In vitro effect of G. glabra and T. Cordifolia plant extracts on Phagocytosis by human neutrophils

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Abstract

The immune system is one of the most complex biological systems in the body. During infection, the immune system is under attack by a large number of viruses, bacteria and fungi. Immune response firstly involves, recognition of the pathogen or foreign material and secondly a reaction to eliminate it. Immune responses are mediated by a variety of cells such as leucocytes and lymphocytes. Alcoholic extracts of Glycyrrhiza glabra and Tinospora cordifolia were used to study their immune modulator activity. These plants are widely used in folk and ayurvedic medicine. Neutrophils when treated with plant extract showed increase in phagocytic activity.

Key words: *Glycyrrhiza glabra, Tinospora cordifolia.* immune modulator, nitroblue tetrazolium test.

Introduction

The immune system is known to be involved in the etiology as well as the patho-physiology of many diseases. Immunology is one of the most rapidly developing areas of biomedical research and promises great rewards in prevention and treatment of a wide range of disorders and diseases [1].

Since, ancient time man has used plants for healing, preventive, curative, rejuvenative and immune-modulating properties. In recent years, this ancient knowledge is gradually gaining global acceptance. Several plants are reported to have immune modulating properties. Among them *Glycyrrhiza glabra* (Jesthmadhu) and *Tinospora cordifolia* (Guduchi) are of great interest. [5]

G. glabra is a legume (related to beans and peas), native to southern Europe and parts of Asia. It is a herbaceous perennial, growing to 1 meter in height, with pinnate leaves about 7-15cm long, with 9-17 leaflets. The flowers are 0.8-1.2cm long, purple to pale whitish blue, produced in a loose inflorescence. The fruit is an oblong pod, 2-3cm long containing several seeds [4]. The flavor of liquorice (root of *G. glabra*) comes mainly from a sweet-testing compound called anethole, an aromatic, unsaturated ether compound. It also consists of another compound called glycyrrhizin, which is mainly an antiviral compound [6]. It also has stimulatory as well as hepato-protective functions [2, 3, 4].

Tinospora cordifolia is a herbaceous vine found in the tropical areas of India, Myanmar and Sri Lanka. The plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests. The leaves are heart shaped. The succulent bark is creamy white to grey in color, with deep clefts spotted with lenticels. It puts out long, slender aerial roots, often growing on mango or neem trees. Flowers are yellow, growing in lax racemes from nodes on wood. Fruits are drupes, turning red when ripe [2, 7]. The alcoholic extract of these plants has shown significant increase in macrophage phagocytic activity [9]. This plant is also known to exhibit anti bacterial activity, anti diabetic activity, hepatoprotective activity, antifungal activity, anti-inflmmatory activity, anti-spasmodic activity as well as anti-leprotic activity [7].

Many synthetic substances such as azathioprin and cyclophosphamide have suppressive and cytotoxic activity affecting the function of the immune system.

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These are alkylating agents which cause cross-linking of DNA thereby inhibiting DNA synthesis. The major drawbacks of these synthetic drugs are undesirable myelosuppresive activity [1]. Immunomodulators of herbal origin appears to be better alternative as they posses none of the side affects of the synthetic drugs.

AIM:

To study utility of the plant extracts of *G. glabra* and *T. cordifolia* as immune modulators by observing their effects on human neutrophils.

Materials and Methods

Plant materials

Root sample of *G. glabra* was obtained from market and that of *T. cordifolia* was collected from the premises of Pravara Institute of Medical Sciences. Both the plant samples were authenticated from Botany department of PVP College Loni Plant were shed dried at room temperature and crushed into fine pieces and grinded prior to use.

Extraction

The ground form of the plant sample was subjected to Soxhlet extraction using 50% ethanol as a solvent [10, 11]. Ratio of 1:5 was maintained of plant: solvent for purpose of extraction [12]. The crude extract of both plant were collected and kept for solvent evaporation at room temperature. The dried form was obtained and stored at 4°C.

