Immunomodulatory Activity of Gymnema sylvestre Leaves
S Gupta, S Pramanik, O Tiwari, N Thacker, M Pande, N Upmanyu

Abstract

To study the immunomodulatory property of the leaves of Gymnema sylvestre R.Br. (Asclepiadaceae). In our present study, the aqueous extract of Gymnema sylvestre leaves was investigated for immunomodulatory activity by assessing Neutrophil locomotion and chemotaxis test, phagocytosis of killed Candida albicans and Nitroblue terazolium tests. The extract was given at does of 10 µg/ml, 25µg/ml, 50µg/ml, 100µg/ml and 1000µg/ml. Results of in-vitro immunomodulatory activity lead to the conclusion that the aqueous extract of Gymnema sylvestre showed predominantly significant activity on in-vitro human neutrophils in all parameters, which is compared to the standard.

INTRODUCTION

Immune system dysfunction is responsible for various diseases like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and infectious diseases (Patwardhan B et al, 1990) ¹. The degree to which the patient becomes abnormally susceptible to infections by this microbial environment depends on the extent of immunosuppression. The suppression of the immune system is characterized by reduction in number and phagocytic function of the neutrophils and macrophages, as well as an impairment of the intracellular bactericidal capacity of these cells. This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infection (Rao CS et al, 1994) ².

This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs (Fulzele SV et al, 2003) ³. Many medicinal plants are known to have Immunomodulatory properties and maintain organic resistance against infection by reestablishing the body’s immune system such as Azadirachta indica (Nat VD et al, 1987) ⁴, Terminalia chebula (Sohni YR et al, 1996) ⁵, Lawsonia alba (Kulkarni SR et al, 1998) ⁶ etc. The phytochemical constituents like terpenoids, steroids, proteins and tannins (Biswas K et al, 2002) ⁷, are considered to exhibit this immunomodulatory property.

A number of in-vitro and in-vivo test system are available for assessing immunomodulatory activity. Phagocytosis is one such widely available for assessing immunomodulatory activity. Phagocytosis is one such widely used method for screening the immune response (Ponkshe CA et al, 2002) ⁸. Phagocytosis is the primary defence mechanism against any foreign bodies entering the body, which is offered by neutrophils and macrophages. The process of phagocytosis consists of sequential stage as motility, adhesion to microorganisms, ingestion of microorganisms, degranulation and intracellular killing of microorganisms (Daniel PS et al 1994) ⁹.

Gymnema sylvestre R.Br. (Asclepiadaceae) leaves, commonly known as Gudmar is a large woody, much branched climber with pubescent young parts in dry forest up to 600 meter height (Arya Vaidya Shala et al, 1997) ¹⁰. Gymnema sylvestre leaf has been widely used in Ayurvedic traditional medicine; Leaves of the plant as anti-diabetes (11), astringent, bitter, acrid, thermogenic, anti-inflammatory, anodyne, digestive and liver toinc (Kokate C.K. 1999) ¹². Tannins are the main chemical constituents present in Gymnema sylvestre and are known to possess anti-inflammatory and immunomodulatory properties (Trease EG, 1993) ¹³.

In our present study, we have attempted to evaluate immunomodulatory potency of the extract using different in-vitro methods for locomotion, phagocytic and intracellular killing potency of neutrophils, which are subsequent events involved in the process of phagocytosis by neutrophils.

MATERIALS AND METHOD
PLANT MATERIAL
The leaves of Gymnema sylvestre was collected in August 2004, Authenticated by Dr.G.R.Hedge. Professor and Head, P.G. Department of Botany, Karnataka University, Dharwad.

PREPARATION OF EXTRACTS
The shade dried leaves were subjected to physical evaluation. The standardized coarse powdered of the leaves was subjected to water extract.

PREPARATION OF THE TEST SAMPLE
Sample for in-vitro study were prepared by dissolving 20mg of crude extract in 20 ml of Distilled water and diluted with normal saline to obtain concentration ranging from 10, 25, 50,100 and 1000µg/ml.

STUDY OF IMMUNOMODULATORY ACTIVITY
NEUTROPHIL LOCOMOTION AND CHEMOTAXIS TEST
Neutrophil cell suspension was prepared in phosphate buffer saline solution (PBS) at about 10⁶ cells/ml. The lower compartment of chemo tactic chamber to a P⁰ of 7.2 e.g. chamber 1-PBS solution (control); chamber 2-casain 1mg/l (standard); and chamber 3, 4, 5, 6, 7 with different concentrations (10, 25, 50, 100 and 1000µg/ml) of test sample. The upper compartment (1ml syringe) was filled with neutrophil cell suspension and the wet filter (Millipore) 3mm pore size was fixed at the bottom of the upper compartment. The upper compartment was placed into the lower compartment and incubated at 37°C for 180 min.

