ANXIOLYTIC ACTIVITY OF LEAF EXTRACT OF *NYCTANTHES ARBOR-TRISTIS* IN EXPERIMENTAL RATS

Shalini Tripathi[a*], P. K. Tripathi[a], M.Vijayakumar[b], Ch.V.Rao[b], P.N.Singh[c]

[a]Mahatma Gandhi College of Pharmacy, Lucknow- 227101,
Uttar Pradesh, India
[b]Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India.
[c]Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi -220015

Summary

Hydroalcoholic extract of leaves of Nyctanthes arbor-tristis Linn (NAT) was Pharmacologically validated for its anxiolytic properties in experimental animals using various models such as elevated zero maze, elevated plus maze, open field exploratory behavior, novelty induced suppressed feeding test and social interaction test. Extract was given orally at different dose levels once daily for three consecutive days, while Lorazepam (Lor) (500 mg/kg i.p.) was administered as positive control. NAT (250 and 500mg/kg) showed significant anxiolytic effects on all the models of anxiety. The result reveals that NAT induced a promising increase in open field ambulation and slight increase in rearings and activity in center whereas grooming and fecal dropping remained unchanged. In elevated plus maze, open arm entries, open arm/closed arm are entries ratio and the time spent on open arms was found to be increased. Both the NAT significantly produced the novelty induced increase in feeding latency test. NAT treated animals also showed increased social interaction in normal environment. The NAT observed under above parameters showed positive anxiolytic activity.

Keywords: Anxiolytic, *Nyctanthes arbor-tristis*, Harsingar, elevated plus-maze test

***C**orresponding author: Shalini Tripathi, Mahatma Gandhi College of Pharmacy, Lucknow- 227101, Uttar Prdaesh India; Email: tripathi.pushpendra@rediffmail.com; Phone: +91-9415087183

Introduction

Nyctanthes arbor-tristis, (Fam. Oleaceae) is commonly known as Parijatham, Harsinghar and Night Jasmine. The leaves, flowers, seeds and bark of *Nyctanthes arbor-tristis* are widely used in traditional remedies and folkloric medicines in India. It is widely distributed throughout India and also cultivated in gardens for its fragrant flowers (1, 2). The fresh juice obtained from the leaves of the plant found to have antimalarial activity (3). The 50% ethanolic extract of the seeds, leaves, roots, flowers and stem of the plant has been proved to posses antiamoebic (4) and antiallergenic properties (5). Leaf extract of the plant showed anti-inflammatory (6), analgesic, antipyretic and ulcerogenic activities (7).

Immunostimulant activity of the leaves, seeds and flowers of the plant has been reported (8). The water soluble fraction of the ethanolic extract has been proved to posses tranquilizing, antihistaminic, purgative effects (9) and depletion of tumor necrosis factor - α (10).The arbortristoside A isolated from the seeds found to have antitumor activity (11).

Many iridoid glycosides have been isolated from the leaves and seeds of the plant. These includes arborside A, arborside B and arborside C (12). The triterpenoides lupeol, oleanolic acid, friedelin and nyctanthic acid were isolated from the leaves of the plant (13). Naringenin-4'-0-β-glucopyrenosyl-α-xylopyranoside (14) has been isolated from the fresh stems of the plant. β-monogentiobioside of α -crocin or crocin 3, β-monogentiobioside-β-D-monoglucoside ester of α-crocin or crocin 2 and β-digentiobioside ester of α-crocin or crocin1 (15) have been isolated from the corolla tubes of flowers of *N. arbor-tristis*. The present study was designed to evaluate anxiolytic activity of leaf extract of *Nyctanthes arbor-tristis*.

