of 0.3% trypan blue and 0.1% eosin (3:1) were added to 0.5 ml of the washed cell suspension, mixed well for 10 min and the hemocytometer chamber was filled with this mixture. The yeast cells looked as single spherical cells or attached doublets; the filamentous forms were absent and 98% of yeast cells were viable, as shown by their ability to exclude the dye (Lehrer et al*.,* 1975). The concentration was then adjusted to 10^8 yeast cells/ml (Cline, 1973).

Assay system

The assay system consisted of 0.1 ml of *C. albicans* suspension, 0.3 ml of macrophage suspension, 0.3 ml of MEM and 0.1 ml of pooled serum obtained from normal volunteers with blood group AB. These quantities were selected to facilitate optimal pelleting of organisms with macrophages. The tubes containing the assay system were incubated at 37°C in a carbon dioxide environment for 90 min with the different solvent extracts and aqueous preparation of the plant at different concentrations; 1, 5, 10, 15 and 20 µg/ml. Smears were prepared using the cytocentrifuge, stained with Giemsa and examined by light microscopy under oil-immersion. Appropriate controls were maintained.

Two hundred macrophages were scanned from each of the test and control smears. The cells with ingested candida were counted. This was expressed as percentage phagocytosis. While counting these macrophages with phagocytosed candida, a record of all intracellular forms of candida was maintained. Three forms were observed; (a) yeast cells with homogenous blue cytoplasm, (b) yeast cells with partially or completely decolorized cytoplasm (ghost cells), and (c) filamentous forms which consist of blue staining yeast cell with one (rarely two) bud, denoting germination within the macrophage. With the given incubation length, the filamentous forms constituted less than 8% of total intracellular forms. The "ghost cells" were considered as killed/digested organisms by macrophages. The intracellular killing capacity (ICK) was expressed as the number of ghost cells present per 100 phagocytosed candidae (Rege and Dahanukar, 1993).

Antibody production

Wistar rats (150 - 200 g) were used for the experiment. Animals were housed under standard conditions of temperature (23±1°C), 12 h light/dark cycle and fed with standard pellet diet (Gold Muhor, Lipton India Ltd.) and water *ad libitum*. The animals were divided into 4 groups consisting of six animals in each group was treated separately with 10 mg/kg body weight of each of the four extracts chosen for the study. Two animals were maintained as control. The extracts at above concentrations were prepared in 5 ml of water and were fed orally to each animal for 14 days.

After 14 days of treatment the Sheep Red Blood Cells (SRBC) (collected in Alsever's solution, were washed three times in large volumes of pyrogen free 0.9% normal saline) at a concentration of 0.5×10^9 cells/ml was injected. The antibody produced against SRBC in each extract treated group and control was estimated by micro-agglutination test. An equal volume of 0.6% washed SRBC was added to doubling dilutions of sera made in microtiter trays, and the pattern of agglutination was read after the trays had stood at room temperature overnight. For the titration of 2-mercapto-ethanol (2-ME) – resistant hemagglutinin, sera were incubated with equal volumes of 0.1 M 2-ME in phosphate-buffered saline at 37°C for 1 h before the addition of SRBC.

RESULTS AND DISCUSSION

Aqueous extract of *T. cordifolia* exhibited boosting of phagocytic ability of macrophage *in vitro* at 5 µg/ml. The above extract did not show any activity at 1 µg/ml and the activity was constant above 5 up to 20 µg/ml. Ethanolic, chloroform and ethyl acetate did not show any activity up to 20 µg/ml and was found to be comparable to the control (Table 1). Treatment with the aqueous and ethanolic extracts of *T. cordifolia* at 10 mg/kg body weight for 14 days significantly increased the antibody production against SRBC in animals when compared to control. Other extracts of the above plant did not show activity (Figures 1 and 2).

