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ACUTE TOXICITY STUDY ON LEAVES OF *Nyctanthes arbor-tristis* L. IN WISTAR ALBINO RATS.

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ABSTRACT

Indian flora is one of the richest in the world. Siddha system of Medicine greatly depends on this enrich flora to treat diseases. Among them one is the *Nyctanthes arbor-tristis* L. (Night Jasmine or *Pavalamalli*), a small tree with its aroma flowers. The leaves of *Nyctanthes* are given for arthritis, fever, laxative in Siddha. The purpose of this study is to evaluate the acute toxicity potential of *Nyctanthes* leaves ethanolic extract (NEE) in Wistar albino rats. NEE is evaluated at multiple dose of 100, 500, 1000, 2000 mg/kgbw among four groups for 24 hours orally. While rats in the control group treated with normal saline (5ml/kg). Observations include changes in body weight, irritability, tremor, laboured breathing, staggering, convulsion and death. At the end of the study, biochemical parameters were also estimated. The results indicates safety of oral administration at 100mg/kg bw dose of *Nyctanthes* leaves and is proved statistically significant ($p>0.05$). No death is observed in any of the animals.

KEY WORDS

Siddha Medicine, *Nyctanthes* leaves ethanolic extract (NEE), Wistar albino rats, acute toxicity, Dose.

1. INTRODUCTION

India is known as the "Emporium of Medicinal plants" due to the availability of several thousands of medicinal plants in the different bioclimatic zones and also for its rich heritage of traditional system of medicines.[1] The use of plants as a source of medicine has been an integral part of life in India from the earliest times. Siddha system of Medicine is as old as mankind, is considered as the mother medicine of ancient Tamils/Dravidians in South India, [2] being a product of several cultural patterns separated by aeons, is full of complexities and intricacies [3] which uses herbs prominently. It, accommodates an entirety of herbs, and is unique in exhibiting fewer side effects [4]. Among them is the, Night Jasmine [5] or *Pavalamalli* [6] *(Nyctanthes arbor-tristis* L.) a small tree with its fragrant flowers [5]. The leaves are given for gout [7], fever, back pain, *Vatha* diseases and as worm infestation in Siddha Medicine [6]. To rule out for any adverse effect, that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge [8] , toxicity studies are essential. So, the purpose of this study is to evaluate the acute toxicity potential of *Nyctanthes* leaves ethanolic extract (NEE) in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Collection of plants and identification

Nyctanthes leaves are collected at Tirunelveli, Tamil Nadu and is identified, authenticated by the botanists of Department of Medicinal Botany, Government Siddha Medical College, Tirunelveli, and Tamil Nadu.

2.2 Siddha literature review of *Nyctanthes* leaves

Parijatham (*Nyctanthes arbor-tristis* L.) is a little tree of 15 – 20 feet high which grows widely in many Indian homes chiefly for its scented flowers. The flowers resembles as like the jasmine (*malli*) in its smell and shape. The pedicel (or stalk) of the flower is yellowish red in colour which is quite similar to the red coral (*pavalam*) and hence the name *Pavala malli* (Coral jasmine). The tree blooms all around the year and quite higher in the rainy season only in the evenings and drops in the mornings, hence also called as Night jasmine. The upper surfaces of the leaves are scabrous and are blackish green in colour. They possess bitter (*Kaippu*) taste (*Suvai*) with heat potency (*Veeriyam*) and attain pungent (*Kaarppu*) as the post absorptive taste (*Privu*) [6] [7]. The leaves contain an alkaloid principle named Nyctanthine; they also contain an astringent principle, a resinous substance, colouring

matter, sugar and a trace of an oily substance, tannic acid, methyl salicylate, carotene, an amorphous resin and traces of volatile oil. They have anti-periodic, alternative, antibilious, expectorant, cholagogue, laxative, mild bitter tonic, anthelmintic, antibacterial, antifungal, anti – inflammatory, antioxidant, anti – pyretic, hepatoprotective, immunopotential pharmacological effect [5] [9]. The infusion of the leaves are given twice daily for Back pain and fever. The tender leaves were grinded with ginger juice and given twice a day for periodic fever. The leave juice is mixed with little amount of honey and salt which is given as a dewormer. The decoction of the leaves is given to cure *Kapha* diseases, constipation and used as an alternative in obstinate cases of sciatica and rheumatism [6].