Materials and Methods

Preparation of plant extracts

The leaves of *Nyctanthes arbor-tristis* were collected from the local garden of the Rajarshi Rananjay Singh College of Pharmacy, Amethi, India in May 2004. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. A voucher specimen (LWG accessions No. 94392) of the collected sample was deposited in the institutional herbarium for future reference. The powdered leaves of *Nyctanthes arbor-tristis* (5 kg) were passed through S.S. sieve (20mesh) before extraction. Plant material was successively extracted with ethanol (50%) in soxhlet apparatus. The crude extract obtained was concentrated in a rotary evaporator under reduced pressure and freeze dried to yield 12.5 %w/w (NAT).

Animals

Adult albino rats (150-180g) and Wister mice (25-35g) of either sex were obtained form the Animal House of the Institute and were randomly distributed into different experimental groups. The rats were housed in groups of six in polypropylene cages at an ambient temperature of 25±10C and 45-55% RH with a 12:12 h light /dark cycle. Animals were provided with commercial food pellets and water ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals.

Drug treatment

In the acute toxicity study no deaths were observed during the period at the doses tested up to 2000mg/kg. Hence, the NAT was administered orally at two different dose levels (250 and 500 mg/kg) once daily for three consecutive days. Control group of animals received suspension of 1% CMC in distilled water. Lorazepam (500 mg/kg, i.p.) was used as standard drug and were administered intraperitoneally to rodents 30 min before experiments for comparison. Experiments were conducted on day 3, one hour after the last drug administration.

Assessment of anxiolytic activity

Open-field test

The open-field apparatus was made of plywood and consisted of squares (61x61 cm). The entire apparatus was painted black except for 6 mm thick white lines, which divided the floor into 16 squares. Open-field was lighted by a 40W bulb focusing on the field from a height of about 100 cm. The entire room, except the open-field was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioural aspects were noted. Ambulation: this was measured in terms of the number of squares crossed by the animal, Rearings: number of times the animal stood on its hind limbs, Self groomings: number of times the animal groomed facial region, and licked/ washed/scratched various parts of its body, *Activity in center*: number of central squares crossed by the animal; and, Fecal droppings: number of fecal droppings excreted during the period (16).

Elevated plus-maze test

The maze had two opposite arms, 50x10 cm. crossed with two enclosed arms of the same dimension but having 40 cm high walls. The arms were connected with a central square, 10x10 cm, giving the apparatus shape of a plus sign. The maze was kept in a dimly lit room and elevated 50 cm above the floor. Native rats were placed individually in center of the maze facing an enclosed arm. Thereafter, number of entries and time spent on the open and closed arms were recorded during the next 5 min. An arm entry was defined when all four paws of the rat were in the arm. A neutral 'blind' observer made observations (17).

Elevated zero-maze test

The maze comprised of a black Perspex annular platform (105 cm in diameter, 10cm width) elevated to 65 cm above the ground level, divided equally into four quadrants. The two opposite quadrants were enclosed by a black Perspex wall (27 cm high) on both the inner and outer edges of the platform, while the remaining two opposite quadrants were surrounded by Perspex "lip" (1 cm high) which served as a tactile guide to animals on these open areas. The apparatus was illuminated by dim white light arranged in such a manner as to provide similar lux levels in open and enclosed quadrants. Rats were placed on one of the enclosed quadrants for a 5 min test period. The maze was cleaned with 5% ethanol/ water solution and dried thoroughly between test sessions. During the 5 min test period time spent on open arms, number of head dips' over the edges of platform, and number of stretched attend postures' from closed to open quadrants were recorded . Animals were scored as being in the open area when all four paws were in the open quadrants and in the enclosed area only when all four paws had passed the open-closed divide (18).

Social interaction test

The rats were first housed individually for 5 days before testing. The apparatus used for the test was a wooden box (60x60x35 cm) with a solid floor and was placed in a dimly lit room. On day 6, the rats were placed individually in the box and given two 7.5 min familiarization sessions at 2 h interval. On day 7, rats were paired on weight and sex basis and placed in the box for 7.5 min.

During this time total time spent by the rat pair in "social interaction", including sniffing, following, grooming, kicking, boxing, biting and crawling under or over the partner, was recorded by a neutral 'blind' observer (19).

