

Immunomodulatory activities of the Non-dialyzable latex fraction (NDL) from *Calotropis procera* (Ait.) R. Br.

Prashant Bagherwal

Institute of Pharmacy, Vikram University, Ujjain (M.P.), India.

Corres.author: prashant_bagherwal@rediffmail.com

Mob.- 09685024006

Abstract: The objective of present study was to scrutinize the immunological potential of the latex of *Calotropis procera* against sheep red blood cells (SRBC) as antigens were investigated in Wistar albino rats by studying cell-mediated, delayed type hypersensitivity reaction (DTH), humoral immune response, macrophage phagocytosis assay were carried out by carbon clearance method and *e. coli* induced bacteremia sepsis induced for survival study, by means of percent mortality in the experimental animals. The latex was fractionated according to water solubility and molecular size of its components. The fractions were named as non-dialyzable latex (NDL) was corresponding to the major latex proteins and other dialyzable latex (DL) corresponding to low molecular size substances and rubber latex (RL) which was highly insoluble in water. The HA titre levels were quantified by primary & secondary humoral immune response in rats. The fractions induced production of antibodies titre level was significantly ($p < 0.05$) increases in response to SRBC. In addition immunostimulation was counteracted by up regulating macrophage phagocytosis in response to carbon particles, when rats received NDL fractions by oral route displayed considerable immunological response. Oral administration of NDL fractions, dose dependently increased immunostimulatory responses. DTH reaction was found to be augmented significantly ($p < 0.05$) by increasing the mean foot pad thickness after 48 hrs. In the survival study the protective effects of NDL as compared to control group I and negative control group II in *E. Coli* induced peritonitis has shown 50% & 66.6% mortality while pretreated groups III, IV and V, with NDL has reduced mortality in rats injected with $1 \times 10^{(8)}$ *E. coli* intraperitoneally from 16.6% to 0.0% and 0.0% respectively. According to the results, the NDL proteins of latex of *Calotropis procera* can provoke immunological activities. The NDL has previously shown to display anti-inflammatory and anticancer activities, it should be relevant to determine whether NDL could induce such activities when assayed by oral route.

Keywords: NDL latex proteins of *Calotropis procera*, Immunomodulation, Phagocytosis, Cellular & humoral immune response.

INTRODUCTION:

There is an increasing interest concerning the plant *Calotropis procera* due to innumerable relevant biological activities found in its latex. It would not be surprising *Calotropis procera* to produce more latex than *Hevea brasiliensis* in a comparative basis. In India many people seems to be inserted in medicinal uses of the crude latex mainly to combat skin infections by topical application, Rasik et al. reported¹ that latex of *Calotropis procera* has healing potential on dermal wounds in Guinea pigs. However, adverse effects of latex of *Calotropis procera* have also been reported as cited before Alencar et. al.² Furthermore, no information is available concerning possible immunological effects caused by latex *Calotropis procera*. We have accumulated efforts to fractionate the NDL fraction from the latex of *Calotropis procera* in attempt to separate interesting activities from undesired properties. The NDL fractions was obtained after executing an ordinary protocol based on centrifugation and dialysis that gave rise to a fraction (NDL) that anti-inflammatory and antinociceptive properties were joined Alencar et al³; Soares et al⁴.

The relevance of study not known yet whether the latex of *Calotropis procera* displays immunological effects and if does, what could be the contribution of the latex proteins in such an undesired event. This question has motivated the present study as well the fact that the latex has been shown to exhibit anti-inflammatory and anti-cancer activity when tested by oral route in rats.⁵ Choedon et al.⁶ As it could be expected more expressive immunological responses were verified in animals that were sensitized by oral route. It has been already determined that all anti-inflammatory potency of the whole latex of *Calotropis procera* is present in this fraction³

We have estimated that rubber fraction and soluble proteins comprises 84% and 9% of latex of *Calotropis procera*. Our recent results suggest that a soluble protein fraction purified from the polyisoprene fraction exhibits anti-inflammatory and analgesic properties while strong inflammatory reaction is induced by another latex fraction.^{2,3,4} It would be thus interesting to investigate the immunological properties and allergenic effects of these fractions since the whole latex of *Calotropis procera* has been previously reported to possess strong inflammatory action⁷ In addition we have determined the presence of peptides in NDL although any further biochemical or functional characterization has been done on latex of *C. Procera*. The molecules in NDL could certainly explain presence of detectable immunological response. Even NDL elicited

antibodies synthesis that evidences the presence of proteins in these fractions able to induce immune responses.