2.3 Preparation of *Nyctanthes* leaves ethanolic extract (NEE)

The fresh leaves of *Nyctanthes* are made to dry in shade for about a week. Then, they are made into coarse powder. About 100 grams of the coarse powder is taken and is soaked in 90% of ethanol and subjected to extraction. Then distill off the solvent and the *Nyctanthes* ethanolic extract concentration is found in water bath is taken for the present study.

2.3 Drug preparation

The *Nyctanthes* leaves ethanolic extract (NEE) was suspended in water and four multiple doses viz; 100, 500, 1000 and 2000 mg kg body weight was prepared.

2.4 Experimental groups

Experimental Wistar strain female Albino rats (8 -12 weeks old, 180- 200 g body weight) of five groups, each consisting of six rats were kept for determination for acute toxicity studies. Amongst five groups, group I was the control group and group II, III, IV& V are test groups. All the rats were accommodated in standard steel cages at the laboratory of Kongu nadu Arts and Science College, Coimbatore. The animals are facilitated with standard environmental condition of illumination (12:12 h dark: light cycle) and temperature (27 ± 2ºC). They were provided with rat feed and water given ad libitum. This experimental model was done according to the ethical guidelines with IAEC approval No. 1012/C06/CPCSEA-Corres-2008-2009. The bioassays were conducted according to the World Health Organization guidelines for the evaluation of the safety and efficiency of herbal medicines (WHO, 1992) [10].

2.5 Acute oral toxicity

Body weight is recorded before the administration of the test drug, NEE. The acute toxicity effect of NEE was assessed by gross behaviour model. The test drug, NEE with multiple doses of 100, 500, 1000, 2000 mg/ bw (TABLE- 1) was administered orally one hour prior to the experiment orally to overnight fasted (food but not water withheld) animals of groups- II, III, IV, V by using an Intra gastric Catheter tube (IGC). While the control group – I receives normal saline vehicle (0.3% w/v CMC – Carboxy methyl cellulose) by using an Intra gastric Catheter tube (IGC) orally. Observations on behaviour changes, includes changes in irritability, tremor, laboured breathing, staggering, convulsion and death. After drug administration, the behaviour modifications were observed once during the first 2 hours and then frequently for next 24 hours and then at 48 hours and 72 hours to observe for toxic signs and symptoms like morbidity and mortality in rats. The mortality was observed for 10 days after treatment. Again body weight is also recorded. The observed result was recorded as the score normal, good, fair, poor, bad relative to the average intensity of the phenomena of behaviour observed.

At the end of the experiment i.e. 24 hours after the dose, blood samples were collected through cardiac puncture under chloroform anaesthesia into non heparinised tube for liver and renal function test. Biochemical profile of Liver and Kidney were carried out by standard procedure [10].

2.6 Statistical analysis

Data are expressed as mean ± S.E.M (Standard error of the mean) and statistically evaluated by one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test.

Table-1

Acute Oral Toxicity of Nee

Group (Dose)	No. of rats	Death	Dose difference	Mean death
Group-I (Normal Saline)	6	-	-	-
Group-II (100mg/kg bw)	6	-	100	-
Group-III (500mg/kg bw)	6	-	500	-
Group-IV (1000mg/kg bw)	6	-	1000	-
Group-V (2000mg/kg bw)	6	-	2000	-

Table-2

Effect on Gross Behavioural Changes Observed with Nee

Group (Dose)	Signs and symptoms (No. of animals)	Score
Group-I (Normal Saline)	Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)	Normal
Group-II (100mg/kg bw)	Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)	Good. Normal activities seen
Group-III (500mg/kg bw)	Irritability (0) Tremor (0) Laboured breathing (0) Staggering (2) Convulsion (1) Death (0)	Fair. Normal activities seen
Group-IV (1000mg/kg bw)	Irritability (4) Tremor (3) Laboured breathing (3) Staggering (1) Convulsion (3) Death (0)	Poor. 50% of animals have shown normal activities
Group-V (2000mg/kg bw)	Irritability (4) Tremor (4) Laboured breathing (4) Staggering (4) Convulsion (5) Death (0)	Bad. Normal activities not seen in this group.

