

## Research Article

# Investigation of Immunomodulatory Potential of *Euphorbia neriifolia* Linn. against Betamethasone Induced Immunosuppression

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### ABSTRACT

*Euphorbia neriifolia* (Linn) family *Euphorbiaceae*, commonly known as Milk Hedge. In this paper we have determined the immunomodulatory activity of 70 % v/v hydro-alcoholic extract of dried leaves of *Euphorbia neriifolia* by oral administration at dose of 400 mg/kg/day of body weight to healthy albino rats divided into four groups consisting of six animals each. The assessment of immunomodulatory activity was done by testing the survival rate of rats against abdominal sepsis caused by *E. coli*, determination of hematological parameters, phagocytic index determined by carbon clearance method and humoral immune responses determined by haemagglutination antibody titre method and cellular immune responses determined by footpad swelling method. The hydro-alcoholic extract of *Euphorbia neriifolia* have to possess significant protection against *E. coli* induced abdominal sepsis, significant increase in total leucocyte count, differential leucocyte count and phagocytic index were determined. It remarkably potentiates haemagglutination antibody titre and cell mediated immunity by facilitating the footpad thickness response in normal and Betamethasone induced immunosuppressed rats.

**Keywords:** Abdominal sepsis, carbon clearance method, haemagglutination antibody titre, phagocytic index.

### INTRODUCTION

Herbal medicine has become an integral part of standard healthcare, based on a combination of time honored traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections. [1] The modulation of immune response with the aid of various bioactive in order to alleviate certain diseases is an active area of interest. The property of any substance to enhance non-specific resistance of body against pathogens is termed "adaptogenic". This is an important area of research in which we do not have any breakthrough, which is used in vaccination program or immunosuppressant and which can be safely used in organ transplantations and autoimmune diseases. A number of plant products are being examined for their immunomodulating activity. [2] A plethora of plant-derived materials (protein, lectins, polysaccharides, etc) have been shown to stimulate the immune system. [3] Some of the plants known as immunomodulatory agents are *Panax ginseng*, *Viscum album*, *Tinospora cordifolia*,

*Boerhaavia diffusa*, *Withania somnifera*, *Ocimum sanctum* and *Curculigo orchoides* etc. [4-16]

*Euphorbia neriifolia* leaves are used as aphrodisiac, diuretic and also used in the treatment of bronchitis, bleeding piles and in ano-rectal fistula. The tribal population of Chattishgarh region uses the milky latex as an ingredient of aphrodisiac mixture. [17-19] The aqueous extract of the latex of *Euphorbia neriifolia* facilitated the wound healing process as evidenced by increase in tensile strength, DNA content, epithelization and angiogenesis. [20] The present study was undertaken to find out the possible actions of *Euphorbia neriifolia* leaves on immune system in rats.

### EXPERIMENTAL

#### Materials

*Euphorbia neriifolia* Linn. leaves were collected from Hoshangabad, MP, India, in the month of September 2006. The plant was identified with the help of available literature and authenticated by Dr. A. P. Shrivastava, Principal, Pandit Khushilal Sharma Govt. Ayurveda College and Institute, Bhopal, India. A voucher specimen was deposited in the herbarium of department (No. 1085).

Antigens, SRBC were collected in Alsever's solution and wash thrice with large volumes of normal saline and the cells were adjusted to a concentration of  $5 \times 10^8$  cells/ml for immunization and challenging.

