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Anti-inflammatory potential of *Nyctanthes arbor-tristis* by carrageenan induced paw edema model in wistar rats

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ABSTRACT

Nyctanthes arbor-tristis (NAT) is a West Indian shrub and an ornamental plant with several medicinal properties in traditional medicine. In the present investigation, NAT leaves extracted in methanol was screened for its anti-inflammatory activity using STD Indomethacin (10 mg/kg) and Carrageenan induced rat paw edema model with a compound dose 250 mg/kg and observed that the methanol extract showed very mild anti-inflammatory activity.

Keywords: Anti-inflammatory, Indomethacin, Carrageenan induced rat paw edema, *Nyctanthes arbor-tristis*.

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INTRODUCTION

A defense mechanism that helps the body to protect itself against allergens, noxious stimuli, infections and so on is generally considered to be inflammation [1]. The release of prostaglandins, bradykinin, & histamine will be observed during these inflammatory reactions. Severe side effects with commercial drugs evoked us to discover the new anti-inflammatory agents from natural sources even though plants have minimal drawbacks. *Nyctanthes arbor-tristis* is distributed throughout the India. The plant generally tolerates moderate shade. The leaves of *Nyctanthes arbor-tristis* are rough, marginated, opposite with several medicinal properties like treatments of chronic fever, diuretic, rheumatism etc., [2]. The literature survey for anti-inflammatory activities revealed only with the activities of the ethanol extract of NAT fruit [3], aqueous alcoholic extracts of NAT leaves [4], orange tubular clayx of NAT extracted in ethanol [5], leaf and fruit extracted in water soluble ethanolic extract [6] and NAT leaves extracted in water and ethanol [7], with this survey this work was extended to anti-inflammatory activity with NAT Leaves extracted in methanol.

MATERIALS AND METHODS

Plant Material and its Extraction

The freshly collected leaves of *Nyctanthes arbor-tristis* were shade-dried and coarsely-powdered. The powdered leaves were extracted by using soxhlet apparatus. The solvent was evaporated to dryness under reduced pressure at 37^0C [8]. The plant was authenticated by Botanical Survey of India, Coimbatore, No. BSI/SRC/5/23/2011-12/Tech. 1443.

Evaluation of Anti-inflammatory Activity

The activity was evaluated by using acute model - Carrageenan induced rat paw edema method. The experiments were conducted after obtaining approval from Institutional Animal Ethical Committee of IICT, Hyderabad, A.P.

Dose:

Compound Dose – 250mg/kg
STD (Indomethacin) -10 mg/kg

Drug:

Chemical name: 1- (4. Chlorobenzoyl)-(-methoxy-2-methylindol-3-yl) acetic acid
Commercial name: Indomethacin

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Experimental animals

Male Albino Wistar rats weighing between 130-150 g were used for the experiments. They were kept in polypropylene cages under standard laboratory conditions (12: 12 hr light/dark cycle at 24^{o}C. Rats were provided with commercial rat diet (NIN, Hyderabad) and water adlibitum. Animals were quarantined and acclimatized to laboratory conditions for 7 days prior to study initiation. Animals were observed for general health and suitability for testing during this period.

Carrageenan induced rat paw edema

The anti-inflammatory activity of the test compounds was evaluated in Wistar rats employing the method of [9]. Animals were fasted overnight and were divided into control, standard and different test groups. Animals in the standard group received indomethacin at the dose of 10 mg/kg, by oral route. Before this all the test compounds were administered by oral route at the dose of 250 mg/kg/day for 10days. Required quantity of the test compound was weighed and made suspension with gum acacia. This suspension was administered orally to all the rats at a specific time each day throughout the period of the study. The rats in the control group received the vehicle solution without test compounds. One hour after test drugs administration on termination day, rats in all the groups were challenged with 0.1 ml of 1% carrageenan in the sub plantar region of right hind paw. Paw volumes were measured before and every one hr up to 3 hrs after the challenge of carrageenan using digital Plethysmometer (Ugo Basile, Italy). The percent inhibition of paw volume for treated groups was calculated by comparing with mean paw volume of control group.

$$\% \text{ Inhibition} = Vc – Vt /Vc$$

(Vc- Control Mean paw volume, Vt – Test paw volume)

Statistical Analysis

The experimental values are mentioned as Mean ± S.E.M (N=6)
Standard Error of the Mean - S.E.M

RESULTS AND DISCUSSION

Test for acute toxicity

No mortality & stereotypical symptoms, such as, diarrhea, convulsions were observed during the 24hr period of acute toxicity studies in animals.

The widely used model for acute inflammation is carrageenan paw induced edema model for the discovery of new anti-inflammatory agents [10]. The oedema development in rat

paw is the biphasic event with the release of histamine and serotonin in the initial phase and second phase to prostaglandins [11]. The body weight index on zero (0) day and on termination day in wistar rats was presented in figure 1. The effect of natural agents and reference drug on carrageeenan oedema upon administration is presented in tables 1 & 2.

The anti-inflammatory activity was represented as paw volumes and percentage inhibitions by NAT extracts at different time intervals in carrageenan-induced wistar rats are presented in tables 1 & 2.