Preparation of neutrophil suspension

Anticoagulated blood was mixed with half its volume in 6% dextran-T500 and kept at room temperature for 45 min or until sedimentation occured. The leukocyte rich plasma was aspirated and centrifuged at 1000 rpm for 10 min at 5°C. The cell pellet was immediately resuspended in 0.9% saline solution. A layer of ficoll-hypaque solution was added beneath the cell suspension thereby maintaining sharp interface between the two layers. These layers were centrifuged at 1400 rpm for 40 min continually. Cell pellet was subjected to hypotonic lysis by resuspending the neutrophil/ RBC pellet in 0.2% cold saline solution for 30 seconds. At the end of this period, isotonicity was restored by adding equal volume of 1.6% cold saline solution. This process was carried out twice by centrifuging at 1000 rpm for 6 min at 5°C. The pellet obtained was resuspended in PBS [Phosphate Buffer Saline] / Glucose solution to obtain a concentration of 1 x 10^7 cells/ ml of solution. The cells obtained were immediately used for further process [13]

Preparation of Reaction Mixture

To determine the Phagocytic index (PI), a reaction mixture was prepared by adding together, 0.5 ml of neutrophil suspension, 1 ml of *Candida albicans* suspension and 0.2 ml of pooled serum. Plant extract dilutions were added in a series of tubes and the mixture was incubated at 37°C, for 30 min. Thick smears were prepared, fixed with methanol and stained with Giemsa stain. Positive control was prepared without adding the plant extract. Slides were observed and immunostimulation was calculated by using following equation [1].

$$\text{Stimulation}(\%) = \frac{\text{PI(test)} - \text{PI(control)}}{\text{PI(control)}} \times 100$$

Phagocytosis assay

Preparation of Candida albicans suspension

Candida albicans culture was incubated in Sabouraud broth overnight and then centrifuged to form a cell button at the bottom with the supernatant being discarded. The cell button was washed with sterile HBSS and centrifuged again. This was carried out 4 times. The final cell button was mixed with a mixture of sterile HBSS [Hank's Balanced Salt Solution] and human serum in a proportion of 4:1. Thus a cell suspension of concentration 1 x 107 was obtained [9].

Nitroblue tetrazolium slide test (NBT)

Dilutions of plant extracts were prepared in Hank's Balanced Salt Solution (pH-7.2). Dilutions were applied on 1cm marked circle on clean grease free glass slides. The positive control used was LPS [lipo-polysaccharide] (500µg/ml).Human blood (0.2ml) was obtained by finger prick method and placed on the respective circled slides. Blood was allowed to clot by incubating at 37oc for 45 minutes in a moist chamber. Clot was carefully removed and slide was gently rinsed with PBS [Phosphate buffer saline] (pH-7.2). NBT drops 3 were added on each circle and incubated at 370c for 20miutes in a moist camber. Slide was rinsed using PBS (pH7.2), fixed in 100% methanol for 1minute and counterstained using safranin. The stained slides were scanned under oil immersion [13].

Evaluation of standard deviation

Three sets of data were collected and their results evaluated. The standard deviation from the mean value was obtained by calculating square root of variance [14].

Results :

Effect of Nitroblue tetrazolium silde test (NBT)

The results of NBT test with the 50% ethanol extracts of root of G.glabra and stem of T. cordifolia is presented in Table 1. The results showed that the reduction in Neutrophils was 73.03 % at 500 µg/ml and 90.19 % at 1000 µg/ml of ethanolic extract of G. glabra while 90.23% at 500 µg/ml and 94.29 % at 1000 µg/ml of ethenolic extract of T. cordifolia respectively.