*Preparation of hydro-alcoholic crude extract*

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**Table 1: Survival study of *E. coli* induced abdominal sepsis in rats**

Group	After 24 h	After 48 h
	% Mortality	% Mortality
Control (0.2 ml 2% CMC suspension)	50.0	66.6
Control + Betamethasone (1 mg/kg i.v.)	66.6	100.0
Treated ( <i>Euphorbia neriifolia</i> extract, 400 mg/kg p.o.)	0.0	16.6
Treated with <i>Euphorbia neriifolia</i> extract + Betamethasone	16.6	33.3

N = 6,

**Table 2: Effect of *Euphorbia neriifolia* extract on hematological parameters**

Group	Total Leukocyte Count on days			Total Lymphocyte Count on days			Total Neutrophil Count on days		
	(Number of cells/ml)			(Number of cells/ml)			(Number of cells/ml)		
	0	7	14	0	7	14	0	7	14
Control (0.2 ml of 2 % CMC suspension)	5210±1 9	5271±2 7 <sup>a</sup>	5141±2 1 <sup>b</sup>	2356± 27	2376±1 1 <sup>a</sup>	2300±1 3 <sup>a</sup>	2703± 22	2760±1 5 <sup>a</sup>	2643±1 4 <sup>a</sup>
Control + Betamethasone (1 mg/kg i.v.)	4907±2 8	5028±3 3 <sup>c</sup>	4027±1 8 <sup>d</sup>	2216± 10	2241±1 1 <sup>a</sup>	1740±2 7 <sup>d</sup>	2540± 13	2585±1 9 <sup>b</sup>	2101±2 1 <sup>d</sup>
Treated [ <i>Euphorbia neriifolia</i> extract] (400 mg/kg p.o.)	5450±2 5	6026±4 2 <sup>d</sup>	7092±1 8 <sup>d</sup>	2518± 11	2845±2 9 <sup>d</sup>	3336±2 5 <sup>d</sup>	2811± 21	3168±1 6 <sup>d</sup>	3606±1 2 <sup>d</sup>
Treated ( <i>Euphorbia neriifolia</i> extract)+ Betamethasone	5411±1 3	5721±3 7 <sup>d</sup>	5261±2 7 <sup>d</sup>	2505± 14	2726±2 8 <sup>d</sup>	2398±2 2 <sup>d</sup>	2768± 16	2910±2 4 <sup>d</sup>	2606±1 1 <sup>d</sup>

Values are expressed as the mean ± SEM (n = 6). Statistics significant vs Control, <sup>a</sup>P>0.05, <sup>b</sup>P<0.05, <sup>c</sup>P<0.01 and <sup>d</sup>P<0.001

Freshly collected *Euphorbia neriifolia* leaves were dried in shade and coarse powder was prepared by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week. The macerated mixture was filtered through muslin cloth and evaporated at 40°C up to one third of initial volume, remaining solvent was completely evaporated at 40°C, using a rotary vacuum evaporator (Superfit, India). The residue was designated as hydro-alcoholic extract and used for further studies. Dose at 100, 200 and 400 mg/kg/day of body weight were selected range from 1/6 to 1/15 of LD<sub>50</sub> based on the preliminary study conducted at our laboratory and data are not shown in this paper. We used three different doses i.e. 100, 200 and 400 mg/kg of *Euphorbia neriifolia* but lower doses don't showed significant immunoprotective effect so we gave the data of 400 mg/kg of body weight in this paper.

#### Animal

Wister albino rats (120-200 g) of either sex supplied from Ravi Chand & Sons, Ahmedabad, India were used. The animals housed under standard laboratory conditions maintained at 25 ± 1°C and under 12 / 12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India.

Animals were divided into four groups viz. Group A received 0.2 ml of 2 % w/v carboxy methyl cellulose suspension orally as a control group, Group B received 1 mg/kg body weight of Betamethasone intravenously as a control immunosuppressed group, Group C received 400 mg/kg of body weight of hydro-alcoholic extract of *Euphorbia neriifolia* daily for 14 days and Group D received 400 mg/kg of body weight of hydro-alcoholic extract of *Euphorbia neriifolia* daily

for 14 days and immunosuppressed with Betamethasone (from 11<sup>th</sup> day to 14<sup>th</sup> day).

