INVESTIGATION OF ANALGESIC POTENTIAL IN FLOWERS OF
NYCTANTHES ARBOR- TRISTIS (L.)

Shalini Tripathi* and P. K. Tripathi,

Department of Pharmacy, Rameshwaram Institute of Technology & Management
Lucknow- 227101, Uttar Pradesh, India

Summary

Water soluble fraction of ethanolic extract of flowers of Nyctanthes arbor-tristis Linn (NAT flower extract) was pharmacologically investigated for its analgesic potential in experimental 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, i.p.) and aspirin (25 mg/kg, i.p.) were administered respectively as positive control. Studies revealed that the flower extract have activity to prevent pains in the rodents. The analgesic effect was dose dependent and found to be statistically significant as compared to the control.

Keywords: Analgesic, Nyctanthes arbor-tristis

*Corresponding author: Department of Pharmacy, Rameshwaram Institute of Technology and Management, Lucknow- 227101, Uttar Pradesh India, Phone: +91-9415087183
Email: shalinitripathi01@rediffmail.com;

Introduction

The investigation on 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 posses antiamoebic (4) and anti allergic properties (5). 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 (6). Earlier, we have reported the anxiolytic activity (7), antidepressant activity (8) and antiaggressive activity (9) in leaf extract and analgesic activity in fruit extract of Nyctanthes arbor- tristis (10). The present study was undertaken to evaluate analgesic activity of flower extract of Nyctanthes arbor- tristis in rodents.
Material and Method

Preparation of plant extracts

The flowers of *Nyctanthes arbor-tristis* (NAT) were collected from the local garden of Lucknow, India in November 2009. 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 flowers of *Nyctanthes arbor-tristis* (2 kg) were passed through S.S. sieve (20 meshes) 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 10.65% w/w water soluble fraction of this extract (NAT flower extract) was taken for the study.

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±10°C 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 NAT flower extract 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. Pentazocine (10 mg/kg) and aspirin (25 mg/kg) were used respectively 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.

Safety evaluation

NAT flower extract 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 (11).

Assessment of Analgesic Activity

The three most widely used rodent models were chosen to evaluate the effect of NAT flower extract 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 (12).

Hot plate reaction in mice

Mice were screened by placing them on a hot plate maintained at 55±1°C and recording the reaction time in seconds for forepaw licking or jumping (13). 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.

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 (13). 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 ± 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

Analgesic activity exhibited by flower extract of Nyctanthes arbor tristis in rodents synergies with the analgesic activity of pentazocine. The extract significantly increased the tail flick reaction time in rats (Figure-1). Tail flick method was originally developed by Wolff et al. (14) for quantitative measurement of pain threshold in man against radiation. This test is very useful for discriminating between centrally acting morphine-like analgesic and non-opiate analgesic. Woolfe and Mac Donald (15) originally described the hot plate method. The validity of this test has been shown even in the presence of substantial impairment of motor performance (16). Mixed opiate agonists-antagonists can be evaluated if the temperature of the hot plate is lowered to 49.5°C (17, 18). It is known that centrally acting analgesic drugs elevate the pain threshold of rodents towards heat. The above findings indicate that NAT flower extract may be centrally acting (Figure-2).
In order to distinguish between the central and peripheral analgesic action of NAT flower extract, 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 NAT flower extract significantly inhibited the acetic acid induced writhing response and potentiated the anti-inflammatory activity of aspirin (Figure-3). The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It may therefore assume that NAT flower extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins (19).

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

![Figure 1](image1)

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

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

![Figure 2](image2)

Data are given as mean ± S.E.M. ($n = 12$). *P* <0.001 as compared to control
Figure 3: Effect of *Nyctanthes arbor-tristis* Flower Extract on Acetic Acid Induced Writhing in Mice.

![Graph showing effect of extract on writhing](image)

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

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**References**


