ANTIDEPRESSANT ACTIVITY OF NYCTANTHES *ARBOR-TRISTIS* LEAF EXTRACT

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Summary

Hydroalcoholic extract of leaves of Nyctanthes arbor-tristis Linn (NAT) was Pharmacologically validated for its antidepressant properties in experimental animals using various models such as Forced swim test, Learned helplessness test, Tail suspension test and Reserpine induced hypothermia **.**Extract was given orally at different dose levels once daily for three consecutive days, while Imipramine (15 mg/kg, i.p.) was administered as positive control. NAT (250 and 500mg/kg) showed significant antidepressant effects on all the models. Immobility time in FST and TST was significantly reduced by NAT. A decrease in number of escape failures in LHT was also observed in NAT treated rats. NAT also completely antagonized reserpine induced hypothermia. Results suggested that NAT showed significant antidepressant activity.

Keywords: Antidepressent, *Nyctanthes arbor-tristis*,

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Introduction

Depression is a heterogeneous disorder that affects a person's mood, physical health and behaviour. Suicidal tendency remains one of the common outcomes of depression, with depressive illness being responsible for 60% of the death toll. The causes of depression vary. Psychosocial factors, such as adverse living conditions, can influence the onset and persistence of depressive episodes. Genetic and biological factors also play a part. It is estimated that 5.8% of men and 9.5% of women will experience a depressive episode in any given year. These prevalence figures can, however, vary across different populations. It is estimated that 121 million people currently suffer from depression and it will become the second leading cause of premature death or disability worldwide by the year 2020 (1). Despite considerable progress made during the last few decades, successful treatment of clinical depression with currently available therapeutic agents can be achieved only in 65-75% of which only 40-50% achieves complete recovery (2). Although there are many effective antidepressants available today, the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated The search for new molecules as target for antidepressants drug discovery, therefore, remains a continuing challenging for modern psychiatry. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly (3).

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 (4, 5). The fresh juice obtained from the leaves of the plant found to have antimalarial activity (6). The 50% ethanolic extract of the seeds, leaves, roots, flowers and stem of the plant has been proved to posses antiamoebic (7) and antiallergenic properties (8). Leaf extract of the plant showed anti-inflammatory (9), analgesic, antipyretic and ulcerogenic activities (10). Immunostimulant activity of the leaves, seeds and flowers of the plant has been reported (11). The water soluble fraction of the ethanolic extract has been proved to posses tranquilizing, antihistaminic, purgative effects (12) and depletion of tumor necrosis factor - α (13). Earlier, we have reported the anxiolytic activity of leaf extract of *Nyctanthes arbor-tristis* (14). The present study was designed to evaluate antidepressant activity of leaf extract of *Nyctanthes arbor-tristis* in experimentally induced depression in rats.

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

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. Positive control Imipramine (15 mg/kg, i.p.) was administered along with its control group (10%, v/v PEG, 10 ml/kg, i.p.) 30 min before the experimentation.

Assessment of antidepressant activity

Forced swim test

The rats were placed in a cylinder (45x20cm) containing 38 cm water (25 ± 2^{o}C), so that the rat could not touch bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pretest, followed by a 5 min test 24 h later. Drugs were administered after pretest. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period was noted (15).

Learned helplessness test

This model is based on the assumption that, exposure to uncontrollable stress associated with repeated experience of failure to escape form the stress produces a helpless situation, which result in performance deficits in subsequent learning tasks. In this experiment electric foot shocks were delivered in 20x10x10 cm chamber with Plexiglas walls and cover. Control rats were placed for 1 h in identical chambers but no shocks were administered. Inescapable shock pretreatment was in the morning. Avoidance training was initiated 48 h after inescapable shock pretreatment. Avoidance sessions performed for 3 consecutive days (day 3, 4 and 5) in the morning, and the number of escape failures, referred as no crossing response during shock delivery, was recorded (16).

Tail suspension test

A mouse was hung on a wire in an upside down posture so that its nostrils just touch the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5 min observation noted. This test is a reliable and rapid screening method for antidepressants, including those involving the serotonergic system (17).