Novelty induced suppressed feeding latency test

The test apparatus was a wooden box (60x60x35 cm) with a solid floor placed in a dimly lit room. The floor of the wooden box was covered with 2cm layer of wooden chips, and laboratory chow pellets were evenly placed on the floor. A similar arrangement was made in the home cages of the rats. Food was removed from the home cage 48 h prior to testing, but water was provided ad libitum. Native rats were placed individually in the test chamber and the latency to begin eating (defined as chewing of the pellet and not merely sniffing of playing with it), was recorded. If the rat had not eaten within 300 sec, the test was terminated and latency score 300 sec was assigned. A neutral 'blind' observer made observations (20).

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad software for the determination of level of significance.

Results and Discussions

For anxiety most of the animal models were developed for benzodiazepines (BDZ) and, since these compounds also exhibit significant muscle relaxant and anticonvulsant effects, evaluation of anxiolytic activity, even with non-BDZ compounds, invariably now includes tests for these neuropharmacological actions (21). The sedative, amnesic and ataxic effects of BDZ and non-BDZ anxiolytic are definite drawbacks when these drugs are used for the treatment of anxiety. The present study, evaluated the anxiolytic activity of leaf extract of *Nyctanthes arbor-tristis* (NAT).

Rats treated with both the dose of NAT showed dose dependent significant increase in open field ambulation, rearings, self grooming and activity in center with compared to vehicle treated control rats, evincing significant anxiolytic activity of NAT. However the open-field fecal droppings remain unchanged. Lorazepam (Lor) also induced significant anxiolytic activity and the effects were found to be more than that of NAT (Table 1). In the open field, animals are in a novel environment, they express decreased ambulation, exploration, freezing, rearing and grooming behaviour, and increased defecation due to anxiety and fear which heightened autonomic activity, these behavioural changes are attenuated due to classical anxiolytics and augmented by anxiogenic agents (22).

Table 1. Effect of NAT on open field exploratory behavior in rats

Treatment (mg/kg)	Ambulation	Rearings	Self groomings	Activity in centre	Fecal droppings
Control	45.01 ± 4.54	665 ± 1.03	6.47 ± 1.01	1.86 ± 0.17	3.52 ± 0.65
NAT 250	65.10 ± 4.46**	9.12 ± 1.12	8.12 ± 1.15	3.56 ± 0.58*	2.12 ± 0.34
NAT 500	78.71 ± 5.32***	12.23 ± 1.48*	10.82 ± 1.10*	4.52 ± 0.68**	1.35 ± 0.26*
Lor 500	84.46 ± 6.42***	14.25 ± 1.89**	12.92 ± 1.24**	5.82 ± 1.02***	2.16 ± 0.21

Values are expressed as mean ± SEM, control n =12 & treatment extract n = 6. *P< .05, **P<0.01, ***P<0.001 as compared to control

Similarly in elevated plus maze and elevated zero maze tests, open closed arm entries and time ratios provide a measure of fear induced inhibition of exploratory activity. These responses are increased by anxiolytic agents (23). NAT treated rats exhibited dose dependent significant increase in time spent in open arms, entries made on open arms and significant decrease in time spent in enclosed arms and entries on enclosed arms in comparison to control rats. The result obtained by open/closed time and entries ratios also indicated significant anxiolytic in rats by NAT. Lorazepam caused more anxiolysis in comparison to NAT. The results have been summarised in Table 2.

Table 2. Effect of NAT extracts on the elevated plus maze behaviors in

Treatment (mg/kg)	Time spent (sec)		No. of Entries	
	Enclosed arms	Open arms	Enclosed arms	Open arms
Control	210. 54 ± 8.89	27.91 ± 2.68	6.84 ± 2.1	2.68 ± 0.81
NAT 250	195. 98 ± 8.48**	36.66 ± 3. 92***	8.14 ± 1.3	3.85 ± 1.11*
NAT 500	172. 32 ± 6.26***	67.23 ± 5. 98***	11.23 ± 2.11**	5.75 ± 1.21***
Lor 500	166. 98 ± 7.11***	71.12 ± 4.15***	9.83 ± 1.91*	6.95 ± 1.53***

Values are expressed as mean ± SEM, control n =12 & treatment extract n = 6. *P< .05, **P<0.01, ***P<0.001 as compared to control

Table 3. Effect of NAT extract on the elevated zero maze behaviors in rats.

