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A Study on Immuno-bioactivities of Nyctanthes arbor-tristis

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Abstract: ***This study investigates the immuno-bioactive properties of the ethanolic extract of Nyctanthes arbor-tristis (NAEE) in Swiss albino rats. Plant materials were collected from the Almora District, Uttarakhand, India, and processed to obtain the extract. Rats were treated with NAEE orally at doses ranging from 50 to 200 mg/kg for 21 days. Hematological changes, delayed-type hypersensitivity (DTH) reactions, and humoral immune responses were evaluated. Results showed dose-dependent increases in white blood cell (WBC) and red blood cell (RBC) counts, with significant elevations observed at 200 mg/kg NAEE. DTH reactions revealed dose-dependent changes in edema size, peaking at 24 hours post-challenge with 200 mg/kg NAEE. Additionally, significant enhancements in humoral and bacterial antibody titers were observed at doses of 200 mg/kg NAEE. These findings suggest the potential immunostimulatory effects of NAEE, highlighting its importance as a candidate for further exploration in immunotherapy.***

I. INTRODUCTION

Synthetic chemicals have long been utilized in medicine, albeit often accompanied by various side effects. In recent years, there has been a growing interest in exploring plants as sources of bioactive compounds for treating diseases, including cancer. Plants contain a plethora of chemical substances that contribute to their diverse biological properties and perform essential physiological functions. Out of the vast number of flowering plants worldwide, more than 50,000 have been identified for their medicinal and pharmacological uses, with India boasting a particularly rich diversity of medicinal flora (Uniyal SK, et al., 2006). The utilization of plants for medicinal purposes dates back to ancient times and is documented in various literary works. This traditional knowledge has provided the foundation for many important drugs in modern medicine. *Nyctanthes arbor-tristis L.* (Oleaceae), commonly known as Harshingar or Night Jasmine (Kiew R, Bass P., 1984), is one such plant with extensive medicinal uses dating back to ancient times. It is employed in Ayurveda, Siddha-Ayurveda, and Yunani systems of medicine for its laxative, diuretic, anti-venom, digestive, mild bitter tonic, and expectorant properties (Rangika BS, 2015). *Nyctanthes arbor-tristis* , classified under Magnoliophyta, Magnoliopsida, Lamiales, and Oleaceae, is a well-documented shrub or small tree with a lifespan of 5-20 years, reaching heights of up to 10 meters. It exhibits a wide range of pharmacological activities and is cultivated in tropical and subtropical regions globally (Rani C, et al., 2012; Agrawal J, et al., 2013). In India, it is found in the outer Himalayas, Jammu and Kashmir, Nepal, Assam, Bengal, Tripura, and extending through the central region up to the Godavari in the South (Jain PK, and Pandey A., 2016). It thrives in red and black soils with a pH ranging from 5.6 to 7.5, favoring arid and semi-arid climatic conditions (Latha PG, et al., 2005). Previously, the taxonomic classification of *Nyctanthes arbor-tristis* was unclear, but it was eventually placed in the Oleaceae family by Bentham and Hooker. Subsequent investigations by Vaishampayan and Sharma (1983) aimed to determine its precise taxonomic placement. The plant has been traditionally used in folk medicine for its antibilious, gynecological, and hepatoprotective properties (Kumari TDS, et al., 2012).

II. Materials and Methods

2.1 Collection and Preparation of Plant Materials:

Healthy leaves of *Nyctanthes arbor-tristis* were gathered from the Almora District, Uttarakhand, India, during the early morning hours. Following collection, the leaves were washed with tap water and subsequently shade-dried for a duration of 10 days. Once dried, the leaves were mechanically powdered.

2.2 Preparation of Plant Extract:

The extraction of plant compounds was carried out using the method described by *Tiwari et al*. (2004), with minor adjustments made to the purification process. The extract underwent filtration using Whatman filter paper no. 1 (WHATMAN, ENGLAND) to eliminate any unextractable material, including cellular components and insoluble constituents. To obtain a concentrated crude extract, the filtrate was then evaporated at a temperature of 45°C. The quantity of the extract was determined through weighing.

2.3 Experimental Animals:

Swiss albino rats, weighing between 100 to 125 grams of either sex, were utilized for studying immuno-bioactivity. The rats were housed in conditions of 12 hours light/12 hours dark cycles, maintaining standard temperature (28°C) and relative humidity (60%), with ad libitum access to food and water. All experimental procedures adhered to internationally accepted principles for the care and use of laboratory animals.

