



Effect of *Achyranthes aspera* extract on phagocytosis by human neutrophils

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Abstract

Objective: To study the effect of hydroalcoholic extract of aerial parts of *Achyranthes aspera* on neutrophil phagocytic function. **Methods:** The different concentrations (25,50 and 100 mg / ml) of extract of aerial parts of *Achyranthes aspera* was subjected to study its effect on different *in vitro* methods of phagocytosis such as neutrophil locomotion and chemotaxis, *in vitro* immunostimulant activity by phagocytosis of killed *Candida albicans* and qualitative nitro blue tetrazolium test using human neutrophils. **Results:** This preliminary study revealed that *Achyranthes aspera* extract has stimulated chemotactic, phagocytic and intracellular killing potency of human neutrophils at the concentration range of 25 - 100 mg / ml. **Conclusion:** From the results obtained it can be observed that the hydroalcoholic extract of *Achyranthes aspera* stimulates cell-mediated immune system by increasing neutrophil phagocytic function.

Key words: *Achyranthes aspera*, neutrophils, phagocytosis, chemotaxis.

1. Introduction

Achyranthes aspera Linn. of family *Amaranthaceae* is an erect herb, found throughout India, Baluchistan, Ceylon and Australia [1]. It is reported to contain triterpenoid, saponins, alkaloids, an insect moulting hormone and long chain alcohol and possess wide range of activities such as hypoglycaemic [2], antifungal [3], antifertility [4, 5], antileprotic [6], antibacterial [7],

antimicrobial [8], diuretic [9], antiperoxide activity [10].

In our present study we have attempted to evaluate immunomodulatory potency of extract of aerial parts of *Achyranthes aspera* 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.

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2. Materials and methods

2.1 Plant material:

The aerial parts of *A. aspera* were collected from the fields near Chopda in October 2002 and identified at the Department of Pharmacognosy and Phytochemistry, Smt. S.S. Patil College of Pharmacy, Chopda, Maharashtra. It was shade dried at room temperature and powdered until able to pass through sieve number 40.

2.2 Preparation of extract:

The powder was then subjected to percolation using 70 % alcohol at room temperature for 24 hours. The dark green filtrate obtained was concentrated, which was then lyophilized and stored at 4°C until further used. The crude alcoholic extract was subjected to preliminary phytochemical investigation.

2.3 Preparation of test sample:

Samples for *in-vitro* study were prepared by dissolving 2.5 gm of crude extract in 25 ml PBS (phosphate buffer solution) to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get a concentration range of 25, 50 and 100 mg/ml. Neutrophils of the blood withdrawn from normal human volunteers were used to study the activity. PBS was used as a vehicle.

2.4 Study of the immunomodulatory activity

2.4.1 Neutrophil locomotion and chemotaxis [11]

Neutrophil cell suspension was prepared in phosphate buffer saline solution (PBS) at about 10^6 cells / ml. The lower compartment of chemotactic chamber (5 ml beaker) was filled with appropriate chemotactic reagents preadjusted to pH of 7.2 e.g. chamber 1 - PBS solution (control), chamber 2-casein 1 mg / ml (standard), and chamber 3, 4 and 5, with

different concentrations (25, 50 and 100 mg/ml) of test sample.

The upper compartment (1 ml syringe) was filled with neutrophil cell suspension and the wet filter (millipore) of 3 mm pore size was fixed at the bottom of the upper compartment. The upper compartment was placed on to 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 2 min and then stained with heamatoxylin dye for 5 min . The fixed filters were observed under microscope using 100 X lens and the number of neutrophil cells reached to the lower surface of the filter was counted .

2.4.2 *In vitro* immunostimulant activity studies by slide method [11]

2.4.2.1 Preparation of *Candida albicans* suspension

The *Candida albicans* culture was incubated in Sabouraud broth overnight and then centrifuged to form a cell button at the bottom and supernatant was discarded. The cell button was 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 cell suspension of concentration 1×10^8 was used for the experiment.

2.4.2.2 Slide preparation

Human blood (0.2 ml) was obtained by finger prick method on a 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 consisting of polymorphonuclear neutrophils (PMNs) was

flooded with predetermined 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 1 h. The slide was drained, fixed with methanol and stained with Giemsa stain. Positive control was tested by preparing the slide in a same way with pooled normal human serum.

