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Original Research Article

Analgesic activity of ethanolic extract of leaves of *Nyctanthes arbor-tristis linn* on experimental animal models

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ABSTRACT

Background: The present study has been undertaken to evaluate the pharmacological efficacy of ethanolic extract of leaves of Nyctanthes arbor-tristis (EENA) LINN as an analgesic agent in comparison with standard non-steroidal anti-inflammatory agents.
Methods: The extract was prepared by soxhlet method and acute toxicity test was performed as per OECD guidelines. The peripheral analgesic activity was assessed using acetic acid induced writhing method. The central analgesic activity was assessed using tail-flick method.
Results: It has been shown that EENA 400mg/kg and aspirin 100mg/kg significantly increased the pain threshold as assessed by increase in the latency period or basal reaction time. EENA (200mg, 400 mg/kg) and aspirin 100 mg/kg also significantly reduced acetic acid induced writhing response showing peripheral analgesic activity.
Conclusions: Since prostaglandins are involved in the pain perception and are inhibited by flavonoids, it could be suggested that the analgesic effect of Nyctanthes arbortristis might be due to its inhibitory action on PG biosynthesis.

Keywords: Analgesic, Flavonoids, Nyctanthes arbortristis

INTRODUCTION

Pain has been defined as "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[1]

Pain is produced when a nociceptive stimulus is applied on the body. The stimulus either causes actual damage or has a partially damaging effect on the tissues. In injured tissues, some endogenous pain producing substances like histamine, 5-HT, kinins, acetylcholine, lactic acid, K+ and pain modulating agents like prostaglandins and leukotriene's are released. Prostaglandins potentiate the aglycogenic power of the pain producing substances and sensitize afferent nerve endings to them, thereby increasing the intensity and duration of pain.[2]

Nyctanthes arbortristis (sewali, sephalika) is a large deciduous shrub or small tree with quadrangular branches, rough all over with an uneven epidermis and stiff white hairs.[3] Leaves are ovate and acuminate with a few large distant teeth. It bears beautiful white flowers in profusion. It belongs to the family of Oleaceae and is widely distributed in tropical East Asia including India and Bangladesh.

Nyctanthes arbortristis is traditionally used as an expectorant, bitter and tonic. It has a mild purgative action. It is used in bilious and obstinate remittent fever, sciatica and rheumatism. It is useful in constipation of children.[4]

METHODS

Experimental animals

Swiss albino Rats (6-8 weeks) of either sex weighing 150-200gm and Swiss mice (weighing 25-30 g, 3-4 month old) of either sex were selected for the experiments. Animals were obtained from central animal house, Guwahati Medical College, Guwahati. Animal studies were performed in accordance with CPCSEA guidelines and the study was approved by Institutional Animal Ethics Committee (IAEC Reg. no. 351/CPCSEA-3/1/2001). They were allowed to acclimatize to the laboratory environment for one week. They were housed in light controlled room (12:12hr) and at constant temperature (22±2°C) conditions and fed with standard laboratory diet and water *ad libitum*.

Plant materials

The leaves of *Nyctanthes arbortristis* were collected in the month of May from different parts of Dibrugarh district and were authenticated by Dr L R Saikia, Professor, Department of Life Science, Dibrugarh University (Voucher specimen. No. DULSc447). The collected plant materials were cleaned, air dried at room temperature. The dried leaves were hand crushed and stored in airtight container.

The dried leaves of *Nyctanthes arbortristis* were finely ground and extracted overnight with ethanol (50:50) in cold. The materials were repeatedly extracted with hot ethanol in soxhlet apparatus and extraction was done by continuous hot percolation using ethanol (95%v/v). The extract was concentrated using a rotary evaporator [5]. It was further concentrated and dried in desiccators. The extract collected as stored in air tight glass container in refrigerator at 2-8˚C for further use in the experiment.

