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Evaluation of Anxiolytic Effect in Flowers of *Nyctanthes arbor-tristis.*

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Abstract
Water soluble fraction of ethanolic extract of flowers of *Nyctanthes arbor-tristis* Linn (NATF) 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 (250 and 500 mg/kg p.o.) once daily for three consecutive days, while Lorazepam (Lor) (0.5 mg/kg i.p.) was administered as positive control. NATF showed significant anxiolytic effects on all the models of anxiety. The result reveals that NATF 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 and the time spent on open arms was found to be increased. Both the doses of NATF significantly produced the novelty induced increase in feeding latency test. NATF treated animals also showed increased social interaction in normal environment. The NATF observed under above parameters showed positive anxiolytic activity.

Key Words
Anxiolytic, Harsingar, *Nyctanthes arbor-tristis*.

Introduction

Anxiety is a feeling of apprehension, uncertainty, nervousness, or fear stemming from the anticipation of imaginary or unreal threat (Kulkarni & Reddy, 1996). The source of this uneasiness is not always known or recognized. An exaggerated or fearful response to an appropriate or inappropriate condition may be observed during anxiety (Blanchard et al, 2001). Anxiety affects one-eighth population worldwide and has become an important research area in the field of psychopharmacology (Yadav et al. 2008). *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 (Kirtikar, 1935; Singh et al, 1995). The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseases (Khatune et al.,2003), in

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the treatment of ophthalmic purposes (Sasmal et al.,2007) and in Antispasmodic activity (Das et al., 2010). The bright orange corolla tubes of the flowers contain a coloring substance nyctanthin, which is identical with ά-Crocetin ($C_{20}H_{24}O_4$) from Saffron. The corolla tubes were formerly used for dyeing silk, sometimes together with Safflower or turmeric (Rout et al., 2007). The leaves, flowers, seeds and barks (600 mg/kg) of *Nyctanthes arbor-tristis* Linn showed significant and dose-dependent prolongation of onset and duration of sleep and was found to cause decrease in dopamine and increase serotonin level. From which it can be concluded that the CNS depressant activity of the ethanol extracts of seeds, leaves and flowers may be due to the decrease in dopamine and increase in serotonin level (Das et al., 2008).The present study was designed to evaluate anxiolytic activity of flower extract of *Nyctanthes arbor-tristis*.

Materials and Methods

Plant Material

The aerial part of *Nyctanthes arbor-tristis* were collected from the local garden of Lucknow, India, in summers. 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.

Preparation of Extracts

The powdered flowers of *Nyctanthes arbor-tristis* were passed through S.S. Sieve (20 mesh) 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 7.5 % w/w of fruit extract (NATF). Water soluble fraction of this extract was used for the evaluation of anxiolytic activity.

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.

Safety Evaluation

NATEF was administered to 10 mice and 10 rats in a dose of 2g/Kg p.o. and observations were made for gross behavioral changes such as locomotion, rearing, respiration, tremors, passivity, righting reflex, lacrimation and mortality for 14 days (Ghosh, 1984).

Drug Treatment

In the acute toxicity study no deaths were observed during the period at the doses tested up to 2000 mg / kg. Single dose administration of NATF did not reveal any significant effect in rodents hence, the NATF 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 (0.5 mg/kg, i.p.) was used as standard drug and was 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 behavioral 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 grooming: 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 (Brostein, 1972).

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 (Pellow, 1986).

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 (Shepherd, 1993).

Social Interection 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 (File, 1978).

Novelty Induced Supressed 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 (Bodnoff, 1988).

Stastical 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

Open Field Test

Rats treated with both the dose of NATF showed dose dependent significant increase in open field ambulation, rearings, self grooming and activity in center as compared to vehicle treated control rats, evincing significant anxiolytic activity of NATF. 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 NATF (Table 1).