In a recent study, we have submitted groups of rats to receive doses of NDL by oral route during the study to evaluate sub-chronic effects. Surprisingly, white blood cell profile was altered in which lymphocytes were found to be augmented. Another previous study had already cited similar observation, although the whole latex was the sample assayed instead of the richest protein fraction of the latex.⁸ these results suggest that latex proteins may act as an immune stimulant. The results found with NDL fraction reinforces the idea that immune adjuvant potentials may be expecting by the responses displayed by NDL fractions.

The present study describes immunological effects induced by NDL a latex fractions of *Calotropis procera* in rats (unpublished results). In this study the NDL was evaluated for the detection of an immune response in following immunomodulatory models such as Humoral & cell mediated immune response, Phagocytic activities, and *E. coli* induced bacteremia for survival study to their ability of stimulating specific antibodies synthesis in rats by oral routes of administration.

MATERIAL AND METODS :

Plant Materials: The Latex of *Calotropis procera* (Ait.) R.Br. was collected from Ujjain (M.P.), INDIA in the month of Nov.2008. The plant was identified with the help of available literature and authenticated by Mr. S.K. Sirsad, Suprintendent / Botanist, A voucher specimen was deposited in the Horticulture & agriculture Department, Ujjain (M.P.)

Latex Preparation:

The crude latex of non-cultivated and healthy plants was collected in distilled water (ratio 1:1) in plastic tubes that were shaken gently, closed and maintained at environmental temperature (25–28 °C) until handled in the laboratory. The samples were initially submitted to centrifugation at 25 °C during 10 min in a bench centrifuge. The precipitated rubber was separated of the soluble phase that was submitted to dialysis against water using membranes of 8000 molecular weight cut-off. During the first hour of dialysis the volume of water used to dialysis was exactly the volume of latex material into dialysis tubes. This water of dialysis was pooled and named dialyzable latex (DL). After additional 60 h of continuous dialysis the membrane

retained material was newly centrifuged as before and the very clean and water soluble material was separated of the new pellet and named non-dialyzable latex (NDL). The new pellet was joined to that of the first centrifugation and named rubber latex (RL). The three latex fractions were thus freeze-dried and used in the further determinations

Toxicity Studies⁹

Acute Toxicity: Acute toxicity test was performed on 3 groups of Wistar albino rats consisting of six animals per group. The NDL fraction from the latex of *Calotropis procera* was administered orally in the doses of 2.5, 0.5 and 10 mg/kg, body weight. The behavioral changes, symptoms of toxicity and mortality were observed for 24 h (WHO, 1967).

Test Animals:

Healthy Wistar albino rats (120-150 g) of either sex were selected for the study. The animals were fed on commercial diet (Hindustan lever pellets, Bangalore) and water *ad libitum*, they were acclimated to laboratory hygienic condition for ten days before starting the experiment. The experimental protocol and animal house has been approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government, (CPCSEA)

Drugs and Treatment

The method of Pyrogallol induced immunosuppression¹⁰ was employed with slight modification to study the Immunomodulatory potential of the extract. Animals were randomly divided in to five groups, consisting of six animals each.

Animals: 5 groups of 6 animals are used.

Treated group I - Control group treated with CMC suspension through orally from Day 1 to Day 22. *sRBC* ($.5 \times 10^9$ cells/100g i.p.) given on day 7 for immunization and day 13 for challenge or untreated with NDL & Pyragallol.

Treated group II - Pyragallol 50 mg/kg, i.p. once daily from day 1 to 7 + CMC suspension through orally from Day 1 to Day 22. *sRBC* ($.5 \times 10^9$ cells/100g i.p.) given on day 7 for immunization and day 13 for challenge.

