



Effect of *Ipomoea batatas* Linn. (Lam) root extracts on phagocytosis by human neutrophils.

K. S. Patil^{1*}, V. V. Chaudhari¹, Sachin Mahajan¹

1. Department of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy Belgaum- 590 010, Karnataka.

Abstract

Objective: To study the effect of methanol extract of *Ipomoea batatas* root on neutrophils phagocytic function. **Methods:** Different concentrations (10, 20,50,100,1000 µg/ml) of methanol extract of *Ipomoea batatas* root was subjected to study its effect on different *in vitro* methods of phagocytosis such as neutrophils locomotion and chemotaxis test, *in vitro* immunostimulant activity by slide method and qualitative nitro blue tetrazolium test using human neutrophils. **Result:** The methanolic extract of *Ipomoea batatas* roots had stimulated chemotactic, phagocytic and intracellular killing potency of human neutrophils at the concentration range of 10-100µg/ml. **Conclusions:** The methanolic extract of *Ipomoea batatas* roots stimulates cell mediated immune system by increasing neutrophil phagocytic function.

Keyword: *Ipomoea batatas*, Immunostimulant activity, neutrophils, phagocytosis.

1. Introduction

Ipomoea batatas of family Convolvulaceae is a slender prostrate vine with tuberous roots, which may be red or whiter in colour. It is cultivated throughout India [1]. It contains protein, thiamine, riboflavin, vitamin, phytosterol, resins, tannins, polysaccharides and coloring matter [2]. Phenolic compounds are also present [3].

It possesses wide range of activities such as antidiabetic [4]. It also shows vasorelaxing property [5]. It also shows antimutagenic activity [6]. It is used as diuretic and used in vitiated conditions of pitha burning sensation,

hyperpiesia, and constipation. In our present study we have attempted to evaluate immunomodulatory potency of *Ipomoea batatas* root extracts 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.

2. Materials and methods

2.1 Plant material

The fresh roots of *Ipomoea batatas* were collected from market of Belgaum in May

* Corresponding author
Email: kalpatil@yahoo.com

2005 and were positively identified by Dr. (Mrs.) M.Vasundara, Associate Professor (Hort.) and Principal Investigator (M&AP), UAS, GKVK, Bangalore - 65, India.

2.2. Preparation of extracts

The roots were shade dried at room temperature and powdered until able to pass through sieve no. 40. The dried roots were subjected to Soxhlet extraction using pet ether, methanol and after that macerated with aqueous extract for 7 days.

Concentrated under reduced pressure at 50°C using rotavapour apparatus to get viscous mass which was then lyophilized and stored at 4°C until used.

The crude extracts were subjected to preliminary phytochemical investigation [7]. It showed the presence of steroids, flavonoids, proteins, glycosides, triterpenoids, polysaccharides and tannins.

2.3. Preparation of test sample

Samples for *in vitro* study were prepared by dissolving 10mg of crude extract in 0.5ml of Dimethylsulphoxide (DMSO) and diluted with normal saline to obtain concentrations ranging from 10, 20, 50, 100, 1000 µg/ml.

2.4. Study of the immunomodulatory activity

2.4.1. Neutrophils locomotion and chemotaxis test [8]

Neutrophil cell suspension was prepared in phosphate buffer saline solution (PBS) at about 10^6 cells/ml. The lower compartment of chemotactic chamber (5ml beaker) was filled with appropriate chemotactic reagents pre-adjusted to a pH of 7.2 e.g. chamber 1-PBS solution (control), chamber 2- Casein 1mg/ml (standard) and chamber 3, 4, 5, 6 and 7 with different concentrations 10, 20, 50, 100, 1000 µg/ml. of test sample.

The upper compartment (1ml syringe) was filled with neutrophil cell suspension and the wet filter (Millipore) of 3mm pore size was fixed at the bottom of the upper compartment. The upper compartment was placed in to the lower compartment and incubated at 37°C for 180min.

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 Haematoxylin dye for 5 min. The fixed filters were observed under microscope using 100x lens and the number of filter was counted.

2.4.2 *In vitro* immunostimulant activity studies by slide method [9].

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 Hanks 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.

Slide preparation

Human blood (0.2ml) 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 1h.

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, 20, 50, 100, 1000 µg/ml.) of test sample. Immunomodulation 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.

A suspension of leucocytes (5×10^6) was prepared in 0.5ml of PBS solution in 7 tubes. 0.1ml of PBS solution (control) and 0.1ml of endotoxin activated plasma (standard) is added to 1st and 2nd tube respectively and to the other 5 tubes added 0.1ml of different concentrations (10, 20, 50, 100, 1000 µg/ml) of test sample. 0.2ml 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 the small volume of PBS solution. A thin film was made with the drop on a slide, dried, fixed by heating, counterstained with dilute carbol-funhsin for 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.