Medicinal plants and their compounds have a special place in the maintenance of health and well-being of humans as well as used as therapeutic purposes (Gerzon, 1980; Jardine, 1980; Kinghorn and Balandrin 1993; Wall and Wani 1993; Cragg et al*.,* 1997). *T. cordifolia* has been extensively used in the treatment of

Table 1. Effect of *Tinospora cordifolia* in boosting phagocytosis against *Candida albicans.*

Extract	Concentration (µg/ml)/the ratio of phagocyte vs. ingested cells (mean ± SD)					
	1	5	10	15	20	Control
Aqueous	1:5 ± 0.06	1:18 ± 0.8	1:19 ± 0.9	1:17 ± 1	1:20 ± 0.8	1:6 ± 0.4
Ethanol	1:4 ± 0.4	1:6 ± 0.5	1:5 ± 0.5	1:8 ± 0.6	1:5 ± 0.9	
Ethyl acetate	1:5 ± 0.06	1:7 ± 0.08	1:7 ± 0.07	1:6 ± 0.04	1:5 ± 0.05	
Chloroform	1:4 ± 0.06	1:6 ± 0.07	1:4 ± 0.06	1:5 ± 0.05	1:7 ± 0.05	

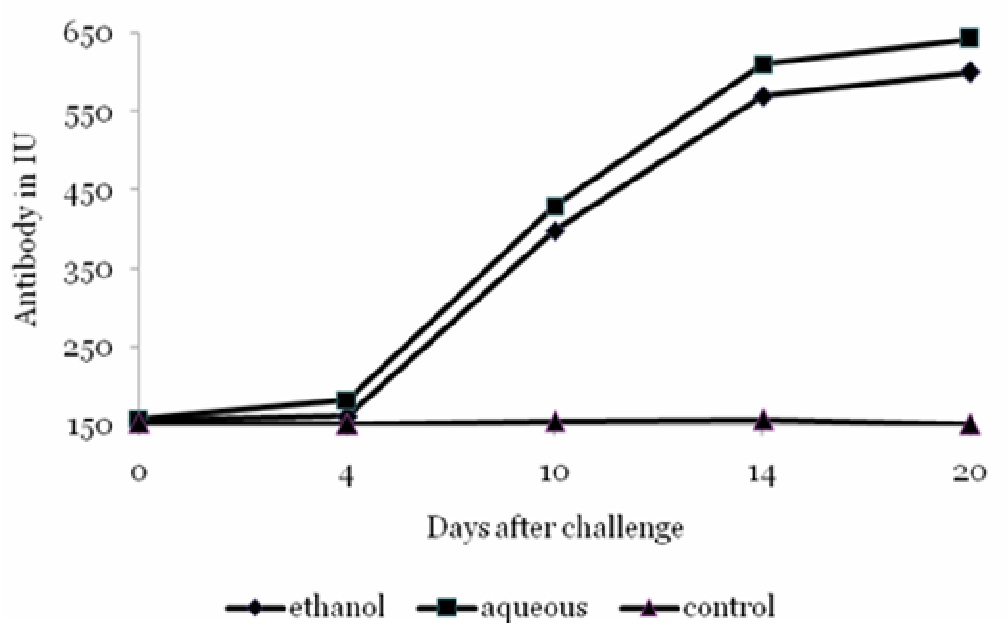

Figure 1. Effect of aqueous and ethanolic extracts of *T. cordifolia* in antibody production.

various types of haematological, hepatic, neurological, diabetic and inflammatory conditions (Bhavmishra, 2002). The immunomodulatory and anti-inflammatory property of *T.cordifolia* has been well documented (Tripathi et al*.,* 1997; Bishayi et al*.,* 2002; Kasture et al*.,* 2001; Subramanian et al*.,* 2002). The present study was aimed at the scientific validation of the immunomodulatory properties of *T. cordifolia*.

In our study, we found that the aqueous extract of *T. cordifolia* was effective in boosting phagocyte mediated immune response *in vitro*. The extract at a concentration of 5 µg/ml showed 200% increase in phagocytic ability of macrophages as compared to control. *T. cordifolia is* reported to benefit the immune system in a variety of ways (Rege et al*.,* 1993; Nagarkatti et al*.,* 1994; Kapil and Sharma 1997).

