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Full Length Research Paper

A study of the immunomodulatory potential of an Indian medicinal plant

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In this study the immunomodulatory potential of an Indian medicinal plant, *Nyctanthes arbor-tristis L.* (Oleaceae), was investigated. The leaf extracts of *N. arbor-tristis* is used to treat arthritis, lung injury and some painful conditions such as cancer, chronic fever and rheumatism. An ethanolic extract of *N. arbor-tristis* (NAEE) was screened in rats for humoral and cell-mediated immune responses. Oral administration of the NAEE to rats at a dose of 50, 100, 150 and 200 mg/kg significantly enhanced the circulating antibody titre when challenged with sheep red blood cells (SRBC) and heat -killed *Salmonella* antigens. The chronic administration of NAEE increased the total counts of white blood cells (WBC) and potentated the delayed-type hypersensitivity (DTH) reactions. The present study confirms the strong immuno-bioactivities in extracts of *Nyctanthes arbor-tristis L*.

Key words: Immuno-bioactivities, *Nyctanthes arbor-tristis,* anti inflammatory, humoral immunity, delayed-type hypersensitivity.

INTRODUCTION

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants in different parts of the country. The vast majority of people on this planet still rely on their traditional materia medica for their every day health care needs. One quarter of all medical preparations are formulations based on substances derived from plants or plant-derived synthetic analogs. In this study, the immuno-bioactivities of the plant *Nyctanthes arbor-tristis,* commonly used by tribal people for treatment of many of their ailments, were investigated.

The modern system of medicine had always been enthusiastic to evoke non-specific defense mechanisms of human physiology, which led to the discovery of active immunization using microbial preparations to enhance the host defense against infection. Recently, the same enthusiasm has taken an important leap towards exploring a novel group of substances from natural resources that modulate the immune response of living systems (Gutali et al., 2002).

The family *Oleaceae* is represented in South Asia by 600 species divided into 29 genera. In folk medicine, these plants are used in combinations and are prescribed mainly for neurotic and chronic lung disorders (Assenov et al., 1989). They are also effective in treating epilepsy, sleeplessness, cardiovascular insufficiency, jaundice, coughing, and possess wound healing activities (Agarwal, 1999). *N. arbor-tristis* species are also used in tribal herbal medicine for treatment of many kinds of acute and chronic inflammatory diseases (Kuvaev and Blinova, 1960). Recent chemical investigations of some of these plants have shown the presence of relatively high amounts of alkaloid constituents, accumulated predominantly in the leaves and roots. Some major alkaloids like quinolinolids have been isolated and are widely used to treat rheumatism and other chronic disorders (Assenov et al., 1989) . The recent phytochemical analysis of *N. arbor-tristis* revealed the presence of tertiary alkaloids, represented mainly by 7-(alpha-anilino-p-nitrobenzyl)-8-quinolinol and quaternary alkaloids, belonging to protoberberi-

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nes and aporphines (Maleki et al., 2004). These substances may influence the immuno-bioactivites of *N. arbor-tristis*.

In the present study, plant extracts from *N. arbor-tristis* were prepared, administered to rats and its anti-inflammatory properties investigated, as well as its effect on antibody synthesis, total WBC count and delayed-type hypersensitivity reactions.

MATERIALS AND METHODS

Collection of plant materials

Healthy plant leaves were collected from South-western Ghats of Tirunelveli range, TamilNadu, India. They were collected in early morning and were washed in tap water. They were shade-dried for 10 days and powdered mechanically. The collected plant materials were botanically authenticated by the Botany Department, Kamaraj College, Thoothukudi.

Preparation of plant extract

Plant extracts were prepared by the method of Tiwari et al. (2004), with slight modification of the method used to purify the extract. Briefly, the extract was filtered though Whatman filter paper no. 1 (WHATMAN, ENGLAND) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. To obtain a concentrated crude extract, the crude extracts were evaporated at 45^{o}C. The quantity was determined by weighing.

Experimental animals

Swiss albino rats weighing 100 – 125 g of either sex were used to study the immuno-bioactivity. Rats were kept in 12 h light/12 h dark cycles under standard conditions of temperature (28^{o}C) and relative humidity (R_H: 60%) with free access to food and water. All protocols performed in this study were conducted in accordance with internationally accepted principles for use and care of laboratory animals.