Table 1: Effect of plant extracts on quantitative Nitroblue tetrazolium test (NBT)

Sr. No	Test Group	Conc. µg/ml	Percent NBT positive cells Mean SD
1	Positive Control (LPS)	500	88 3
2	Negative control (PBS)	—	14.45 7.5
3	G. glabra	500	73.03 4.368
4	G. glabra	1000	90.19 4.41
5	T. cordifolia	500	90.23 1.96
6	T. cordifolia	1000	94.29 4.293

Phagocytosis assay:

The result of Phagocytosis assay with ethanol extracts of root of G.glabra and stem of T. cordifolia are presented in Table 2. The results show that the activity of phygocytosis in Neutrophils was 62.27 % at 500 µg/ml and 105% at 1000 µg/ml of ethanolic extract of G. glabra while 67.92 % at 500 µg/ml and 92.15 % at 1000 µg/ml of ethanolic extract of T. cordifolia respectively.

Table 2: Effect of plant extracts on phagocytosis by neutrophils.

Sr. No.	Test Group	Conc. µg/ml	Percent stimulation Mean SD
1	Control	—	—
2	G. glabra	500	62.27± 2.8
3	G. glabra	1000	105 ± 7.7
4	T. cordifolia	500	67.92 ± 2.32
5	T. cordifolia	1000	92.15 ± 0.54

Discussion:

The immune system comprises of two mechanisms, primary innate response and secondary adaptive immune response. Though adaptive response is highly specific to the antigens, non specific innate responses play an important role in the initial stages of defense, and one of its main attributes is phagocytosis. Phagocytic cells engulf and destroy foreign substances. Cells which possess such activities are neutrophils, eosinophils, macrophages and monocytes.

The root extract of G. glabra and stem extract of T. cordifolia individually show significant increase in stimulation of phagocytosis of Candida albicans by neutrophils (Fig 1c) and also increase in the percentage of NBT positive cells containing the reduced NBT dye (Fig 1b)

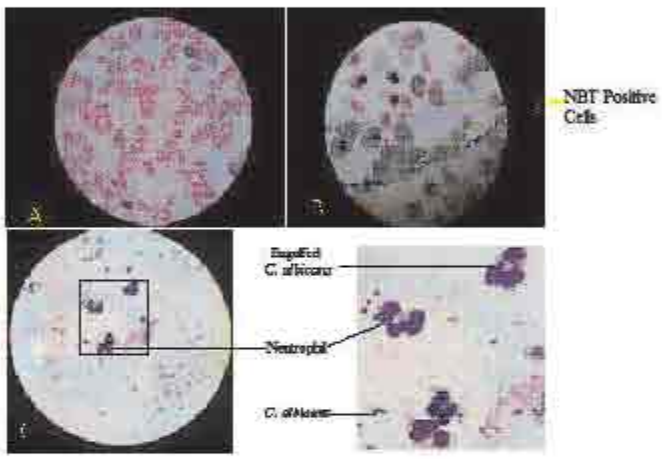

Figure1: NBT assay of human Neutrophils and Phagocytosis of *C. albicans* by human Neutrophils. A. Cells without NBT dye reduction, B. Cells with NBT dye reduction after treated with plant extracts, C. Effect of plant extracts on phagocytosis by neutrophils

when compared to control samples. The active components in G. glabra plant extracts include triterpenoids saponin, isoflavones, coumarins, triterpene sterols, glycyrrhizin, glycyrrhizinic acid, glycyrrhetol, glabrolide and isoglabrolide [4]. Whereas the active components in T. cordifolia plant extract includes alkaloids, glycosides, diterpenoid lactones, steroids, seaquiterpenoids, phenolics, and aliphatic compounds [9]. These active components are responsible for the above mentioned activity.

The evaluation of phagocytic index alone cannot prove the immune modulator activity of the plants extracts, but it gives an insight into their role as immune-modulators. The study done has showed that ethanol extract of plants, G. glabra and T. cordifolia have some role in immune modulation.

Conclusion

The results obtained showed that the root extract of G. glabra and stem extract of T. cordifolia show significant increase in phagocytic activity by human neutrophil.

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