

## **In vitro immunomodulatory effects of some commonly used herbs and cow urine on human blood phagocytosis**

**Madhura Mukadam**

Department of zoology, Gogate Jogalekar College, Ratnagiri(MS) 415612, MS, India

### **Abstract**

Immunomodulation relates to potentiation or suppression of the immune responses of the host, depending on the requirement of the situation. An immunomodulator is a substance which stimulates or suppresses the components of immune system including both innate and adaptive immune responses. The modulation of immune system by various medicinal plant products has become a subject for scientific investigations currently worldwide. Keeping this in view, the present article highlights the importance of herbal extracts and cow urine distillate as potent immunomodulators. Present investigation was planned to evaluate the effect of aqueous extract of leaves of *Tridax procumbens*, *Ocimum sanctum*, *Aloe vera*, flowers of *Catharanthus roseus*(*Vinca rosea*), bulbs of Garlic (*Allium sativum*) and cow urine using *in vitro* method such as phagocyte locomotion, chemotaxis and immunostimulant activity by phagocytosis of killed *Candida albicans*.

**KEY WORDS:** Immunomodulation, phagocytosis, *Candida albicans*

### **INTRODUCTION:**

In modern medicine immunology, plays an important and increasing role in understanding and diagnosis of the diseases. The immune system helps an individual toward establishing an infection free state. Disturbances in the internal state of order of the host immune system results in hyperactivity, hypo-activity or loss of ability to distinguish self from non-self that would lead to diseased state. Such multidimensional response would require proper control through immunomodulation. **Immunomodulation** is a process, which alters the immune system of an organism by interfering with its functions. The immune system can be stimulated and suppressed by various physical, chemical and biological agents. These include steroids, cytotoxic drugs and various natural products of microbial, animal and plant origin (Ziauddin M. and Phansalkar N., 1996). An immunomodulator is substance that helps to regulate the immune system. In terms of medicines this approaches to therapy so much similar to prohost therapy which aims in augmenting host defense against infection by improving immunomodulator effect. Chemotherapeutic agents available today have mainly immunosuppressive activity. Most of them are cytotoxic and exerts a variety of side effects. This has given rise to stimulation in the research for locating natural resources showing immunomodulatory activity (Patil *et al.*, 2008) These are becoming very popular in the natural health were as these do not tend to boost immunity, but to normalize it. Many medicinal plants are known to have immunomodulatory properties and maintain organic resistance *against infection by re-establishing the body's immune system such Azadirachta indica* (Nat V.D., 1987), *Terminalia chebula* ( Sohni Y. R., 1996), *Lawsonia alba* (Kulkarni S. R., 1998) *etc.* the phytochemical constituents like terpenoids, steroids, proteins (Pallabi D. E., 1998) and tannins (Biswas, K., 2002) are considered to exhibit

this immunomodulatory property. The present study was therefore, undertaken to explore the immunomodulatory potency of aqueous extracts of various plant materials and cow urine using *in-vitro* methods for locomotion, phagocytic and intracellular killing potency of neutrophil through the process of phagocytosis.

A number of *in vitro* and *in vivo* test systems are available for assessing immunomodulatory activity. Phagocytosis is one such widely used method for screening the immune response (Ponkshe, C. A., 2002). 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 stages such as motility, adhesion to microorganisms, ingestion of microorganisms, degranulation and intracellular killing of microorganisms (Daniel, P. S. 1994).

Neutrophils play an important role in host immune mechanism system. The neutrophilic phagocyte system has many advantages. They are attracted by a limited number of stimuli, which generally signal the presence of tissue injury of unknown reason. Even the foreign bodies, thermal or chemical burns, bacterial infections and other types of injuries can provoke an intense neutrophil response. Moreover neutrophils are more effective at killing certain bacteria and their ability to digest cellular debris and exogenous particulate matters provide an important step in the host defense mechanism (Berne and Levy, 1988)

## MATERIALS AND METHODS:

### Plant material:

The leaves of *Tridax procumbens*, *Ocimum sanctum*, *Aloe vera*, flowers of *Catharanthus roseus*(*Vinca rosea*) and bulbs of Garlic (*Allium sativum*) collected from the nearby areas of Ratnagiri and were positively identified from the Botany Department of Gogate Jogalekar College, Ratnagiri.