Reserpine induced hypothermia

On the day before testing rats were dosed with 2 mg/kg reserpine (sigma, USA) subcutaneously. Rats had free access to food and water. Eighteen h after reserpine administration, the animals were placed into individual cages. The initial rectal temperature was determined by insertion of an electric thermometer (telethermometer) to a constant depth of 5 cm. Following administration of the NAT extract, the rectal temperature was measured at 18^{th}, 20^{th}, 22^{nd} and 24^{th} h after the resperine administration (18).

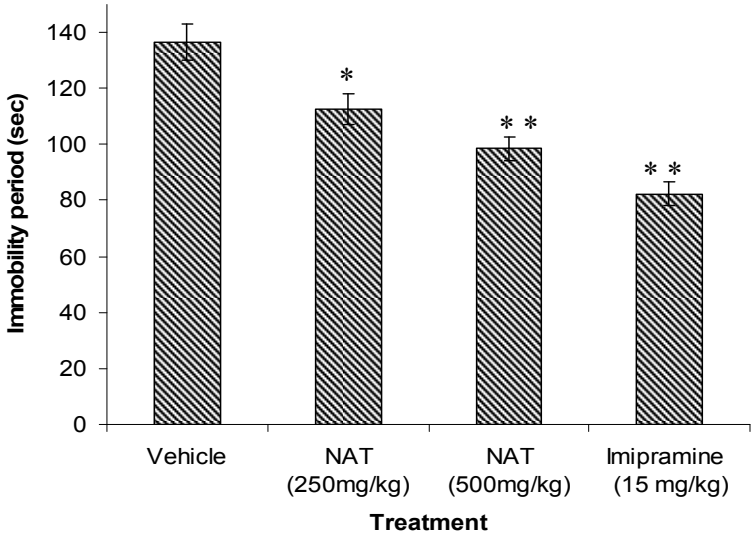
Statistical analysis

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

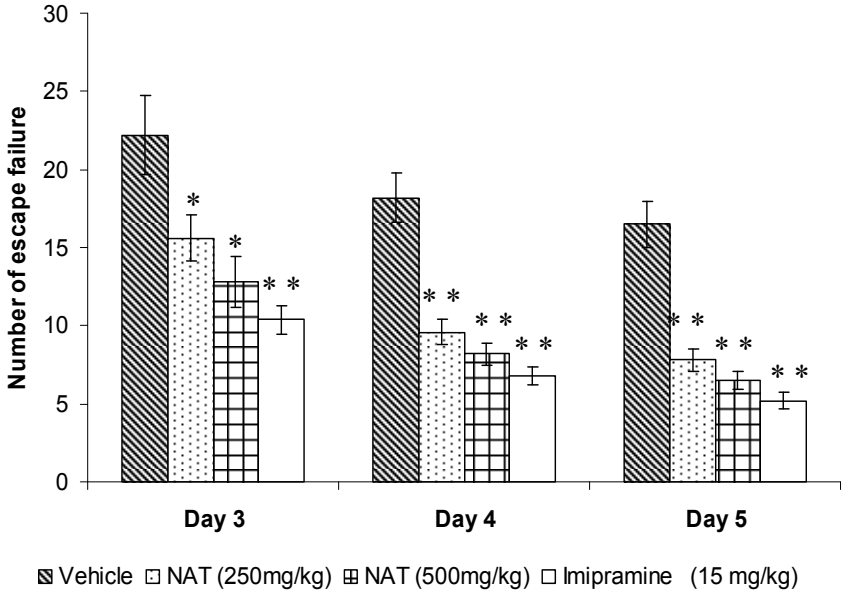
Behavioral despair test, in the initial acute administration of even high dose of NAT leaf extract did not reveal any antidepressant like effect in this test. Repeated oral administration of NAT extract for three consecutive days, however, dose dependently reduced the immobility time in rats. Imipramine also showed similar activity and the effect was comparable to that of higher doses of NAT (Fig. 1). In behavioral despair test mice or rats forced to swim in a restricted space from which they can not escape, exhibit a characteristic immobility. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli (19).