Treated group III - Pyragallol 50 mg/kg, i.p. once daily from day 1 to 7 + oral doses of 2.5 mg/ml of NDL given through orally from Day 1 to Day 22. *sRBC* ($.5 \times 10^9$ cells/100g i.p.) given on day 7 for immunization and day 13 for challenge.

Treated group IV- Pyragallol 50 mg/kg, i.p. once daily from day 1 to 7 + oral doses of 5 mg/ml of NDL given through orally from Day 1 to Day 22. *sRBC* ($.5 \times 10^9$ cells/100g i.p.) given on day 7 for immunization and day 13 for challenge.

Treated group V- Pyragallol 50 mg/kg, i.p. once daily from day 1 to 7 + oral doses of 10 mg/ml of NDL given through orally from Day 1 to Day 22. *sRBC* ($.5 \times 10^9$ cells/100g i.p.) given on day 7 for immunization and day 13 for challenge.

IMMUNOLOGICAL METHODS :

Humoral immune response¹¹

On Day 13 and 20, blood was withdrawn from the retroorbital plexus of all antigenically challenged rats. Twenty-five μ l of serum was serially diluted with 25 μ l of phosphate-buffered saline. SRBC (0.025×10^9 cells) were added to each of these dilutions and incubated at 37°C for one hour. The rank of minimum dilution that exhibited hemagglutination was considered as an antibody titer. The level of antibody titer on Day 13 of the experiment was considered as the primary humoral immune response and the one on Day 20 of the experiment was considered as the secondary humoral immune response.

Cellular immune response¹¹

This was assayed by the footpad reaction method. The edema was induced in the right paw of rats by injecting SRBC (0.025×10^9 cells) in the sub-plantar region on Day 20. The increase in the paw volume in 48 h, i.e. on Day 22 was assessed on digital plethysmo-meter. The mean percentage increase in paw volume was considered as delayed type of hypersensitivity and as an index of cell-mediated immunity. The volume of the left hind paw, injected similarly with phosphate-buffered saline, served as a control.

Phagocytosis by Carbon clearance assay in mice¹²

On 22th Day, this assay was carried out according to the method. Mice were divided into four different groups of six each. Group I, II, III, IV & Vth on day, 22. All animals were injected with 0.25 ml of Indian ink [which was diluted with PBS (pH 7.4) to eight times before use] into the tail vein on 22th day after a warm up period of 15 min at 37°C. Two drops of blood was collected at different time interval of 5, 10, 15 & 20 minutes by tail nipping. Blood was lysed with 3 ml distilled water and centrifuged ($2000 \times g$, 10 min, and

10C). Absorbance was recorded at 650 nm by spectrophotometer [UV 1601, Shimadzu, Japan]

Survival studies¹³

30Wistar rats (150–200 g) were divided into 4 groups. On the test day, 1×10^{18} viable E.coli were injected intraperitoneally. A clinical isolate of E.coli maintained on nutrient agar in our laboratory was used for all studies. For each experiment, E. coli was inoculated in nutrient broth for 18 hours at 35°C in a shaking water bath at 50 rpm. Bacterial cell count was adjusted to give 1×10^{18} org/ml. Survival was monitored hourly during the first 24 hours and daily after that for a further 10 days.

RESULTS:

Pyrogallol induced suppression of humoral as well as cell mediated immune response were significantly attenuated by daily oral treatment with NDL fractions from the latex *Calotropis procera* 2.5, 5, 10 mg/ml orally treated group exhibited similar attenuation of the suppression in immune responses. NDL fractions at the dose of 10 mg/kg on T-lymphocytes and other cell types required for

expression of humoral response to SRBCs, as evidenced by marked increase in haemagglutination titres in rats was also observed the effect of NDL on primary and secondary antibody response on HA titre is shown in [Table 1]. Primary antibody response on day 13th in (10 mg/ml/orally) treated group with normal immune status shown by group II significant increase ($P < 0.01$) in HA titer titre when also compared with the control group I. A significant decrease ($P < 0.01$) in the antibody titre was observed in the Pyragallol treated (Negative control) group II when compared with the control group I. In immunosuppressed groups, where the immunity was suppressed by administration of Pyragallol(50 mg/kg/i.p.) NDL administration produced a significant ($P < 0.01$) rise in the antibody titre when compared with the Pyragallol treated negative control group II. Secondary antibody titre on 20th day in NDL treated groups with normal immune status group showed a significant rise ($P < 0.01$) in the antibody titre when compared with the control group I. In the immunosuppressed groups III,IV and V groups where the immunity was suppressed by Pyragallol, On 20th day NDL showed a significant rise ($P < 0.01$) in HA titer when compared with the I and II group.(Table 1)