*Tridax procumbens*



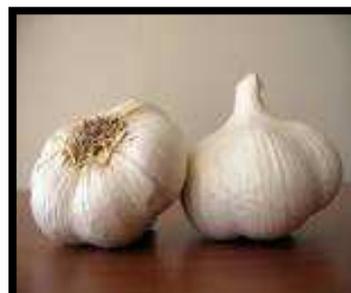
*Ocimum sanctum*(Tulsi)



*Aloe vera*



*Catharanthus roseus*  
(*Vinca rosea*)



*Allium sativum*(Garlic)

### **Preparation of extract:**

The Fresh leaves of the plant were shade dried and then milled to coarse powder by mechanical grinder and then used for extraction. The powdered material was then subjected to exhaustive extraction using 95% ethanol in a soxhlet apparatus. The dark green liquid extract so obtained was concentrated under vacuum and the resulting dried extract was lyophilized and preserved in desiccators until further use. The crude ethanol extract was then subjected to preliminary investigations. Sample for *in vitro* study were prepared by dissolving 2.5 gm of crude extract in 25 ml PBS (Phosphate buffer solution) to obtain a 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.

### ***In vitro* immunostimulant activity studies by slide method. (Wilkinson, 1981)**

#### **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 (HBBS) and centrifuged again. This was done 3-4 times. The final cell button was mixed with a mixture of sterile HBBS and human serum in proportion of 4:1. The cell suspension of concentration  $1 \times 10^8$  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  $37^{\circ}\text{C}$  for 25 min to allow clotting. The blood clot was removed very gently and slide was drained with sterile normal saline. The slide consisting of Polymorphonuclear Neutrophils (PMNs) was flooded with predetermined concentration of test sample and incubated at  $37^{\circ}\text{C}$  for 15 min. The PMNs were covered with *Candida albicans* suspension and incubated at  $37^{\circ}\text{C}$  for 1 hour. 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.

#### **Phagocytosis evaluation**

The mean number of *Candida* cells phagocytosed by polymorphonuclear neutrophils (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 the control. This procedure was repeated for different concentration (25, 50 and 100 mg/ml) of test sample.

Immunostimulation in % was calculated by using following equation:

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

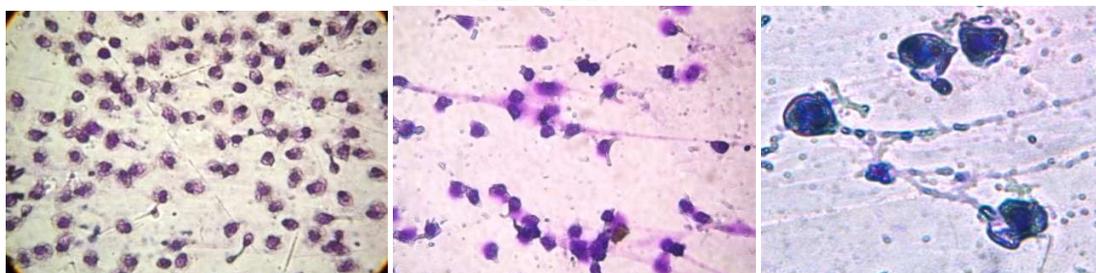
### Qualitative nitroblue tetrazolium test (NBT) (Wilkinson, 1981)

A suspension of leucocytes ( $5 \times 10^6/\text{ml}$ ) was prepared in 0.5 ml of PBS solution in 5 test 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 of test sample. 0.1 ml of different concentration (25, 50 and 100 mg/ml) of test samples were added. 0.2ml of freshly made 0.15% NBT solution was added to each tube and incubated at 37° C for 20 min. Centrifuged at 400 rpm for 3-4min 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, counter stained by dilute Carbol-fuchsin for 15 sec. The slide was washed under tap water, dried and observed under 100X oil emulsion objective. 200 neutrophils were counted for the % of NBT positive cells containing blue granules/lumps.

### Statistical analysis

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

### RESULTS:



Magnification(40X)

(40X)

(100X)

### Phagocytic activity of Neutrophils

Table 1 : Effects of various plant extracts on neutrophil phagocytosis

Sr. No.	Name of Extract	Concentration (mg/ml)	% Stimulation
1	Control(Pooled plasma serum)	-	4.80 ± 0.80
2.	<i>Tridax procumbens</i>	25	18.65±1.23
		50	20.14±1.66
		100	22.65±0.98
3.	<i>Ocimum sanctum(Tulsi)</i>	25	29.39±1.10*

