

ANALGESIC ACTIVITY OF *NYCTANTHES ARBOR-TRISTIS* FRUITS IN RODENTS

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Summary

Water soluble fraction of ethanolic extract of fruits of *Nyctanthes arbor-tristis* Linn (NATEF) was pharmacologically validated for its analgesic properties in experimental animals using various models such as Tail flick latent period, Hot plate reaction in mice and Acetic acid induced writhing response in mice for analgesic activity. Extract was given orally at two different dose levels (250 and 500 mg/kg) once daily for three consecutive days, while Pentazocine (10 mg/kg) and Aspirin (25 mg/kg) were administered as positive control. Studies have shown that the fruit extract have activity to prevent pains in the rodents respectively. The analgesic effect was dose dependent and found to be statistically significant as compared to the control.

Keywords: Analgesic, *Nyctanthes arbor-tristis*

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Introduction

Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (1). *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. Widely distributed throughout India and also cultivated in gardens for its fragrant flowers (2, 3). The 50% ethanolic extract of the seeds, leaves, roots, flowers and stem of the plant has been proved to possess antiamebic (4) and anti allergic properties (5). The arbortristoside A isolated from the seeds found to have antitumor activity (6). Many iridoid glycosides have been isolated from the leaves and seeds of the plant. These include arborside A, arborside B and arborside C (7). Earlier, we have reported the anxiolytic activity (8) and antidepressant activity (9) of leaf extract of *Nyctanthes arbor-tristis*. The seeds are used as anthelmintics and in alopecia. It is antibilious and an expectorant, and is also useful in bilious fevers (10). The powdered seeds are used to cure scurfy affections of scalp, piles and skin diseases (11).

The indigenous people of Chittoor district, Andhra Pradesh (India) widely use the whole plant for treatment of cancer, root for fever, sciatica, anorexia; bark as expectorant (12).

The present study was designed to evaluate analgesic activity of fruit extract of *Nyctanthes arbor-tristis* in rodents.

Material and Method

Preparation of plant extracts

The fruits of *Nyctanthes arbor-tristis* were collected from the local garden of Lucknow, India. 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 fruits of *Nyctanthes arbor-tristis* (5 kg) 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 10.8% w/w. Water soluble fraction of this extract (NATEF) was taken for the study.

Animals

Adult albino rats (150-180g) and Wister mice (25-35g) of either sex were obtained from 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±10C⁰ 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 2000 mg/kg. Hence, the NATEF 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. Standard drugs were administered intraperitoneally to rodents 30 min. before experiments for comparison. Experiments were conducted on day 3, one hour after the last drug administration.

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 (13).

Assessment of Analgesic activity

The three most widely used rodent models were chosen to evaluate the effect of NATEF on analgesic behavior such as, Tail flick latent period, Hot plate reaction in mice, and Acetic acid induced writhing response in mice

Tail flick latent period:

The technique used was described by Devis and co-workers (1946), using a techno analgesiometer. The rat was placed in a rat holder, with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesiometer, called jacket with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current of 6 mA. Through the water jacket, cold water was continuously passed, so that the bridge did not heated and tail could be conveniently placed over the bridge. The time taken for the withdrawal of the tail after switching on the current, was considered as latent period, in sec, of ‘tail flicking’ response. This latent period was the index of nociception. The cut off time for determination of latent period was taken as 30 sec to avoid injury to the skin (14).

Hot plate reaction in mice:

Mice were screened by placing them on a hot plate maintained at $55\pm 1^{\circ}\text{C}$ and recording the reaction time in seconds for forepaw licking or jumping (15). Only mice which reacted with in 15 sec and which did not show large variation when tested on four separate occasions, each 15 min apart, were taken for the test. Pentazocine (10 mg/kg, i.p.) was used as a reference standard. The time for forepaw licking or jumping on the heated plated of the analgesiometer maintains at 55°C was taken as the reaction.

Acetic acid induced writhing response in mice:

Acetic acid solution (15mg/ml) at the dose of 300 mg/kg body weight was injected and the number of writhing in the following 30 min period was observed (15). A significant reduction in number of writhing by any treatment as compared to vehicle treated animal was considered as a positive analgesic response. The percent inhibition of writhing was calculated. Aspirin (25 mg/kg, i.p.) was used as a reference standard.

Statistical analysis

The values were represented as mean \pm 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.