Table 1. Influence of NDL fractions from the latex *Calotropis procera* on the humoral immune and Phagocytosis responses to sheep RBC

Group/Treatment	Antibody Titer Level		Phagocytic Index	
	Primary on 13 th day	Secondary on 20 th day	After 15 min.	After 30 min.
Gr. I. Control group. CMC suspension (Untreated with NDL & Pyragallol)	7.66± 0.577	11.33± 0.88	0.086 ± 0.015	0.0688 ± 0.016
.57.Gr.II (Untreated with NDL) but immunosuppressed with Pyragallol 50 mg/kg	3.33± 0.580**	7.66± 0.34**	0.0648 ±.039	0.0573±.043
Gr. III (Treated) with oral doses of 2.5 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	5.33 ± 0.38	9.35 ± 0.39	0.135 ±0.013	0.118 ±0.023
Gr. IV (Treated) with oral doses of 5 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	5.66 ± 0.33	9.66 ± 0.43	0.138 ±.012	0.122±.018
Gr. V (Treated) with oral doses of 10 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	7.33 ± 0.57 ^{##}	11.33 ± 0.47 ^{##}	0.154 ±.017	0.129±0.021

(n=6) ± Standard deviation, $p < 0.05$, when compared with control group in all cases (statistics; one way ANOVA followed by Dunnett's t test)

Table 2. Influence of NDL fractions from the latex *Calotropis procera* on the Cellular immune response to sheep RBC and Survival study to *E. coli*. Induced bacteremia sepsis.

Group/Treatment	Cellular immune response		Survival study.	
	% DTH \pm SEM After 24 hrs.	% DTH \pm SEM After 48 hrs.	% Mortality After 24 hrs	% Mortality After 48 hrs
Gr. I. Control group. CMC suspension (Untreated with NDL & Pyragallol)	22.51 \pm 0.5	12.13 \pm 0.3	16.6	50
Gr.II (Untreated with NDL) but immunosuppressed with Pyragallol 50 mg/kg	16.28 \pm 0.7***	11.24 \pm 0.4*	16.6	66.6
Gr. III (Treated) with oral doses of 2.5 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	18.68 \pm .49***###	11.34 \pm .43	00	16.6
Gr. IV (Treated) with oral doses of 5 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	18.08 \pm .55***###	10.19 \pm .52***#	00	00
Gr. (Treated) with oral doses of 10 mg/kg of NDL and immunosuppressed with Pyragallol 50 mg/kg.	17.17 \pm .78***	10.26 \pm .64***#	00	00

(n=6) \pm Standard deviation, $p < 0.05$, when compared with control group in all cases (statistics; one way ANOVA followed by Dunnett's test)

It reveals effect of drug at the dose of 2.5, 5, 10 mg/ml was found to suppress delayed time hypersensitivity reaction induced by SRBCs in rats. The effect of NDL on cell mediated immune response by DTH induced footpad edema is shown in (Table 2). On 20th day Pyragallol treated (Negative control) group II showed significant ($p < 0.01$) decrease in the mean difference of paw thickness when compared to control group I. In the all groups of mice with normal immune status, of NDL (5 mg/ml, 10 mg/ml/orally) showed significant ($p < 0.01$) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with control group I. In the all groups of rats NDL treated and immunosuppressed with Pyragallol groups III, IV & V showed significant ($p < 0.01$) potentiated DTH response in terms of increase in the mean difference of paw thickness when compared with Pyragallol (negative control) group II.