		50	34.15±1.30*
		100	38.67±1.28*
4.	<i>Aloe vera</i>	25	11.47±1.56
		50	13.29±1.34
		100	15.56±1.22
5.	<i>Catharanthus roseus</i> ( <i>Vinca rosea</i> )	25	14.33 ±1.26
		50	16.12±1.45
		25	18.34±1.23
6.	<i>Allium sativum</i> (Garlic)	25	23.66±1.45*
		50	25.33±1.66*
		100	29.65±1.24*

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

### DISCUSSION:

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 need to subject such medicinal plants to systematic studies to substantiate the therapeutic claims made with regard to their clinical utility. 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. In the present study, the immunomodulatory activity of five different plant extract was evaluated by using qualitative NBT test and neutrophil phagocytosis methods. The engulfment of microorganisms by leucocytes called phagocytosis and which is one of the main defence mechanisms of an organism (Daniel, P. S., 1987). The movement of neutrophils towards the foreign body is the first and most important step in phagocytosis. The *Ocimum sanctum* leaves extract has significantly increased the neutrophil chemotactic movement as indicated by the increase in number of cells, reached the lower surface of filter, thereby *O. sanctum* leaves extract acts as chemo attractant. The ingestion of micro organisms after coming in contact with them, studied by slide method, provides a rapid and simple means of assessing the overall phagocytic process by the neutrophils. The *O. sanctum* leaves extract 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 micro organisms by the neutrophils, which is dependent on metabolic thrust generated through the hexose monophosphate shunt activation, an activation which is also necessary for normal microbicidal activity ( Daniel, 1994). The *O. sanctum* leaves extract 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.

*Allium sativum*, *Tridax procumbens* and *Catharanthus roseus* (*Vinca rosea*) showed decreased phagocytic function respectively. *Aloe vera* extract has exhibited less significant activity. Hence, from the result obtained, it can be concluded that the aqueous extract of the leaves of *Ocimum sanctum* has exhibited significant effect on phagocytosis

by human neutrophils. Thus the plant can be further explored for its phytochemical profile to identify the active constituents responsible for above mentioned activity.

#### REFERENCES:

- Antos L.B.**, Yamada F.T. and Scheinberg M.A. (1985). Monocytes and lymphocyte interaction in patients with advanced cancer, evidenced for deficient IL-1 production, *Cancer*, 56, 1553-1558.
- Berne R. M** and Levy M. N. (1988). Blood components. 2<sup>nd</sup> Ed. The C. V. Mosby Co., Washington D. C. 1988.
- Biswas K., Chattopadhyay I., Banjee R. K. and Bandyopadhyay U. (2002). *Current Science*, 82(11), 1136- 1345.
- Daniel P. S.** (1987). Basic and Clinical Immunology Vol 6 Appleton and Lange USA.
- Daniel P. S.** (1994) In: Danierl P.S., Abba I. T. and Tristram G. P. (Eds.). Basic and Clinical Immunology, Appleton and Lange : USA; 195-215.
- Devasgayam T.P.A.** and Sainis K.B. (2002). Immune system and antioxidants, especially those derived from Indian Medicinal Plants, *Indian Journal of Experimental Biology*; 40, 639-655.
- Kulkarni S. R.** and Karande V. S.(1998). *Indian Drugs*, 37(7), 427-433.
- Mayer K.H.** and DeTorres O.H. (1985). Current guidelines on the use of antibacterial drugs in patients with malignancies, *Drugs* 29, 262-279.
- Nat V. D.**, Klerx J. P., Dijk V. H. and Desilva K. T.(1987). *Ethnopharmacol*, 19, 125-131.
- Pallabi D. E.** and Dasgupta S. C.(1998). Immunopotentiality and immunoprophylactic activities of immune 21-A herbal products. *Indian Journal of Pharmacol.* 30(3), 163-168.
- Patil K. S.** Majumdar P. and Wadekar, R. R. (2008). Effect of *Enhydra fluctuans* Lour. Leaf extract on phagocytosis by human neutrophils *J. Nat Rem* 8:76-81.
- Ponkshe C. A.** and Indap M . M. (2002). *Indian J. Exp. Biol.*, 40, 1399-1402.
- Sohni Y. R.** and Bhatt, V. S. (1996). *J. Ethnopharmacol*, 54, 119-124.
- Thatte U.M. and Dahanukar S.A.(1986). Ayurveda and Contemporary Scientific thought, *Trends Pharmacology Sciences*, 7, 2.
- Ziauddin M. and Phansalkar N. (1996). Studies on the immunomodulatory effects of Ashwangandha, *Journal of Ethnopharmacology*, 50: 69-76.