Experimental design

In each experiment, the animals were randomly divided into five groups and each group consisted of six animals. The animals in Group I served as a test control. Animals in Groups II, III, IV and V, respectively, received an aqueous dose of NAEE of 50, 100, 150 and 200 mg/kg orally for 21 days.

Preparation of sheep red blood cell (SRBC) antigen

Sheep blood was collected from a local slaughter house in sterilized container in the presence of an anticoagulant. SRBC were obtained by centrifugation and the cells were washed three times in Phosphate Buffer Saline (PBS) (pH 7.8) . The SRBC antigen was prepared in PBS at a dose level of $1x10^{8}$ cells/ml.

Preparation of bacterial antigen

A loopful of *Salmonella typhi* growth was streaked onto Mueller-Hinton agar and the plate incubated at 37^{o}C for 24 h. A single colony was then inoculated into Mueller-Hinton broth and incubated in a shaker at 37^{o}C for 24 h. The cultured bacteria were attenuated at 80^{o}C for 30 min. Bacterial cells were then collected by centrifugation, and the pellet was washed and resuspended in PBS to the desired concentration ($1x10^{6}$ cells/ml).

Hematological changes

Group II, III, IV and V animals, respectively, received NAEE orally at a dose of 50, 100, 150, and 200 mg/kg for 21 days. On the 21^{st} day, blood samples were collected from the orbital plexuses of individual animals and the total WBC and RBC counts were determined with a haemocytometer (ROHAM, India).

Delayed-type hypersensitivity (DTH) reactions

The method described by Doherty (1981) was used. Rats of either sex were divided into five groups of six animals in each group. NAEE (50, 100, 150 and 200 mg/kg, p.o.) was administered on day 0 and continued untill the day of challenge. The rats were primed with 0.1 ml of the SRBC suspension containing $1x10^{8}$ cells, i.p., on day 7 and challenged on day 14 with 0.05 ml of $2x10^{8}$ SRBC in the right hind foot pad. The control left hind paw received an equal volume of saline. The thickness of the foot pad was measured at 0, 12, 24, 36, and 48 h after challenge. The difference in the thickness of the right hind paw and the left hind paw was used as a measure of delayed-type hypersensitivity reaction.

Humoral immune response

The acclimatized rats of either sex were divided into two sets, consisting of four groups of six animals in each group. NAEE (50, 100, 150 and 200 mg/kg, p.o.) was administered on day 0 and continued until the 21^{st} day of the experiment. On day 7, Group I rats were immunized with 0.1 ml of $1x10^{8}$ SRBC, i.p. The Group II rats received a single dose of 0.05×10^{6} cells/ml, s.c, of *Salmonella* (heat-killed) antigen. Blood samples were collected from the orbital plexuses of individual animals on day 14 and the antibody titres were determined (Puri et al., 1994). Briefly, an aliquot (25 µl) of two-fold diluted sera in isotonic saline was challenged with 25 µl of a 0.1% (v/v) SRBC suspension in microtitre plates for Group I rats. The plates were incubated at 37^{o}C for 1 h and then observed for haemagglutination. Similarly, the sera of Group II rats were evaluated using a WIDAL tube agglutination test kit (SPAN, India). The highest dilution of agglutination was taken as the antibody titre. The antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1. The mean ranks of different groups were statistically compared.

Statistical analysis

The results are presented as mean ± standard deviation (S.D.). Statistical significance between the groups was analyzed by the Student's t-test and $P<0.05$ was considered to be statistically significant.

RESULTS

For the evaluation of immuno-bioactivities, rats were treated with NAEE at doses ranging between 50 to 200 mg/kg for 21 days. Dose-related increases in the WBC and RBC counts were observed, the rats are treated at

Table 1. Effect of ethanolic extract of *N. arbor-tristis* (NAEE) on total blood cell count.

Group	Dose (mg/kg)	WBC X 10^3	RBC X 10^6
I	Control	11.30 ± 1.9*	9.12 ± 0.9
II	50	14.86 ± 2.1	12.89 ± 0.5
III	100	16.27 ± 1.7	15.36 ± 1.2
IV	150	17.31 ± 1.5	16.23 ± 1.8
V	200	17.98 ± 1.1*	16.87 ± 1.9*

Values are expressed as Mean ± S.D. of six observations $P < 0.05$ as compared to control.