2.5 Statistical analysis

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 *Ipomoea batatas* roots extracts has caused a significant ($p < 0.001$) dose dependant, increase in movement of number of neutrophils from the upper compartment to lower surface of filter (Table 1), stimulation of phagocytosis of *Candida albicans* by neutrophils (Table 3) and also increase in % of NBT positive cells containing the reduced NBT dye (Table 2) when compared with control samples containing PBS solution. In neutrophils locomotion and chemotaxis test and qualitative

Table 1. Effects of methanolic extract of *Ipomoea batatas* roots on neutrophils locomotion and chemotaxis

Treatment	Concentration µg/ml	Mean number of neutrophils per field
Control (PBS)	-	15.25 \pm 0.25
Casein	-	156.0 \pm 2.175*
Methanolic extract of <i>Ipomoea batatas</i>	10	110.8 \pm 0.48*
Methanolic extract of <i>Ipomoea batatas</i>	20	114.3 \pm 2.175*
Methanolic extract of <i>Ipomoea batatas</i>	50	120.3 \pm 0.48*
Methanolic extract of <i>Ipomoea batatas</i>	100	130.3 \pm 0.25*
Methanolic extract of <i>Ipomoea batatas</i>	1000	12.25 \pm 0.48

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

NBT test, the results obtained with *Ipomoea batatas* root extract treatment were comparable with that of standard. These effects were observed with the concentration range of 10, 20, 50, 100 µg/ml of test sample, however it failed to show any significant effect at 1000 µg/ml concentration.

Table 2. Effect of methanolic extract of *Ipomoea batatas* roots on neutrophil phagocytosis.

Treatment	Concentration µg/ml	% stimulation
Control (PBS)	-	10.75 ± 0.47
Methanolic extract of <i>Ipomoea batatas</i>	10	18.10 ± 0.41*
Methanolic extract of <i>Ipomoea batatas</i>	20	20.5 ± 0.29*
Methanolic extract of <i>Ipomoea batatas</i>	50	22.5 ± 0.65*
Methanolic extract of <i>Ipomoea batatas</i>	100	23.50 ± 0.29*
Methanolic extract of <i>Ipomoea batatas</i>	1000	6.66 ± 0.78

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

Table 3. Effect of methanolic extract of *Ipomoea batatas* roots on qualitative NBT test.

Treatment	Concentration µg/ml	% NBT Positive cells
Control (PBS)	-	10.75 ± 0.4
Endotoxin activated plasma	-	41.49 ± 0.41*
Methanolic extract of <i>Ipomoea batatas</i>	10	34.50 ± 0.65*
Methanolic extract of <i>Ipomoea batatas</i>	20	34.75 ± 0.45*
Methanolic extract of <i>Ipomoea batatas</i>	50	38.75 ± 0.48*
Methanolic extract of <i>Ipomoea batatas</i>	100	43.33 ± 0.85*
Methanolic extract of <i>Ipomoea batatas</i>	1000	19.33 ± 0.88

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

4. Discussions and Conclusion

Immunomodulatory agents of plant and animal origin increase the immune responsiveness of the body against pathogens by activating the non-specific immune system. However there is a specific need to subject such medicinal plant

to systemic studies to substantiate the therapeutic claims made with regard to their clinical utility.

In the present study, *Ipomoea batatas* roots extract significantly increased the phagocytic function of human neutrophils, when compared to control indicating the possible immunostimulating effect. The movement of neutrophils towards the foreign body is the first and most important step in phagocytosis. The methanolic extract of roots of *Ipomoea batatas* has significantly increased the neutrophil chemo tactic movement as indicated by the increase in number of cells, reached the lower surface of filter, thereby methanolic extract of roots of *Ipomoea batatas* acts as chemo attractant. The ingestion of microorganisms after coming in contact with them, studies by slide method, provides a rapid and means of assessing the overall phagocytic process by the neutrophils. Methanolic extract of roots of *Ipomoea batatas* displayed significant increase in ingestion of *Candida albicans* by neutrophils, thereby enhancing the phagocytic process of neutrophils. 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 which is also necessary for normal microbicidal activity [10]. The methanolic extract of roots of *Ipomoea batatas* has 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. On the basis of results obtained

from the study we can conclude that methanolic extract of roots of *Ipomoea batatas* stimulates cell-mediated immune system as evident by the increase in neutrophil phagocytic activity in dose dependent manner. It is logical to suggest that it may be useful as an adjuvant in several immuno-suppressed clinical conditions.

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Reference

1. Arya Vaidya S, (1994-2002) "*Indian Medicinal Plants-A Compendium of 500 species*", Orient Longman Ltd., Madras Vol III, 218 pp.
2. Chadha YR, (1952) *The Wealth of India – A Dictionary of Indian Raw Materials and Industrial Products*, Vol V, Publication and Information Directorate, CSIR: New Delhi; 244-245 pp.
3. Yoshimoto YS. (2002) *Natural medicines*; 56(3): 121 pp.
4. Juglans R, (2003) *Phytopharm* 4(1) 126 pp.
5. Abeywardena MY, (2004) *J. Ethanopharmacol.* 92:311-316.
6. Yoshimoto M, Okuno (2002) *J. Agricultural and Food Chemistry*. 50(13): 3718-3722.
7. Khandelwal KR. (2002) *Practical Pharmacognosy, Techniques and Experiments*, IX Edn., Nirali Prakashan, Pune; 149-159pp.
8. Wilkinson PC, (1981) In: Thomson RA (Ed). *Technique in Clinical Immunology*, Blackwell Scientific Publication: London; 273-293.
9. Ponkshe CA, Indap M Madhavi. (2002) *Indian J. Exp. Biol.* 40: 1399-1402.
10. Daniel PS, (1994) In: Daniel PS, Abba IT, Tristram GP. (Eds). *Basic and clinical Immunology*, Appleton and Lange: USA; 195-215.