In the present study; phagocytic index were determined by carbon clearance method. When the carbon suspension is injected intravenously, the rate of clearance of carbon from blood by macrophage is

governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. This study demonstrates that NDL fractions from the latex *Calotropis procera* treatment is potentiated more the phagocytosis of reticulo endothelial system increase in clearance response reveals stimulation of the reticuloendothelial and macrophages activity. The effect of NDL (5, 10 mg/ml/orally) on the phagocytic activity by the carbon clearance test is shown in [Table 1]. The phagocytic activity of the reticulo-endothelial system is generally measured by the rate of removal of carbon particles from the blood stream. In carbon clearance test, NDL treated immunosuppressed, all groups III, IV & V, exhibited significantly high phagocytic index. The phagocytic index of NDL (5 mg/ml, 10 mg/ml/orally) showed significant ($p < 0.01$) increased in phagocytic index when compared to control & negative control group.

DISCUSSION

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppressant implies mainly to reduce resistance against infections,

The results obtained in the present study indicate that NDL is a potent immunostimulant, stimulating specific and non-specific immune mechanisms. The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effectors' of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells.

To evaluate the effect of NDL on humoral response, its influence was tested on sheep erythrocyte specific HA titre in mice¹⁴ Pyragallol showed significant inhibition in antibody titre response, while NDL doses counteract the suppression of both primary and secondary humoral responses induced by pyragallol.² This indicates the enhanced responsiveness of macrophages, T and B lymphocyte subsets involved in antibody synthesis. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines).¹⁵ DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induce vasodilatation, macrophage accumulation, and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing. When activated TH1 cells encounter certain antigens, viz. SRBCs. They secrete cytokines that induce a localized inflammatory reaction called delayed type hypersensitivity. DTH comprises of two phases, an initial sensitization phase after the primary contact with SRBC antigen. During this period TH1 cells are activated and clonally expanded by APC (antigen presenting cells) with class II MHC molecule (eg. langerhans cells and macrophages are APC involved in DTH response). A subsequent exposure to the SRBCs antigen induces the effector phase of the DTH response, where TH1 cells secrete a variety of cytokines that recruits and activates macrophages and other non specific inflammatory mediators. The delay

in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages.¹⁶ Therefore, increase in DTH reaction in mice in response to T cell dependent antigen revealed the NDL fractions on T cells.

The role of phagocytosis is the removal of microorganisms and foreign bodies, dead or injured cells. The increase in the carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by the opsonisation of parasites with antibodies and complementing C3b, leading to a more rapid clearance of parasites from the blood.¹⁷ NDL appeared to enhance the phagocytic function by exhibiting a clearance rate of carbon by the cells of the reticulo-endothelium system. Cytokines are secreted by activated immune cells for marinating and extravasations of the phagocytes mainly polymorphonuclear neutrophils. Significantly evoked increase in the adhesion of neutrophils to nylon fibers which correlates to the process of margination of cells in blood vessels.¹⁸

In the present study, NDL evoked a significant increase in percent of neutrophils. This may help in increasing immunity of body against microbial infections. Since NDL was found to have a significant immunostimulatory activity on both the specific and non-specific immune mechanisms. The significant increase in the immunostimulatory activity of NDL could be attributed to the presence of protein analysis of NDL suggests that still retain residual proteins found in NDL that could explain its Immunological and allergenic responses induced by latex fractions (NDL) of *Calotropis procera* contains three types of proteins PI, PII, and PIII which is biologically active constituents have been identified compounds³. Therefore, the latex of plant holds promise for being used as an immunostimulating agent and an in-depth study on NDL fractions from the latex *Calotropis procera* effective as immunomodulating entities from the plant is warranted to determine the potent immunostimulating fraction from *Calotropis procera*. Thus, the study validates the traditional use of *Calotropis procera* as in medicine system.

CONCLUSION

In conclusion, the results obtained in the present study show those NDL fractions from the latex *Calotropis procera* produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals and suggest its therapeutic

usefulness in disorders of immunological origin. Further studies using *in vivo* and *in vitro* models of immunomodulation are needed to confirm the

Immunomodulatory activity of NDL fractions from the latex *Calotropis procera* and its mechanism of action.