2.4.2.3 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 (25, 50, and 100 micro gram/ml) of test sample. Immunostimulation in % was calculated by using following equation :

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

2.4.3 Qualitative nitroblue tetrazolium (NBT) test [11]

A suspension of leucocytes (5×10^6 / ml) was prepared in 0.5 ml of PBS solution in 5 tubes. 0.1ml of PBS solution (control) and 0.1 ml of endotoxin activated plasma (standard) is added to the 1st and 2nd tube respectively and to the other 3 tubes added 0.1 ml of different concentrations (25, 50 and 100 mg / ml) of test sample. 0.2 ml of freshly made 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 a small volume of PBS solution.

A thin film was made with the drop on a slide, dried and fixed by heating,

counterstained by dilute carbol-fuchsin for 15 sec. The slide was washed under tap water, dried and observed under 100 X oil emulsion objective. 200 neutrophils were counted for the % of NBT positive cells containing blue granules/lumps.

2.5 Statistical analyses

The values are expressed in mean \pm SEM (n =3). The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's t - test to determine the statistical significance.

3. Results

The crude hydroalcoholic extract of *A.aspera* showed presence of triterpenoids, saponins, sterols and alkaloids. The extract of aerial parts of *A.aspera* has caused a significant increase in movement of number of neutrophils from the upper compartment to lower surface of filter in a dose dependent manner (Table.1), stimulation of phagocytosis of *Candida albicans* by neutrophils (Table.2) and also increase in percentage of NBT positive cells containing the reduced NBT dye (Table.3). When compared with control samples containing PBS solution.

Table 1.
Effect of extract of aerial parts of *Achyranthes aspera* on neutrophil locomotion and chemotaxis.

Sl.No.	Group	Concentration (mg/ml)	Mean number of neutrophil/Field
1	Control (PBS)	—	5.68 \pm 0.73
2	Standard (casein)	01	69.28 \pm 1.24*
3	<i>A.aspera</i> extract	25	44.26 \pm 1.31*
4	<i>A.aspera</i> extract	50	46.58 \pm 1.43*
5	<i>A.aspera</i> extract	100	49.21 \pm 1.48*

Values are mean \pm SEM (n=3), * P< 0.001 compared to control group.

Table 2.
Effect of extract of aerial parts of *Achyranthes aspera* on neutrophil phagocytosis.

Sl.No.	Group	Concentration (mg/ml)	Mean number of neutrophil/Field
1	Control (pooled plasma serum)	—	4.88 ± 0.84
2	<i>A.aspera</i> extract	25	28.34 ± 1.09*
3	<i>A.aspera</i> extract	50	30.12 ± 1.22*
4	<i>A.aspera</i> extract	100	32.68 ± 1.26*

Values are mean ± SEM (n=3), * P< 0.001 compared to control group.

Table 3.
Effect of extract of aerial parts of *Achyranthes aspera* on qualitative NBT test.

Sl.No.	Group	Concentration (mg/ml)	% NBT positive cells
1	Control (PBS)	—	21.32 ± 1.06
2	Endotoxin activated plasma	—	75.02 ± 0.92*
3	<i>A.aspera</i> extract	25	60.46 ± 0.84*
4	<i>A.aspera</i> extract	50	64.32 ± 1.15*
5	<i>A.aspera</i> extract	100	80.16 ± 1.07*

Values are mean ± SEM (n=3), * P< 0.001 compared to control group.

In neutrophil locomotion and chemotaxis test and qualitative NBT test, the results obtained with *A.aspera* extract were comparable with that of standard.

4. Discussion

Immunomodulatory agents can influence any constituent or a function of the immune system in a specific or non-specific manner including either innate or adoptive arms of the immune response. Recently there is an enthusiasm

towards exploration of novel group of compounds from natural sources that modulate the immune response of living systems and influence the disease process[12, 13].

In the present study extract of aerial parts of *A.aspera* significantly increased the phagocytic function of human neutrophils when compared to control indicating the possible immuno stimulating effect. The *A.aspera* extract has significantly increased the neutrophil chemotactic movement as indicated by the increase in number of cells, reached the lower surface of filter.

The ingestion of micro-organisms after coming in contact with them studied by slide method which provides a rapid and simple means of assessing the overall phagocytic process by the neutrophils.

The extract of aerial parts of *A.aspera* has significantly increased in ingestion of *Candida albicans* by neutrophils. The alcoholic extract of aerial parts of *A.aspera* has significantly increased the intracellular reduction of NBT dye to formazan (deep blue compound) by the neutrophils confirming the intracellular killing property of phagocytosing neutrophils.

From the results obtained it can be concluded that the extract of aerial parts of *A.aspera* has exhibited significant effect on phagocytosis by human neutrophils and chemotactic locomotion of neutrophils. Thus the plant can be further explored for its phytochemical profile to identify the active constituents responsible for the above-mentioned activities.

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