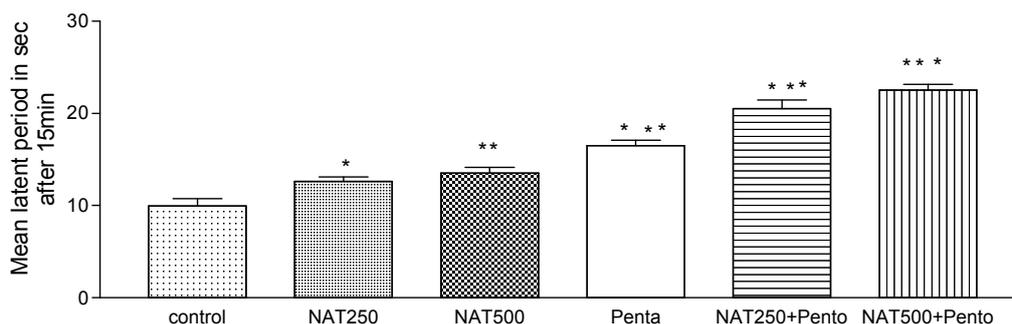
Result and Discussion

NATEF exhibited analgesic activity in rodents and synergies with the analgesic activity of pentazocine. The extract was found to significantly increase the tail flick reaction time in rats (Figure-1). Originally tail flick method was developed by Wolff et al. (16) for quantitative measurement of pain threshold in man against radiation and for analgesic opiates. Later on, the procedure has been used by many authors to evaluate analgesic activity in animal experiments by measuring drug-induced changes in the sensitivity of mice or rats to heat stress applied to their tails. This test is very useful for discriminating between centrally acting morphine-like analgesic and non-opiate analgesic. Woolfe and Mac Donald (17) originally described the hot plate method. This test has been found to be suitable for evaluation of centrally but not of peripherally acting

analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance (18). Mixed opiate agonists-antagonists can be evaluated if the temperature of the hot plate is lowered to 49.5⁰ C (19, 20). It is known that centrally acting analgesic drugs elevate the pain threshold of rodents towards heat. The above findings indicate that NATEF may be centrally acting. (Figure-2)

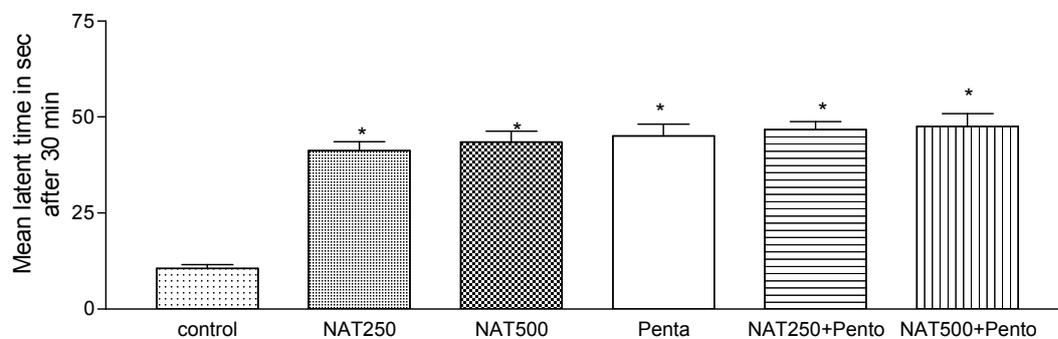
In order to distinguish between the central and peripheral analgesic action of NATEF, acetic acid induced writhing response in mice was used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animal reacts with characteristics stretching behavior, which is called writhing. It was found that NATEF significantly inhibited the acetic acid induced writhing response and potentiated the anti-inflammatory activity of aspirin as well (Figure-3). The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that NATEF exerts an analgesic effect probably by inhibiting synthesis of action of prostaglandins and leukotrienes (21).

Figure 1: Effect of *Nyctanthes arbor - tristis* Fruit Extract on Tail Flick Latent Period in Rats.



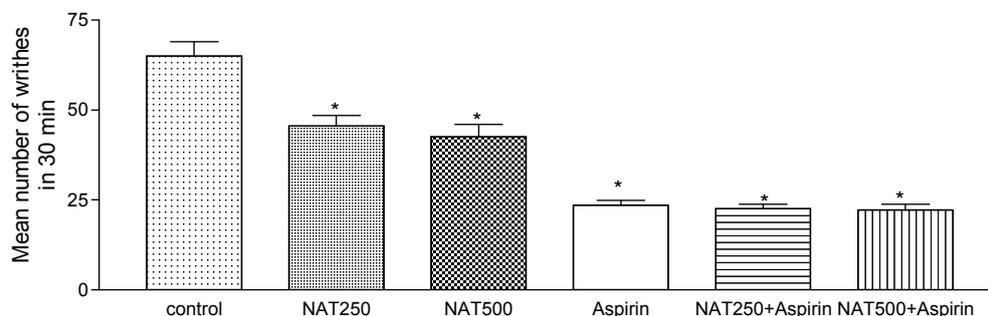
Data are given as mean \pm S.E.M. ($n = 12$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control

Figure 2: Effect of *Nyctanthes arbor - tristis* Fruit Extract on Hot Plate Reaction Time in Mice.



Data are given as mean \pm S.E.M. ($n = 12$). * $P < 0.001$ as compared to control

Figure 3: Effect of *Nyctanthes arbor - tristis* Fruit Extract on Acetic Acid Induced Writhing in Mice.



Data are given as mean \pm S.E.M. ($n = 12$). * $P < 0.001$, as compared to control

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