REFERENCES:

1. Rasik, A.M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M.P., Srivastava, S., Jain, H.K., Kulshrestha, D.K., 1999. Healing potential of *Calotropis procera* on dermal wounds in Guinea pigs. *J. Ethnopharmacol.* 68, 261–266.
2. Alencar, N.M.A., Figueiredo, I.S.T., Vale, M.R., Bitencurt, F.S., Oliveira, J.S., Ribeiro, R.A., Ramos, M.V., 2004. Anti-inflammatory effect of the latex from *Calotropis procera* in three different experimental models: peritonitis, paw edema and hemorrhagic cystitis. *Planta Med.* 70, 1144–1149.
3. Alencar et al., Alencar, N.M.N., Oliveira, J.S., Mesquita, R.O., Lima, M.W., Vale, M.R., Etchells, J.P., Freitas, C.D.T., Ramos, M.V., 2006. Pro-and antiinflammatory activities of the latex from *Calotropis procera* (Ait.) R. Br. is triggered by compounds fractionated by dialysis. *Inflamm. Res.* 55, 559–564.
4. Soares, P.M., Lima, S.R., Matos, S.G., Andrade, M.M., Patrocínio, M.C.A., Freitas, C.D.T., Ramos, M.V., Criddle, D.N., Cardi, B.A., Carvalho, K.M., Assreuy, A.M.S., Vasconcelos, S.M.M., 2005. Antinociceptive activity of *Calotropis procera* latex in mice. *J. Ethnopharmacol.* 99, 125–129.
5. Arya, S., Kumar, V.L., 2005. Antiinflammatory efficacy of extracts of latex of *Calotropis procera* against different mediators of inflammation. *Mediat. Inflamm.* 2005, 228–232.
6. Choedon, T., Mathan, G., Arya, S., Kumar, V.L., Kumar, V., 2006. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. *World J. Gastroenterol.* 12, 2517–2522.
7. Shivkar, Y.M., Kumar, V.L., 2004. Effect of anti-inflammatory drugs on pleurisy induced by latex of *Calotropis procera* in rats. *Pharmacol. Res.* 50, 335–340.
8. Dada, Y.O., Lamidi, M.T., Eghianruwa, K.I., Adepaju, F., 2002. Effects of oral administration of the latex of *Calotropis procera* on weights, hematology and plasma biochemistry of rats. *Trop. Vet.* 20, 218–225..
9. W.H.O. Report of a scientific group. Principles for preclinical testing of drug safety. World Health Technical report series. 341, W.H.O., Geneva, Switzerland, 1967.
10. Joharapurkar AA, Wanjari MM, Dixit PV, Zambad SP, Umathe SN. Pyrogallol: A novel tool for screening immunomodulators. *Ind J Pharmacol* 2004; 36(6): 355-359.
11. Joharapurkar AA, Zambad SP, Wanjari MM, Umathe SN. *In vivo* evaluation of antioxidant activity of alcoholic extract of *Rubia cordifolia* Linn. And its influence on ethanol-induced immunosuppression. *Indian J Pharmacol* 2003; 35:232-6.
12. Stossel TP. Phagocytosis: Part I. *N Engl J Med* 1974; 290:717-23.
13. Thatte UM, Chhabria S, Karandikar SM, Dahanukar SK. Immunotherapeutic modification of E.coli abdominal sepsis & mortality in mice by Indian Medicinal Plant. *Ind. Drugs* 1987; 25:95-97.
14. B. Benacerraf. A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and B lymphocytes. *J of Immunology*, 1978; 120: 1809-1812.
15. L.E. Miller, H.R. Ludke, J.E. Peacock, R.H. Tomar, *Manual of Laboratory Immunology*. Lea and Febiger, London, 1991; 1-18.
16. C.V. Rao, *Immunology*. Introduction, India: Rajkamal Electric press; 2006; 1-12.
17. D.E, Pallabi, S.C. Dasgupta, A. Gomes. Immunopotentiating and immuno-prophylactic activities of Immune 21, a polyherbal product. *Indian J Pharmacology*, 1998; 30: 163-8.
18. V. Gayathri, V.V. Asha, A. Subramoniam. Preliminary studies on the immunomodulatory and antioxidant properties of *Selaginella species*. *Indian J Pharmacol*, 2005; 37:381-5.