#### Determination of abdominal sepsis caused by *E. coli*

All the animals on day 14<sup>th</sup>, after 3 h of the last dose of *Euphorbia neriifolia* extract, 1 ml of *E. coli* cell suspension was injected intraperitoneally for all groups and the percent mortality were observed up to 48 h. The survivors, if any, were observed for further 3 days. [21-22]

#### Determination of hematological parameters

After scheduled treatment as given in 2.3, blood samples were collected from retro orbital plexus on day 0, 7<sup>th</sup> and 14<sup>th</sup> for the determination of total leukocyte count (TLC) and differential leukocyte count (DLC).

#### Determination of Phagocytic index

All groups were administered with 0.2 ml/animal of carbon suspension (Pelikan Tuschea Ink, Germany) intravenously through tail vein on seventh day. Blood samples were collected from retro-orbital plexuses immediately before and 5, 10, 15 and 20 min after the injection of carbon suspension. An aliquot of each and 25 µl of blood sample lysed with 2 ml of 0.1 % acetic acid and absorbance was observed at 675 nm. The graph of absorbance against time was plotted for each animal and its respective test groups. The phagocytic index was determined by the slope of time concentration curve. [23]

#### Determination of humoral immune response

The animals were immunised with 0.1 ml of 1×10<sup>8</sup> SRBC, intraperitoneally on day 0. Blood samples were collected from individual animals from the retro-orbital plexuses on day 7. Antibody levels were determined by the haemagglutination technique. [24-25] Two-fold dilutions sera in saline (0.025 ml) were mixed with 0.025 ml of 0.1 % v/v SRBC suspension in microtitre plates. The plates were incubated at 37°C for 1 h and then inspected for haemagglutination. The highest dilution giving rise to macroscopic haemagglutination was taken as antibody titre.

**Table 3: Effects of *Euphorbia nerifolia* extract on haemagglutination antibody titre, delayed type hypersensitivity and phagocytic index**

GROUP	Haemagglutination Antibody Titre		Delayed Type Hypersensitivity (footpad thickness)			Phagocytic Index
	Primary	Secondary	0 h	24 h	48 h	
Control (0.2 ml of 2 % CMC suspension)	7.24±0.40	10.7±0.2	18.6±0.01	22.9±0.01	15.7±0.01	-
Control + Betamethasone (1 mg/kg i.v.)	5.18±0.20 <sup>c</sup>	8.63±0.35	22.9±0.01 <sup>a</sup>	25.7±0.01 <sup>a</sup>	24.3±0.01 <sup>b</sup>	0.0127
Treated [ <i>Euphorbia nerifolia</i> extract] (400 mg/kg p.o.)	14.7±0.26 <sup>c</sup>	20.7±0.28 <sup>c</sup>	41.4±0.01 <sup>c</sup>	25.1±0.01 <sup>a</sup>	5.7±0.01 <sup>c</sup>	0.0286
Treated ( <i>Euphorbia nerifolia</i> extract)+ Betamethasone	10.2±0.29 <sup>c</sup>	14.9±0.28 <sup>c</sup>	37.2±0.01 <sup>c</sup>	17.2±0.01 <sup>a</sup>	11.4±0.01 <sup>a</sup>	0.0252

Values are expressed as the mean ± SEM (n = 6). Statistics significant vs Control, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 and <sup>c</sup>P<0.001

Antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significance.

#### Determination of cell mediated immune response

The animals were immunized by injecting 0.1 ml of SRBC suspension containing  $1 \times 10^8$  cells, intraperitoneally, on day 0 and challenged on day 7 with 0.05 ml of  $2 \times 10^8$  SRBC in the right hind foot pad. The contra lateral paw received an equal volume of saline. The foot thickness was measured at 0, 24, 48 and 72 h after challenge using Mitutoyo Dial Caliper (Mitutoyo Manufacturing Company, Japan).<sup>[26]</sup> The difference in the thickness of the right hind paw and left hind paw was used as a measure of DTH reaction.

#### Statistical analysis

Results were expressed as mean ± SEM, statistical significance was calculated by applying one way ANOVA. P<0.05 was considered as significant.