Acute toxicity study

Acute toxicity test was done for the ethanolic extract of *Nyctanthes arbortristis* following OECD 425 guidelines. As the LD50 of EENA is found to be more than 2000mg/kg, 100mg/kg, 200mg/kg and 400mg/kg were selected for the study.[6,7]

Phytochemical analysis

EENA was subjected to qualitative phytochemical analysis of alkaloids, flavonoids, tannins, saponins, sterols, and terpinoids as per standard methods.[8]

Methods for central analgesic activity

Healthy young albino rats of either sex weighing 150-200gm and showing a normal reaction time of 4-5 seconds were used for the study. They were divided into five groups with six animals in each group.

In tail flick method, latencies (reaction time) of the animals were assessed by the analgesiometer (Labotech, India).[9] The strength of the current passing through the naked nichrome wire was kept constant at 5 Amps. The distance between the heat source and tail skin was 1.5 cm. The site of application of the radiant heat in the tail had been maintained at 2.5 cm, measured from the root of the tail for all rats. The time taken by the animals to withdraw (flick) their tail from the hot wire were noted and taken as the 'reaction time'. The cut off reaction time was fixed at 10 seconds to avoid tissue damage.

Each rat of the groups is tried on the analgesiometer at 30, 60, 120 and 180 minutes after the administration of the drugs. Aspirin was taken as the standard drug at the dose of 100mg/kg per orally.

Group A: Control, vehicle normal saline (NS) 5ml/kg orally

Group B: EENA 100mg⁄kg orally

Group C: EENA 200mg⁄kg orally

Group D: EENA 400mg⁄kg orally

Group E: Aspirin 100mg/kg orally

Methods for peripheral analgesic activity

Healthy albino mice of either sex weighing 20-30 gm were taken and divided into five groups with six animals in each group.

Group A: Control, normal saline 5 ml/kg orally

Group B**:** EENA 100mg⁄kg orally

Group C**:** EENA 200mg⁄kg orally

Group D**:** EENA 400mg⁄kg orally

Group E: Aspirin 100 mg/kg orally

The peripheral analgesic activity was tested with acetic acid (1ml/100gm body weight of 0.6% acetic acid) induced writhing response.[10] One hour after administration of drugs, induction of writhing was done in mice by giving intra-peritoneal injection of acetic acid at a dose of 5 ml/kg body weight. The number of writhing responses were counted and recorded for 20 minutes in each group and percentage protection was noted. Aspirin was used as the standard drug at the dose of 100mg/kg per orally.

Statistical analysis

Statistical analysis was done using one way ANOVA followed by Dunnett's multiple comparison tests.[11] Significance level of <0.05 was considered as significant.

RESULTS

The EENA showed significant central analgesic activity as compared to control ($p < 0.01$; Table 1) as evidenced by significant increase in the latency time.

Significant peripheral analgesic action was also observed with EENA and aspirin as compared to control ($p < 0.05$; table 2) as evidenced by inhibition of abdominal writhes produced by acetic acid.

Table 1: Central analgesic activity of the ethanolic extracts Nyctanthes arbortristis on tail flick response in albino rat.

Group	Drug Dose	Pre-drug reaction time in sec (Mean±sd)	Reaction time in seconds (Mean ± SD) 30 min	60 min	90 min	120 min
A	N/S10ml/kg	4.49±0.37	4.67±0.51	4.67±0.51	4.68±0.47	4.66±0.51
B	EENA100mg/kg	4.16±0.02	4.64±0.41	5.53±0.37	6.16±0.64	6.65±0.46
C	EENA200mg/kg	4.23±0.10	5.25±0.55	6.12±0.27	7.42±0.66	7.81±0.94
D	EENA400mg/kg	4.34±0.09	6.22±0.44	6.31±0.72	7.56±0.81	8.18±0.58
E	Aspirin100mg/kg	4.44±0.29	6.27±0.88	6.85±0.87	7.61±1.08	7.75±1.15

One-way F (df) 12.4 (4, 25), 16.79 (4, 25), 21.37 (4, 25), 9.56 (4, 25); ANOVA P <0.05 <0.05 <0.05 <0.05; n = 6 in each group.