The upper compartment was removed and inverted to empty the fluid. The lower surface of the filter was fixed with 70% ethanol for 2min and then stained with Haematoxylin dye for 5 min. The fixed filters were observed under microscope using 100x lens and number of neutrophil cells reached to the lower surface was counted.

PHAGOCYTOSIS OF KILLED CANDIDA ALBICANS
PREPARATION OF SUSPENSION
The Candida albicans culture was incubated in Sabouraud broth overnight and then centrifuged to form a cell bottom and supernatant was discarded. The cell button washed with sterile Hank's Balanced Salt Solution (HBSS) and centrifuged again. This was done 3-4 times. The final cell button was mixed with a mixture of sterile HBSS and human serum in proportion of 4:1. The final cell suspension of concentration 1x10⁶ was used for the experiment.

SLIDE PREPARATION
Human blood (0.2ml) was obtained by finger prick method on sterile glass slide and incubated at 37°C for 25 min to allow clotting. The blood clot was removed very gently and slide was drained slowly with sterile normal saline, taking care not to wash the adhered neutrophils (invisible). The slide consisted of polymorphonuclear neutrophils (PMNs) was flood with concentration of test sample and incubated at 37°C for 15 min. The PMNs were covered with Candida albicans suspension and incubated at 37°C for 1hr. The slide was drained, fixed with methanol and stained with Giemsa stain.

PHAGOCYTOSIS EVALUATION
The mean number of Candida cells phagocytosed by PMNs on the slide was determined microscopically for 100 granulocytes using morphological criteria. This number was taken as Phagocytic Index (PI) and was compared with basal PI of control. This procedure was repeated for different concentrations (10, 25, 50, 10 and 1000µg/ml) of test sample. Immunostimulation in % was calculated by using following equation:

Stimulation (%) = PI (test) - PI (control) x 100/PI (control)

QUALITATIVE NITROBLUE TERAZOLIUM (NBT) TEST
A suspension of leucocytes (5x10⁶ /ml) was prepared in 0.5ml of PBS solution in 7 tubes. 0.1ml PBS solution (control) and 0.1ml of endotoxin activated plasma (standard) is added to 1st and 2nd tube respectively and to the other 4 tubes added 0.1ml of different concentrations (10, 25, 50, 100 and 1000µg/ml) of the test samples; 0.2 ml of freshly prepared 0.15% NBT solution was added to each tube and incubated at 37°C for 20 min. Centrifuged at 400g for 3-4 min to discard the supernatant.

The cells were resuspended in the small volume of PBS solution. A thin film was made with the drop on the slide, dried, fixed by heating, counterstained with dilute carbol-fuchsin for the 15sec. The slide was washed under tap water, dried and focused under 100x oil immersion objective; 200 neutrophils were counted for the % of NBT positive cells containing blue granules /lumps.

RESULTS
Preliminary phytochemical investigation reveals the presence of tannins (Fayez E.K et al, 1998). Aqueous extract have showed significant activity in the some of the
parameters at higher concentrations. However highly significant were obtained in all the parameter in all the concentrations of aqueous extract.

The neutrophil locomotion and chemotaxis showed significant activity at all concentrations; extract have showed significant activity at 20µg/ml concentrations (table1). In case of phagocytosis of killed Candida albicans, extract showed significant activity even at low concentration of 10µg/ml concentrations (table2). In Nitro Tetrazolium qualitative tests, extract showed significant activity. The extract showed predominantly very good at 10µg/ml concentrations (table3)

Figure 1
Table No.1 Neutrophil Locomotion and Chemo tactic Activity

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Figure 2
Table No -2 hagocytosis of Killed Candida albicans

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DISCUSSION

Immunomodulatory activity of Gymnema sylvestre leaves significantly increased the phagocytic function of human neutrophils, when compared to control indicating the possible immunostimulating effect.

The engulfment of microorganism by leukocytes called phagocytosis and which is one of the main defence mechanisms of an organism (Daniel P.S, 1987). The Gymnema sylvestre leaves extract have significantly increased the neutrophil chemo tactic movement as indicate by the increase in number of cells reached the lower surface of filter, there by extracts acts as chemo attractant.

The final step of phagocytosis is the intracellular killing of microorganisms by the neutrophils, which is dependent on metabolic thrust generated through the hexose monophosphate shunt activation, and activation which also necessary for the normal microbicidal activity (Daniel PS et al 1994).

The extracts have significantly increased the intracellular reduction of NBT dye to formazan (deep blue compound) by the neutrophils confirming the intracellular killing property and overall metabolic integrity of phagocytosing neutrophils.

Tannins obtained from the leaves are found to possess anti-inflammatotory and immunomodulatory properties. Thus it can be also be concluded that immunomodulatory activity may be due to the presence of tannins in the Gymnema sylvestre.
References

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