Fig. 1 Effect of NAT on immobility time in forced swimming test



Control n =12 & treatment extract n = 6. *P< .01, **P<0.001 as compared to control

Fig. 2 Effect of NAT on number of escape failure in the learned helplessness test

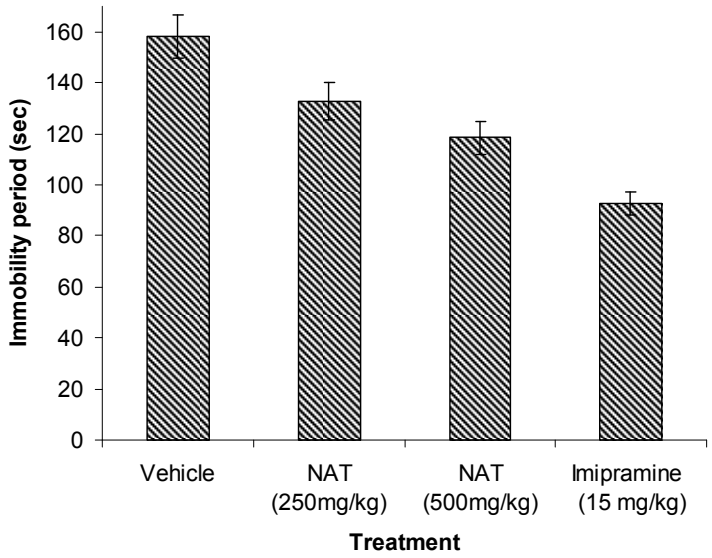


Control n =12 & treatment extract n = 6. *P< .01, **P<0.001 as compared to control

In learned helplessness test, the escape failures significantly and dose dependently decreased in rats treated with both the dose of NAT extract Imipramine showed more activity than that of NAT extract (Fig. 2). In learned helplessness test, rodents are exposing to inescapable and unavoidable electric shocks in one situation later fail to escape shock in a different situation when escape is possible (20). This phenomenon was evaluated as a potential model of depression (16). The learned helplessness test is a well-validated and reliable animal model of depression. In this model, induction of learned helplessness produces broad-ranging behavioural deficits in affect, cognition, sleep and motor performance that closely resemble many of the symptoms of depression (21).

In tail suspension test, NAT leaf extract caused a significant and dose dependent decrease in immobility time in tail suspension test. This effect is regarded as indicative for antidepressant activity (Fig. 3). In tail suspension test, immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothermia to reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempt to escape when suspended by tail (22).

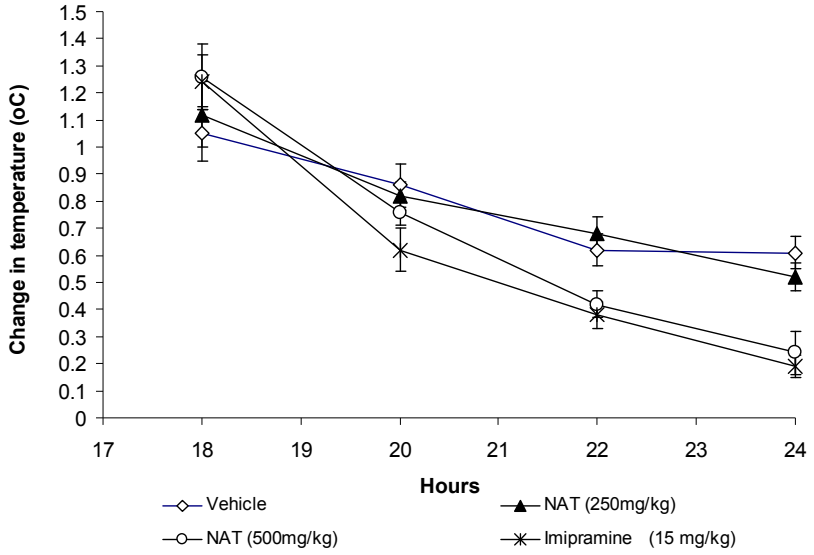
Fig. 3 Effect of NAT on immobility time in tail suspension test



Control n =12 & treatment extract n = 6. *P< .01, **P<0.001 as compared to control

Reserpine induced hypothermia test, the results demonstrate that NAT leaf extract completely antagonized reserpine induced hypothermia. Imipramine also showed complete antagonism of reserpine induced hypothermia and its effects were comparable to that of NAT leaf extract (Fig. 4). Reserpine induced hypothermia test has been proven as a simple and reliable method to detect antidepressants activity.

Fig. 4 Effect of NAT on reserpine induced hypothermia



Results suggested that NAT extracts possess significant anti depressant activity when tested against various models of depression, the observed antidepressant activity of *Nyctanthes arbor-tristis* leaf extract was qualitatively comparable to that induced by Imipramine.

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