Table 2. Effect of ethanolic extract of *N. arbor-tristis* (NAEE) on delayed-type hypersensitivity (DTH) edema (in mm)

GROUP	Dose(mg/kg)	Edema size in different time intervals (Size in mm)				
		oh	12 h	24 h	36 h	48 h
I	Control	-	0.2±0.01	0.4±0.03	0.2±0.01	-
II	50	-	0.4±0.03	0.7±0.08	0.5±0.04	0.45±0.03
III	100	-	0.53±0.04	0.93±0.08	0.73±0.05	0.61±0.04
IV	150	-	0.82±0.62	1.01±0.94	0.91±0.82	0.84±0.06
V	200	-	1.07±0.01	1.57±0.01*	1.07±0.98	1.01±0.9

Values are expressed as Mean ± S.D. of six observations. * $P < 0.05$ as compared to control.

Table 3. Effect of ethanolic extract of *N. arbor-tristis* (NAEE) on antibody titre of SRBC and *Salmonella* antigen.

Group	Dose (mg/kg)	SRBC Antibody titre	*Salmonella* Antibody titre
I	Control	2.7 ± 0.5	2.12 ± 0.9
II	50	4.6 ± 0.3*	3.89 ± 0.5
III	100	5.2 ± 1.7	5.36 ± 0.9
IV	150	7.83 ± 1.5	5.83 ± 1.8
V	200	8.08 ± 1.4*	6.07 ± 1.9*

Values are expressed as Mean ± S.D. of six observations. * $P < 0.05$ as compared to control.

the concentration of 200 mg/kg NAEE shows significant increases in WBC and RBC count (Table 1). In delayed-type hypersensitivity (DTH) reactions, using SRBC as an antigen, dose-related changes were observed in the edema size. Edema achieved a peak after 24 h and the size of the edema 1.57 mm in 200 mg/kg NAEE treated rats (Table 2).

A significant dose related increases in humoral and bacterial antibody titres was observed in rats treated with NAEE (50–200 mg/kg, p.o.) In SRBC and bacterial agglutination attained a peak at a dose of 200 mg/kg it showed 8.08 ± 1.4 and 6.07 ± 1.9 titer respectively (Table 3).

DISCUSSION

In the present study, the immuno-bioactivity of *N. arbor-tristis*, an important plant in indigenous medicinal practice, was explored. Administration of *N. arbor-tristis* increased total counts WBC and RBC cells significantly, indicating that the extract could stimulate the haemopoetic system. Moreover, there was an increase in positive bone marrow cells, indicating that NAEE treatment could also enhance the differentiation of stem cells.

Delayed-type hypersensitivity (DTH) is a part of the process of graft rejection, tumor immunity, and most importantly, immunity to many intracellular infectious microorganisms, especially those causing chronic diseases such as tuberculosis (Elgert, 1996). 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 (Descotes, 1999) and activation, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more

effective killing (Kuby, 1997). In the present study, the SRBC chemical sensitizer, which in combination with skin proteins acquires antigenicity (Asherson and Ptak, 1968), were used to elicit contact hypersensitivity reactions in rats. It was found that NAEE dose-dependently potentated the DTH reaction induced by SRBC. Increase in DTH reaction in rats in response to thymus-dependent antigen suggests the stimulatory effect of NAEE on T- lymphocytes and accessory cell types required for the expression of the reaction (Luster et al., 1982).

NAEE extract was found to increase the circulating antibody titre and antibody-forming cells. In fact, anti-body-forming cells were found to be stimulated much earlier (seventh day) than the maximum antibody titre obtained (14^{th} day). However, an increased titre remained several days thereafter, indicating that the immunological activity could be sustained for several days.

The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibodies function as the effectors of the humoral response by binding to the 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 *N. arbortristis* on the humoral immune response, its influence was tested on sheep erythrocyte-specific haemagglutination and *Salmonella* antigen-specific antibody titres in rats. Higher doses of NAEE were found to significantly enhance the production of circulating antibody titres. This indicates the enhanced responsiveness of macrophages and T- and B-lymphocyte subsets involved in antibody synthesis (Benacerraf, 1978).

The present preliminary investigation suggests that *N. arbortristis* stimulates both the cellular and the humoral immunity. Further studies to elucidate the exact immunobioactive mechanism of *N. arbortristis* are in progress.

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