In carrageenan-induced paw oedema rats, the anti-inflammatory activity was evaluated by using methanol leaf/fruit/stem extracts derived from NAT plant. The reference drug used in this study belongs to the class of non-steroidal anti-inflammatory drug, Indomethacin. Due to the agent formed sustained oedema volume, the crucial time observed during anti-inflammatory activity in Carrageenan-induced rat paw oedema model is 3rd h. Hence IC_{50} values were calculated for each sample at 3rd h observation.

The animals were divided into three groups with 6 wistar rats each.
Group 1 : Control (vehicle solution 10ml/kg)
Group 2 : Extract (250 mg/kg)
Group 3 : STD (10 mg/kg)

The body weight index of the carrageenan induced wistar rats with control and NAT methanol leaf extract were compared on the 1st and the 11th day and found no significant change. On the 1st day the control is 154.80±2.63 and on the termination day 159.95 ±2.42 whereas the extract is 156.68±2.94 on the 1st day and 158.82±0.03 on the termination day.

A dosage of 10 mg/kg of STD/Indomethacin showed lower inhibitory activity during the 1st h, that is, 34.14±6.99. However, a significant effect of inhibition has been observed during the 2nd and the 3rd hour as 61.07± 6.69 and 60.47±4.29.

A dose level of 250 mg/kg of dried leaves extracted in methanol of NAT plant showed a non-significant inhibitory response at all time periods. During the 1st h the percentage of inhibition observed was 8.83±0.03 followed with a slight incline during the 2nd h. as 9.33±1.94 and 10.14±2.17 of inhibitory response during the 3rd h. As the phytochemical study of NAT Methanolic leaf extract showed less number of phytochemicals, it can be concluded that results of the above research with methanol leaf extract of NAT plant showed very mild activity for the treatment of inflammation.

Table 1: Body Weight Index

Compound Code	1st DAY	11th DAY
CONTROL	154.80±2.63	159.95 ±2.42
EXTRACT	156.68±2.94	158.82±0.03

Values are mentioned as Mean ± S.E.M (n=6).

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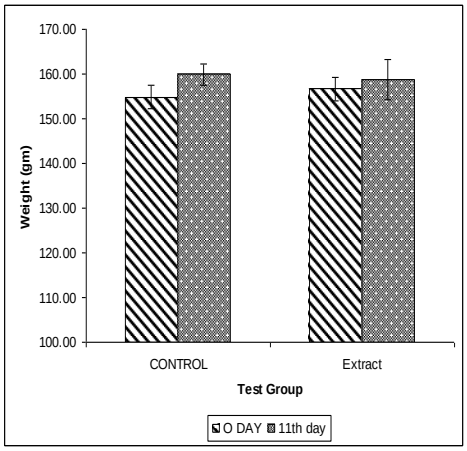
Figure 1: Represents Body Weight Index on 0 day and on Termination Day in Carrageenan Induced Model in Wistar Rats.



CARRAGEENAN INDUCED PAW EDEMA MODEL

CONCLUSION

The present study on carrageenan induced oedema indicated that NAT methanol leaf extracts exhibited very mild anti-inflammatory effect (upto 10%). Body weight increase of the extract group of rats was comparable with control group of rats. As a part of the research work highly polar NAT extracts were selected for anti-inflammatory activity among which *Nyctanthes arbor-tristis* leaves extracted in methanol possess less number of active chemical constituents which help in anti-inflammatory drug research.

Table 2: Paw Volumes and Percentage Inhibition at different time intervals in Carrageenan Induced Model in Wistar Rats.

Rat No	Group	O Hr	1st hr	% Inhibition	2nd hr	% Inhibition	3rd hr	% Inhibition
1		0.92	1.8		2.45		2.48	
2		1.1	1.56		2.03		2.13	
3		1.17	1.52		2.31		2.49	
4	Control	1.1	1.7		2.29		2.52	
5		1.18	1.5		2.1		2.08	
6		1.09	1.76		2.37		2.45	
Mean ± S.E.M		**1.09±0.03**	**1.64±0.05**		**2.26±0.07**		**2.36±0.20**	
7		1.28	1.8	4.87	2.28	8.26	2.38	13.04
8		1.1	1.6	8.53	2.01	16.51	2.30	5.14
9	Extract	1.07	1.59	4.87	2.14	1.83	2.28	4.35
10		1.24	1.7	15.84	2.24	8.26	2.34	13.04
11		1.23	1.75	4.87	2.20	11.01	2.27	17.79
12		1.12	1.59	14.01	2.10	10.09	2.29	7.51
Mean ± S.E.M		**1.17±0.04**	**1.67±0.04**	**8.83±0.03**	**2.06±0.06**	**9.33±1.94**	**2.31±0.02**	**10.14±2.17**

25		1.22	1.55	39.63	1.52	72.48	1.64	66.80
26		1.05	1.44	28.65	1.50	58.72	1.44	69.17
27	**STD**	1.05	1.5	17.67	1.77	33.94	1.72	47.04
28		1	1.43	21.33	1.53	51.38	1.63	50.20
29		1.13	1.32	65.24	1.41	74.31	1.48	72.33
30		0.94	1.31	32.31	1.21	75.23	1.48	57.31
Mean ± S.E.M		**1.06±0.04**	**1.42±0.06**	**34.14±6.99**	**1.49±0.07**	**61.07±6.69**	**1.57±0.05**	**60.47±4.29**

Values are mentioned as Mean ± S.E.M (n=6).

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