The alcoholic and aqueous extracts of *T. cordifolia* have been tested successfully for immunomodulatory properties (Thatte et al*.,* 1987; Dahanukar et al*.,* 1988; Thatte and Dahanukar 1989; Rege et al*.,* 1989; Rege et al*.,* 1999; Dikshit et al*.,* 2000; Manjrekar et al*.,* 2000). It has been also observed that extracts of several medicinal plants stimulate the macrophages (Broker and Bhatt 1953, Atal et al*.,* 1986, Thatte et al*.,* 1994). Interestingly in our study, none of the other extracts of the same plant showed above activity. We presume that active compounds responsible for enhancing phagocytosis in *T. cordifolia* may be highly polar in nature and hence present in the aqueous extract. Previous authors have reported similar findings that most of the therapeutic compounds in plants are polar in nature and require polar solvents for their extraction. However, the ethanolic extract failed to record significant activity in our present study. The active principle in *T. cordifolia* includes alkaloids, glycosides, diterpinoid lactones, steroids, sesquiterpenoids, phenolics and aliphatic compounds that may be responsible for the activity (Singh et al*.,* 2003).

To understand whether this plant possesses any other immunomodulatory properties other than phagocytosis we studied its effect on humoral antibody production. The

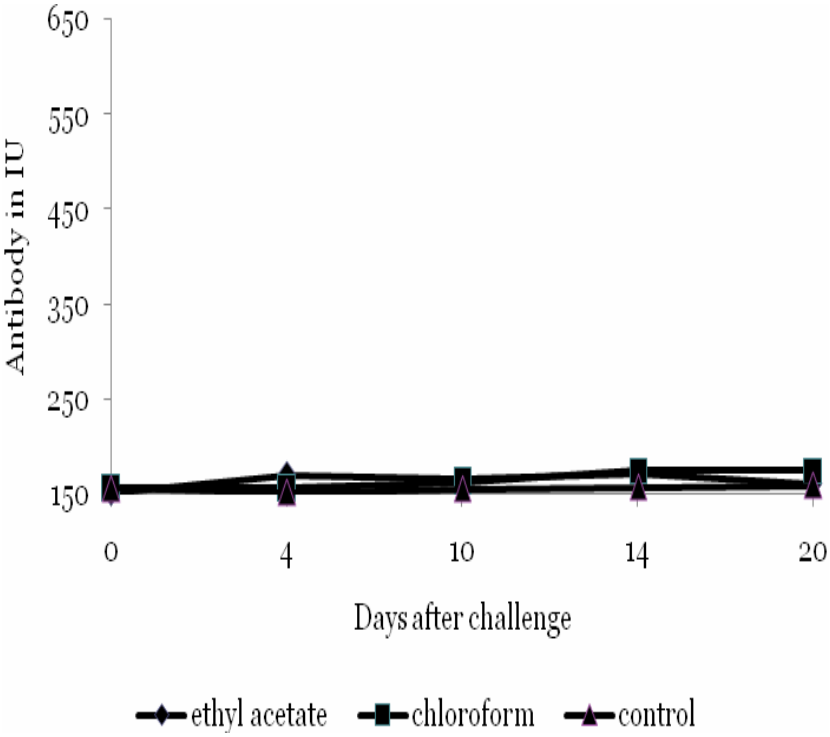

Figure 2. Effect of chloroform and ethyl acetate extracts of *T. cordifolia* in antibody production.

study reveals that aqueous and ethanolic extracts of *T. cordifolia* enhanced antibody production when SRBC was used as an antigen. In our present study, we have revalidated the immunomodulatory properties of the extracts of this plant.

Boosting of macrophage mediated innate (non-specific) immunity coupled with increased antibody production (acquired specific immunity) using a safe plant based compound could emerge as an alternate way in preventing various diseases per se. The present study reveals the multifaceted immunomodulatory potential of *T. cordifolia.* In this era of immunosuppression, steroid treatment, organ transplantation, HIV/AIDS, chemotherapy, etc, there is an inevitable need to judiciously exploit and utilize the immunomodulatory properties of the unique medicinal plants like *T. cordifolia* in modern medicine.

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