TABLE-3

EFFECT OF NEE ON LIVER & RENAL FUNCTION

Parameters	Units	Treatment Groups (Dose)				
		Group-I (Normal Saline)	**Group-II (100mg/kg bw)**	**Group-III (500mg/kg bw)**	**Group-IV (1000mg/kg bw)**	**Group-V (2000mg/kg bw)**
Total bilirubin	mg/dl	0.76±0.05	1.03±0.05	2.81±0.61*	3.38±0.34**	3.94±0.16**
Total protein	g/dl	8.09±0.16	7.84±0.13	6.73±0.12**	6.61±0.24**	6.56±0.18**
Albumin	g/dl	4.36±0.31	4.08±0.11	3.89±0.15	3.78±0.13*	3.68±0.15*
Globulin	g/dl	3.73±0.56	3.76±0.43	2.84±0.78*	2.83±0.31*	2.88±0.43*
ALT	U/L	14.85±0.18	31.54±1.36	48.54±1.16*	81.54±3.91**	86.84±3.18**
AST	U/L	15.93±0.64	29.56±2.11	54.36±1.84**	62.16±1.86**	74.93±3.11***
ALP	U/L	116.16±2.15	131.36±1.36	190.56±2.86**	221.36±3.64***	236.16±4.06***
Urea	mg/dl	12.16±0.13	16.54±0.92	31.54±1.13*	38.54±1.31**	41.15±1.56**
Creatinine	mg/dl	0.91±0.06	0.83±0.03	1.98±0.54	3.96±1.13*	4.86±1.84*

Data represented as mean ±SEM (Standard error of the mean), (N=6). *P<0.05; **P<0.005; ***P<0.005 compared Group Vs Other groups.

(ALT – Alanine transaminase, AST – Aspartate transaminase, ALP – Alkaline phosphatase

NEE - *Nyctanthes* leaves ethanolic extract)

3. RESULTS AND DISCUSSION

During the 24 hours of acute oral toxicity study, there is no significant change in the gross behaviour changes and Good score and normal activities were seen at the dose of 100 mg/ kg bw. At the dose of 500mg/kg bw behaviour changes like staggering and convulsion were noted with fair score, normal activities seen. Dose of 1000mg/ kg bw shows behavioural changes like irritability, tremor, laboured breathing, staggering and convulsion with poor score. Only 50% of the animals showed normal activities. At the dose of 2000 mg/kg bw behavioural changes like irritability, tremor, laboured breathing, staggering and convulsion are found increasing in numbers with bad score. Normal activities are not seen in this group (TABLE 1&2). No other abnormal toxic signs or mortality were found.

During the experimental study period, there is no significant changes in biochemical parameters; bilirubin, total protein, albumin, globulin, ALT, AST, ALP, Urea and Creatinine revealing the safety profile of *Nyctanthes* ethanolic extract (NEE) at the dose of 100 mg/kg bw ($p > 0.05$). But at the doses of 500, 1000, 2000 mg/ kg bw, there were variation from normal values in liver and renal function tests (TABLE 3) with varying level of significance.

The above results thus gave evidence for the total safety profile of ethanolic extract of *Nyctanthes arbor-tristis.*L. leaves, suggesting its safe use even in single dose treatment at 100mg/kg bw for therapeutic use would not produce any toxic effects.

4. CONCLUSION

The acute toxicity studies of *Nyctanthes arbor-tristis.*L. leaves ethanolic extract (NEE) at the dose of 100 mg/ kg bw did not possess any toxic effect in Wistar albino rats and is need of evaluation in chronic toxicity studies for long term standard effective use in future.

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