## RESULTS AND DISCUSSION

Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy.<sup>[27]</sup> Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation.<sup>[28]</sup> The presence of immunostimulatory compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such products, if well tolerated by the patient, may be developed into alternative adjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc.<sup>[29]</sup>

In the present study, the immunomodulatory activity of *Euphorbia nerifolia* Linn., an important plant of indigenous system of medicine was explored. Measuring the mortality rate in *E. coli* induced abdominal sepsis can assess the positive immuno prophylactic efficacy of an immunomodulatory property of the plant extracts (Table 1). Intra-abdominal sepsis contributes to be a major cause of morbidity and mortality following trauma and abdominal surgery for bowel perforation. Treatment of this condition has always focused on appropriate surgery, antibiotics and

nutritional support. But in spite of this, fatal complications have been reported. A factor that influences the recovery from such an infective process is the host defense mechanism. This study clearly indicates that *Euphorbia nerifolia* extract at 400 mg/kg of body weight might have enhanced the capacity of monocyte-macrophage system in both normal and immunosuppressed rats (Table 1). A high degree of cell proliferation renders the bone marrow a sensitive target particularly to cytotoxic drugs. In fact, bone marrow is the organ most affected during any immunosuppression therapy with this class of drugs. Loss of stem cells and inability of the bone marrow to regenerate new blood cells results in thrombocytopenia and leucopenia. Administration of the extract of leaves of *Euphorbia nerifolia* was found to increase the total leukocyte count and differential leukocyte count in normal and immunosuppressed animals. The results of the present study indicate that the extract can stimulate the bone marrow activity (Table 2). In view of the pivotal role played by the macrophages in coordinating the processing and presentation of antigen to B-cells, the fraction was further evaluated for its effect on macrophage phagocytic activity. 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. In this study, it clearly indicates that the rate of elimination of carbon particles is more in *Euphorbia nerifolia* treated group when compared with immune suppressed animals. This study demonstrates that *Euphorbia nerifolia* potentiate the phagocytic index in normal and immune suppressed animals (Table 3).

The haemagglutination antibody titre was used to assess humoral immune response. At the selected dose both primary and secondary antibody titre was observed in rats treated with hydro-alcoholic extract of *Euphorbia nerifolia*. The augmentation of the humoral immune response to SRBCs by hydro-alcoholic extract is evidenced by increase in the antibody titres in the blood of rats (Table 3). Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins, etc.<sup>[30]</sup> The haemagglutination antibody titre was used to assess humoral immune response. The results of this experiment demonstrated that *Euphorbia nerifolia* extract

administration significant rise the primary antibody levels. More or less similar results were observed for with secondary antibody titres. The augmentation of the humoral immune response to SRBCs by hydro-alcoholic *Euphorbia nerifolia* extract is evidenced by increase in the antibody titres in the blood of rats (Table 3).

In the present investigation, SRBC-induced delayed-type hypersensitivity was used to assess the effect of the fraction on cell-mediated immunity. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defense against infectious organisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions.<sup>[30]</sup> Therefore, increase in DTH reaction in rats response to T cell dependent antigen revealed the stimulatory effect of hydro-alcoholic extracts of *Euphorbia nerifolia* on T cells (Table 3). *Euphorbia nerifolia* showed the protective activity against impaired DTH conditions. The immediate hypersensitivity in this group was found due to prevention the damage of short lived suppressor T cell against Betamethasone treatment. This study clearly indicates the immunoprotective activity of *Euphorbia nerifolia* in normal and immunosuppressed rats (Table 3).

Thus, the immunostimulatory effect produced by hydro-alcoholic extract of *Euphorbia nerifolia* in betamethasone-induced immunosuppression may be due to cell mediated and humoral antibody mediated activation of T and B cells. It can therefore be concluded that *Euphorbia nerifolia* is a potential immunostimulants against immunosuppressant and can be used as a complimentary therapeutic agent.

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