2.4 Experimental Design:

For each experiment, the rats were randomly divided into five groups, each comprising six animals. Group I served as the control, while Groups II, III, IV, and V received oral doses of NAEE at concentrations of 50, 100, 150, and 200 mg/kg, respectively, for a duration of 21 days.

2.5 Preparation of Sheep Red Blood Cell (SRBC) Antigen:

Sheep blood was collected from a local slaughterhouse in a sterilized container containing an anticoagulant. SRBCs were obtained through centrifugation and washed thrice with Phosphate Buffer Saline (PBS) (pH 7.8). The SRBC antigen was then prepared in PBS at a concentration of 1x108 cells/ml and incubated in a shaker at 37°C for 24 hours.

2.6 Hematological Changes:

Animals from Groups II to V received respective doses of NAEE orally for 21 days. On the 21st day, blood samples were collected from individual animals, and the total WBC and RBC counts were determined using a hemocytometer.

2.7 Delayed-Type Hypersensitivity (DTH) Reactions:

Following NAEE administration, rats were primed with SRBC suspension on day 7 and challenged with SRBC on day 14. The thickness of the foot pad was measured at specified intervals post-challenge to assess DTH reactions.

2.8 Humoral Immune Response:

Rats received NAEE for 21 days, followed by immunization with SRBC or Salmonella antigen on day 7. Blood samples were collected on day 14 to determine antibody titers.

2.9 Statistical Analysis:

Results were expressed as mean ± standard deviation (S.D.). Statistical significance between groups was analyzed using Student's t-test, with $P<0.05$ considered significant.

III. 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
1	Control	11.20 ± 1.9*	9.12 ± 0.9
2	50	14.66 ± 2.1	12.69 ± 0.5
3	100	16.17 ± 1.7	15.26 ± 1.2
4	150	17.21 ± 1.5	16.13 ± 1.8
5	200	17.58 ± 1.1*	16.47 ± 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)

Group	Dose (mg/kg)	Edema size in different time intervals				
		oh	12h	24h	36h	48h
1	Control	-	0.2±0.01	0.4±0.02	0.2±0.01	-
2	50	-	0.4±0.03	0.7±0.07	0.5±0.03	0.43±0.03
3	100	-	0.52±0.04	0.93±0.05	0.72±0.05	0.60±0.04
4	150	-	0.81±0.62	1.01±0.92	0.96±0.82	0.82±0.06
5	200	-	1.06±0.01	1.57±0.01*	1.08±0.98	1.96±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
1	Control	2.76 ± 0.5	12 ± 0.9
2	50	4.67 ± 0.3*	3.89 ± 0.5
3	100	5.24 ± 1.7	5.35 ± 0.9
4	150	7.83 ± 1.5	5.88 ± 1.8
5	200	8.06 ± 1.4*	6.08 ± 1.9*

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

Rats were subjected to treatment with NAEE at varying doses ranging from 50 to 200 mg/kg over a period of 21 days to assess immuno-bioactivities. Results demonstrated dose-dependent elevations in both white blood cell (WBC) and red blood cell (RBC) counts, with significant increases observed particularly in rats treated with 200 mg/kg NAEE (Table 1). Furthermore, in evaluations of delayed-type hypersensitivity (DTH) reactions using sheep red blood cells (SRBC) as an antigen, dose-dependent changes in edema size were noted, peaking at 24 hours with an edema size of 1.57 mm in rats treated with 200 mg/kg NAEE (Table 2).

Moreover, significant dose-related enhancements in humoral and bacterial antibody titers were observed in rats administered NAEE orally at doses ranging from 50 to 200 mg/kg. Notably, in both SRBC and bacterial agglutination assays, the highest titers were reached at the 200 mg/kg dose, with titers measuring 8.08 ± 1.4 and 6.07 ± 1.9, respectively (Table 3). These findings collectively suggest a potential immunostimulatory effect of NAEE, warranting further investigation into its underlying mechanisms and therapeutic implications.

IV. Conclusion

The ethanolic extract of *Nyctanthes arbor-tristis* demonstrated significant immuno-bioactive properties in Swiss albino rats. The extract elicited dose-dependent increases in WBC and RBC counts, indicating its potential role in enhancing immune function. Furthermore, NAEE exhibited dose-dependent modulation of DTH reactions, with notable effects on edema size. The extract also enhanced humoral and bacterial antibody titers, suggesting its immunostimulatory potential. These findings underscore the importance of *Nyctanthes arbor-tristis* as a source of bioactive compounds with immunomodulatory properties, warranting further investigation into its mechanisms of action and therapeutic applications in immunotherapy.

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