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ANTISPASMODIC AND ANTHELMINTIC ACTIVITY OF *NYCTANTHES ARBORTRISTIS* LINN.

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ABSTRACT

Antispasmodic activity of the ethanolic extracts of different parts of *Nyctanthes arbortristis* Linn. was estimated using guinea pig ileum preparation against acetylcholine. Anthelmintic activity was tested following the method described by Kailashraj and Kurup, using earthworm (*Pheretima posthuma*). The extracts exhibited antispasmodic activity, which was less than that of piperazine citrate. The ethanolic extracts were found to have concentration-dependent paralytic activity, whereas its seeds and flowers showed lethal effect on the worms. It was also observed that the paralytic and lethal effects of respective ethanolic extracts were potentiated by the presence of atropine. It reveals that the anthelmintic activity of the extracts is due to the inhibition of motility by relaxing and depressing responsiveness to contractile action of acetylcholine.

INTRODUCTION:

Nyctanthes arbortristis Linn. (Fam. Oleaceae), commonly known as Harsingar or Night Jasmine, is a common wild hardy large shrub or small tree. It is a native of India, distributed wild in sub-Himalayan regions and southwards to Godavari. It is also found in Indian gardens for ornamental purposes[1-2]. Its different parts are known to possess different pharmacological activities in Indian systems of medicines. Several phytochemical and pharmacological investigations have also been done on this plant[4-12]. The antispasmodic[4] and anthelmintic[1-3] activities of the leaves of *Nyctanthes arbortristis* L have been reported. The present study is carried out with the ethanolic extracts of its different parts like flowers, barks, seeds and leaves to study and confirm its anthelmintic activity.

MATERIALS AND METHODS:

Plant material: The flowers, barks, seeds and leaves of *Nyctanthes arbortristis* L. were collected from the gardens and forests of Orissa. The herbarium of the plant (CNH/I-I(20)/2005-Tech-II/254) was authenticated as *Nyctanthes arbortristis* (Fam.Oleaceae) from Botanical Survey of India, Kolkata. The dried leaves, barks and seeds were powdered coarsely and than were extracted successively with petroleum ether, chloroform and ethanol (90%) in soxhlet apparatus[10-12]. Its fresh flowers were extracted with ethanol (50%)[10-11]. The ethanolic extracts of leaves, barks, seeds and flowers were evaporated to dryness to get dark gummy masses, having yield value 14%, 12.5%, 26.5% and 13% respectively. The water soluble-portions of the extracts were subjected to further pharmacological screening.

Animals: Guinea pig weighing 400-600 g was obtained from the animal house of B.I.T., Mesra, Ranchi. It was kept under controlled environmental conditions allowing free access to food and water and acclimatized for at least a week before the commencement of the experiment. The Institutional Animals Ethics Committee (Registration No. 62/02/ac/CPCSEA) approved the experiments.

Antispasmodic activity: The antispasmodic activity was estimated using guinea pig ileum preparation against acetylcholine, used as the spasmodic agent[13-14]. Effect of the isolated compound on the concentration response curve of acetylcholine was observed. Then keeping the concentration of acetylcholine constant in the bath solution, responses were observed with the increasing concentrations of arbortristoside-A to estimate the amount, required to block the contraction that was produced by acetylcholine.

Anthelmintic activity: The anthelmintic activity was observed according to the method described by Kailashraj and Kurup (1962)[15]. Six earthworms, *Pheretima posthuma* of nearly equal size (8±1cm) were placed in each petridishes containing 15ml of normal saline and different dilutions (0.1%, 0.2%, and 0.5%) of the extracts and the standard piperazine citrate with normal saline. The

time taken by the worms to become motionless, considered as to paralysis was recorded.

The lethal time was recorded by observing the time taken to become motionless on repeated application of external stimuli by pricking a pin. In the similar manner the experiment was repeated with all the extracts at different dilutions along with 0.1 ml atropine and the time taken by the worms for paralysis and the lethal time were recorded.

Statistical analysis: The results were subjected to statistical analysis, using ANOVA to determine the significance of the present study, where $p<0.01$ and $p<0.001$ were considered to be significant.

RESULTS:

Antispasmodic activity: The ethanolic extracts of different plant parts of *Nyctanthes arbortristis* Linn. were found to reduce the concentration dependent responses produced by acetylcholine. The contractile response of 0.0002 mg of acetylcholine was inhibited by 90, 132, 120 and 72 mg of ethanolic extracts of the seeds, leaves, barks and flowers of *Nyctanthes arbortristis* Linn. Respectively, whereas piperazine citrate at 16 mg (Table 1).