Table 2: Peripheral analgesic activity of the ethanolic extracts Nyctanthes arbortristis on writhing test in albino mice.

Groups	Drug dose (MG/KG) per orally	No. of writhing movements (MEAN±SEM)	Percentage of protection
A	Normal saline 10ml/kg	30.5±7.94	0
B	EENA 100mg/kg	28.0±6.50	8
C	EENA 200mg/kg	12.4±8.20	59
D	EENA 400mg/kg	9.0±4.29	71
E	Aspirin 100 mg/kg	16.0±7.46	48
One Way Anova	F 10770 Df (4, 25) P < 0.05		

DISCUSSION

The present study was conducted to study the analgesic activity of the ethanolic extracts of the leaves of *Nyctanthes arbortristis* linn in albino rat and mice and was compared with the standard analgesic drug aspirin.

Our study showed that EENA produced significant analgesia, both centrally and peripherally. The mean reaction time in seconds after 30 min, 60 min, 90 min, and 120 minutes of drug administration for EENA (100mg/kg) was 4.64±0.41, 5.53±0.37, 6.16±0.64, and 6.65±0.46 respectively.

The increase in the mean reaction time in seconds with the EENA (200mg/kg) were 5.25±0.55, 6.12±0.27, 7.42±0.66, 7.81±0.94 at 30 min, 60 min, 90 min, and 120 min respectively. For EENA (400mg/kg), the mean reaction time were found to be increased by 6.22±0.44, 6.31±0.72, 7.56±0.81 , 8.18±0.58 at 30 min, 60 min, 90 min, and 120 min respectively ($P<0.05$).

For standard drug aspirin (100mg/kg), the mean reaction time were found to be increased by after 30 min: 6.27±0.88, 60 min: 6.85±0.87, 90 min: 7.61±1.08, and 120 min: 7.75±1.15 when compared to the control at the respective hour. It has been observed that the EENA leaves in a dose of 200 mg/kg and 400mg/kg increased the pain threshold significantly at 30 min, 60 min, 90 min, and 120 minutes of administration when compared to the control group at that particular minute. This shows that the EENA leaves possess central analgesic activity as seen in the present animal model of analgesia.

The effect of EENA on the acetic acid- induced abdominal constrictions in mice is presented in Table 2. The result shows that the extract (100, 200, 400 mg/kg), and the reference drug aspirin (100 mg/kg) significantly ($P <0.0001$) reduced abdominal writhing in mice when compared to the negative control group reducing the mean number of writhing from 30.5±7.46 in the negative group to 9.0±4.29 at the dose of 400 mg/kg. The reduction was in a dose dependent manner. Also the extract caused a dose dependent increase in inhibition of abdominal writhing, increasing it from 0% in negative control group to 71% at the dose 400 mg/kg.

Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, prostaglandins, bradykinin and substance P which

stimulate pain nerve endings. Local peripheral receptors are postulated to be partly involved in the abdominal constriction (writhing response). The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins.[12]

The analgesic action of *Nyctanthes arbortristis* leave extracts suggests an NSAID like action.[13] It has been noted that test drug exhibited significant nociceptive activity in the mouse writhing test which is particularly sensitive to analgesic antipyretic agents. The results found in the present study are in corroboration with those found in the various studies mentioned above.

Elaborate chemical analysis of the leaves *Nyctanthes arbortristis* of has revealed the presence of tannic acid, methyl salicylate, amorphous glucosider, mannitol, amorphous resin, ascorbic acid; carotene and a trace of volatile oil.[14] Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce significant anti-inflammatory effect.[15,16]

Since prostaglandins are involved in the pain perception and are inhibited by flavonoids, it could be suggested that the analgesic effect of *Nyctanthes arbortristis* might be due to its inhibitory action on PG biosynthesis.

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