Elevated Plus Maze Test

NATF 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 NATF. Lorazepam caused more anxiolysis in comparison to NATF. The results have been summarized in Table 2.

Elevated Zero Maze Test

The rats treated with NATF 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 response of stretched attend postures remains unchanged. The results have been summarized in Table 3.

Social Interection Test

The rats treated with NATF spent significantly more time in social interaction in comparison to control rats and effect of NATF extract was found to be dose dependent. Lorazepam also caused significant increase in social interaction (Fig. 1).

Novelty Induced Supressed Feeding Latency Test

NATF 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 NATF extract (Fig 2).

Discussion

For anxiety most of the animal models were developed for benzodiazepines (BDZ) (Wada et al, 1989) and the sedative, amnesic and ataxic effects of BDZ and non-BDZ anxiolytic are major drawbacks of these drugs when used for the treatment of anxiety. Water soluble fraction of ethanol extract of flowers of *Nyctanthes arbor-tristis* (NATF) was evaluated in various behavioral animal models viz. Open- field, Elevated plus-maze, Elevated zero maze test, Social Interection test, Novelty induced supressed feeding latency test for the anxiolytic activity. These are animal models of preliminary pharmacological test of activities on central nervous system, which provide information about action upon psychomotor performance, motor behavior and anxiolysis. In the open field, animals are in a novel environment, they show decreased ambulation, freezing, exploration, rearing and grooming behavior, and increased defecation due to anxiety and fear which heightened autonomic activity, these behavioral changes are attenuated due to classical anxiolytics and augmented by anxiogenic agents (Bhattacharya et al, 1997). Lorazepam acts on benzodiazepine receptors and increases the activities in open field when compared to control. The extract also significantly increased open field activities with respect to control group. The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA A benzodiazepine complex (Dhonnchadha et al, 2003). This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines (Maribel et.al, 2006). 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 (Pellow et al, 1985). In elevated plus maze, mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards

open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics (Hellion et.al, 2006). Extract shows the similar effects of the anxiolytics as shown by the lorazepam. In social interaction test the extract shows the positive response for the anxiolytic activity as compared to the control. In novelty induced suppressed feeding latency test the feeding latency decreases in both lorazepam and extract as compared to the control. 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 (Bhattacharya et al, 1997). Novel enviornment gives a fear response, a state of anxiety in experimental animals which decreses their normal social behaviour and this can be overcome with the treatment of anxiolytics. The present studiy was undertaken to evaluate the anxiolytic activity of *Nyctanthes arbor-tristis* fruit which was found comparable to Lorazepam which is a GABA agonist but involvement of similar mechanism i.e. GABAergic activity of the extract can not be ruled out. We conclude that water soluble fraction of ethanolic extract of flowers of *Nyctanthes arbor tristis* linn produced significant anxiolysis in experimental animals; however the anxiolytic activity of extract were found to be less as compared to standard anxiolytic agent lorazapam.

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Fig. 1: Effect of *Nyctanthes arbor - tristis* Flower Extract on Social Interaction in Rats.

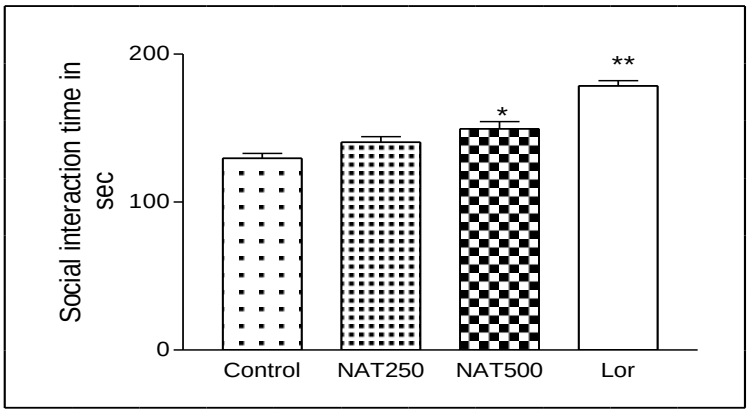

Fig. 2: Effect of *Nyctanthes arbor - tristis* Flower Extract on Latency to Feed in Rats.