Anthelmintic activity: The results are summarized in Table 2. The ethanolic extracts exhibited a concentration dependent paralytic effect. The seeds and flowers showed to cause lethal effect at the concentration of 2 and 5 mg/ml, whereas the leaves and barks showed lethal effect on the worms at 5mg/ml only. Anthelmintic activity of *Nyctanthes arbortristis* L is less than that of the standard; Piperazine citrate. It was also observed that the paralytic and lethal effects of the ethanolic extracts of the seeds, barks and flowers were observed to be potentiated by the presence of atropine and so of the standard drug piperazine citrate. But the ethanolic extract of the leaves showed to cause lethality at higher concentration of 5mg/ml.

Table 2: Effect of ethanolic extracts of different plant parts of *Nyctanthes arbortristis* Linn. on the response obtained by 0.0002 mg of Ach on isolated guinea pig ileum preparation

Isolated guinea pig ileum preparation:

2 ml of Ach + Drug treatment	Amount (mg)required to block the contraction produced by 0.0002 mg of acetylcholine
Atropine (1 μ g/ml)	0.0003
Piperazine citrate (10mg/ml)	16
Flowers extract (60 mg/ml)	72
Barks extract (60mg/ml)	120
Seeds extract (60 mg/ml)	90
Leaves extract (60mg/ml)	132

Table 1: Anthelmintic activity of different parts of *Nyctanthes arbortristis* Linn

Treatment	Percentage Concentration	Time in min.			
		For paralysis		For death	
		Without Atropine	With Atropine	Without Atropine	With Atropine
Control	15 ml	-	-	-	-
Piperazine Citrate	0.1	73.0 ± 0.408	10.5 ±0.289	-	18.5 ± 1.259
	0.2	65.25 ± 0.947	65.25±0.947	-	9.75 ± 0.354
	0.5	55.0 ± 0.817	55.0 ± 0.814	-	7.75 ± 0.25
NAF	0.1	166.25 ± 1.75	15.25 ± 0.479	-	22.25 ± 0.629
	0.2	144.5 ± 1.051	10.25 ± 0.629	252.75 ± 1.53	20.0 ± 0.408
	0.5	111.25 ± 1.493	3.5 ± 0.289	236.25 ± 1.75	16.0 ± 0.823
NAB	0.1	94.75 ± 1.25	29.0 ± 1.354	-	37.25 ± 1.023
	0.2	81.0 ± 1.732	23.75 ± 0.75	-	27.25 ± 0.25
	0.5	67.0±2.45	13.0 ± 0.812	262.0 ± 2.102	20.25 ± 0.344
NAS	0.1	143.25 ± 2.136	6. 25 ± 0.479	-	16.75 ± 0.479
	0.2	116.5 ± 1.553	4.25 ± 0.335	242.75 ± 2.287	9.0 ± 0.408
	0.5	88.5 ± 0.646	3.75 ± 0.479	213.75 ± 1.75	6.0 ± 0.408
NAL	0.1	142.5 ± 0.5	125.0 ± 3.536	-	-
	0.2	115.5 ± 0.646	120.0 ± 3.536	-	-
	0.5	90.5 ± 2.102	43.0 ± 2.0	266.25 ± 1.75	101.5 ± 3.67

Values are expressed as Mean ± SEM NAF, NAB, NAS and NAL: Ethanolic extracts of the flowers, barks, seeds and leaves of *Nyctanthes arbortristis* L respectively

DISCUSSION:

The present study was undertaken on the antispasmodic and anthelmintic activity of the ethanolic extracts of different plant parts of *Nyctanthes arbortristis* Linn; on the basis of inhibition of contractile effect of acetyl choline by various dilutions of the ethanolic extracts, it initiated the study that the potentiation of the activity of the atropine on the motility of anthelmintics. Out of which flowers and seeds of this plant possess good antispasmodic activity. The ethanolic extracts of the seeds and flowers of *Nyctanthes arbortristis* L have more potent anthelmintic activity than that of the barks and leaves, but less than that of the standard drug piperazine citrate.

It has been observed that the ethanolic extracts of seeds, barks and flowers of *Nyctanthes arbortristis* L inhibited the contractile response of acetyl choline. Further, potentiation of the anthelmintic activity of atropine by the ethanolic extracts of different plant parts of the native plant might be due to inhibition of motility by relaxing and depressing responsiveness to contractile action of acetyl choline.

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