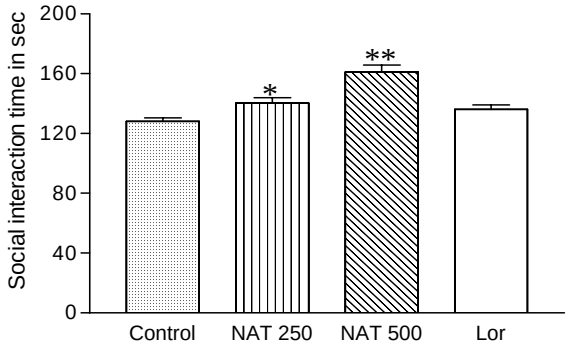
Treatment (mg/kg)	Time spent on open arms (sec)	Head dips(N)	Streched attend postures (N)	Entries in open arms (N)
Control	49.25 ± 3.45	07.81 ± 1.70	3.75 ± 0.82	4.65 ± 1.32
NAT 250	54.11 ± 3.52**	11.84 ± 3.24**	2.99 ± 0.71	7.28 ± 1.65**
NAT 500	71.98 ± 2.21**	12.44 ± 4.02**	2.54 ± 0.85*	10.24 ± 1.26***
Lor 500	72.14 ± 1.75***	15.12 ± 1.92***	3.68 ± 0.74*	12.33 ± 2.62***

Values are expressed as mean ± SEM, control n =12 & treatment extract n = 6.
*P< .05, **P<0.01, ***P<0.001 as compared to control

The rats treated with NAT showed anxiolysis in terms of significant increase in time spent in open arms, entries in open arms and number of head dips on elevated zero maze. However the responses stretched attend postures remains unchanged. The results have been summarised in Table 3. The rats treated with NAT spent significantly more time in social interaction in comparison to control rats and effect of NAT extract was found to be dose dependent. Lorazepam also caused significant increase in social interaction (Fig. 1). NAT caused dose dependent significant attenuation of novelty induce feeding latency in rats in comparison to vehicle treatment. Lorazepam also induced similar effects, however, it was observed to be more than that of NAT extract (Fig 2). Likewise, anxiolytics increase the social interaction and decrease the feeding latency respectively in the social interaction and novelty induced suppressed feeding latency tests in a novel environment (22).

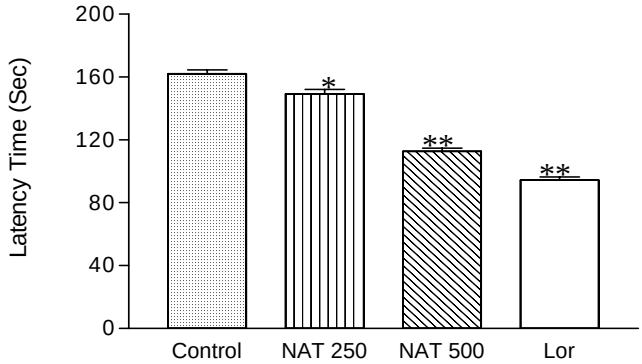
Overall, the results of the present study indicate that ethnolic extract of NAT treatment caused significant dose related anxiolysis in rats tested on all the behavioural paradigms.

Fig.1. Effect of NAT on social interaction in rats



Control n =12 & treatment extract n = 6. *P< .05, **P<0.001 as compared to control

Fig.2. Effect of NAT on latency to feed in rats



Control n =12 & treatment extract n = 6. *P<0.01, **P<0.001 as compared to control

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