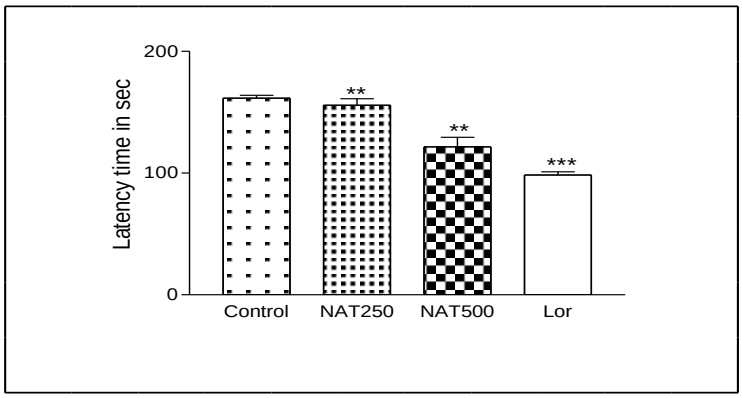

Table 1: Effect of *Nyctanthes arbor - tristis* Flower Extract on Open Field Exploratory Behaviour in Rats.

Treatment	Ambulation	Rearing	Self grooming	Activity in centre	Faecal dropping
Control	45.01 ± 4.54	6.65 ± 1.03	6.47 ± 1.01	1.86 ± 0.17	3.52 ± 0.65
NAT 250	60.56 ± 4.76*	7.98 ± 0.99	7.78 ± 1.15	3.48 ± 0.48*	1.98 ± 0.45
NAT 500	68.71 ± 3.75**	11.65 ± 0.98*	10.71 ± 0.56*	4.47 ± 0.56**	1.40 ± 0.20
Lor. 5	4.46 ± 6.42***	14.25 ±1.89**	12.92 ± 1.24**	5.82 ±1.02***	2.16 ± 0.21

Values are expressed as means ± S.E.M., control n= 12 & treatment extract = 6.
*P < 0.05, **P < 0.01 and, ***P < 0.001 as compared to control.

Table 2: Effect of *Nyctanthes arbor - tristis* Flower Extract on the Elevated Plus Maze in Rats.

Treatment (mg/kg)	Time spent (sec)		No. of Entries	
	Enclosed arms	Open arms	Enclosed arms	Open arms
Control	210.54 ± 8.89	27.10 ± 2.68	6.84 ± 2.10	2.68 ± 0.81
NAT 250	199.82 ± 9.56	29.80 ± 2.97	7.67 ± 1.25	2.89 ± 0.98
NAT 500	182.35 ±7.24*	59.86 ±4.56***	10.89 ± 2.13	4.47 ± 1.34
Lor. 5	166.98±7.11***	71.12±4.15***	9.83 ± 1.91*	6.95 ±1.53***

Values are expressed as means ± S.E.M., control n= 12 & treatment extract = 6.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control.

Table 3: Effect of *Nyctanthes arbor - tristis* Flower Extract on Elevated Zero Maze Behaviour in Rats.

Treatment (mg/kg)	Time spent on open arms (sec)	Head dips (N)	Stretched attend posture (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	48.56 ± 3.68	9.94 ± 3.78	3.12 ± 0.8	4.12 ± 0.67*
NAT 500	61.58 ± 2.98	10.46 ± 4.56	2.98 ± 0.9	8.96 ± 1.26**
Lor. 5	72.14±1.75***	15.12 ±1.92**	3.68 ± 0.7	12.33±2.62***

Values are expressed as means ± S.E.M., control n= 12 & treatment extract = 6.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control.

Conflict of Interest: Not Declared

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