SCREENING OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *NYCTANTHES ARBOR-TRISTIS* LINN.

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Summary

The alcoholic extract of *Nyctanthes arbor-tristis* bark was investigated for analgesic and anti-inflammatory activity at the doses of 10, 20, 50, 100 and 200mg/kg, body weight. The experimental paradigms used were Tail immersion and acetic acid induced writhing methods for analgesic activity, while Carrageenan and formalin induced paw edema were used to assess anti-inflammatory activity. It shows a significant inhibition of paw edema in both models. In analgesic activity alcoholic extract of *Nyctanthes arbor-tristis* bark shows a significant increase in withdrawal time in Tail immersion method and also shows a significant decrease in number of writhing in acetic acid induced writhing method at different doses.

Key Words: Nyctanthes arbor-tristis, AENA, Analgesic, Anti-inflammatory.

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Introduction

Nyctanthes arbor-tristis Linn. (Family: Oleaceae) commonly known as "Parijataka" or "Night flowering" is a well documented plant. The infusion of leaves is useful in fever and rheumatism as disphoretic and diuretic.-(Chakradatta) (1). Extracted juice of leaves is given to children for the expulsion of round and threads worms. (2). The barks are eaten with betel nut and leaf to promote expectoration of thick phlegm (Dymock). (1, 3). The plant has anti-malarial (4) and anti-tumor (5). The leaf extract shows good hepato-protective activity (6), tranquilizing, anti-histaminic and purgative activity (7), analgesic, antipyretic and ulcerogenic activity (8) and anti-inflammatory activity (9). It also shows antibacterial activity (10).

The present study showed that alcoholic extract of *Nyctanthes arbor-tristis* bark (AENA) exhibit analgesic activity by Tail immersion test and acetic acid induced writhing method and anti-inflammatory activity against carrageenan and formalin induced paw edema.

Materials and Methods

Collection of plant:

Nyctanthes arbor-tristis bark was collected from the local area of Ashok nagar, Satpur, Nashik (Maharashtra) and authenticated by Mrs. Athre R.R. (Dept. of Biology, SNJB's Junior College, Neminagar, Chandwad).

Chemical and reagents:

The chemicals used in the present study were acetic acid (Research, Lab Mumbai), Carrageenan (Sigma Lab Mumbai), Formalin (Research, Lab Mumbai), Aspirin (Research Lab, Mumbai).

Preparation of extract:

The dried powdered bark was extracted with Pet. ether and ethanol successively in soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi-solid mass was obtained. (Yield-0.54% and 4.37% respt.)

The phytochemical profile was performed (11). The presence of alkaloids (Dragendroff's and Mayer's reagent), flavonoids (Shinoda test) and Steroids (Liberman-burchard test) were analyzed. The extract shows positive test for alkaloids, steroids and tannins. The extract at the different doses at 10, 20, 50, 100 and 200 mg/kg was suspended in 2% gum acacia.

Animals:

Albino mice (20-25 gm) and wistar rats (100-150gm) were obtained from Yash farm, Pune. Animals were housed in groups of five at an ambient temperature of 25 ± 1 °C. Animals had free access to food and water. Animals were deprived of food but not water 4 h before the experiment. The Institutional Animal Ethical Committee approved the protocol of this study.

Evaluation of Analgesic activity:

Acetic acid induced Writhing response in mice:

In this method, mice in groups of five each were treated with vehicle, AENA (10, 20, 50,100 and 200 mg/kg, p.o.). Analgesic activity of AENA (10, 20, 50,100 and 200 mg/kg, p.o.) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.) (12, 13). Number of writhes per animal was counted in the following 20 min. Aspirin (20 mg/kg, p.o.) was used as a reference standard. Percentage protection against writhing was taken as an index of analgesia.

It is calculated as:

$$\frac{\text{No of writhing in control group} - \text{No of writhing in treated group}}{\text{No. of writhing in control group}} \times 100$$

Tail immersion test:

Mice in groups of five each were treated with vehicle, pentazocine (17.5 mg/kg, i.p.) and AENA (10, 20, 50, 100 and 200 mg/kg, p.o.). The distal 2-3 cm portion of Mouse-tail was immersed in hot water maintained at 55 ± 0.5 ºC (13). The time taken by the mouse to withdraw the tail from hot water was noted as reaction time.

Carrageenan induced paw edema:

The method of winter (14) was used to study acute inflammation. Rats in groups of five each were treated with vehicle, AENA (10, 20, 50, 100 and 200 mg/kg, p.o.) one hour prior to Carrageenan injection. 0.1ml of 1% Carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of Carrageenan injected foot were measured at 0, 1, 2, 3, hr. using Plethysmometer (UGO Basile, Italy) (15). The right hind paw was injected with 0.1 ml of vehicle. Aspirin (20 mg/kg p.o.) was used as reference agent.

Formalin induced paw edema:

Rats in groups of five each were treated with vehicle, AENA (10, 20, 50, 100 and 200 mg/kg, p.o.) one hour prior to formalin injection. 0.05ml of 1%w/v solution of formalin was injected into the sub plantar tissue of left hind paw of each rat. Swelling of formalin injected foot was measured at 0, 1, 2, 3, hr using Plethysmometer (UGO Basile, Italy) (16, 17). The right hind paw was injected with 0.1 ml of vehicle. Aspirin (20 mg/kg, p.o.) was used as reference agent.

Statistical Analysis:

Data are expressed as mean ± SEM. Statistical analysis was done by using one way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ were considered significant.

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Results

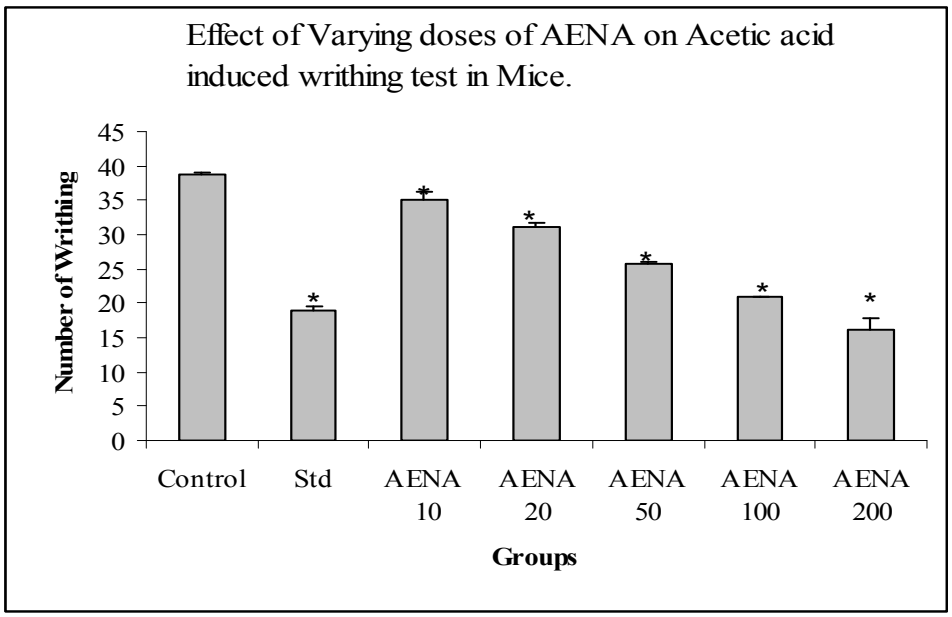
Acute toxicity studies:

No mortality was observed following oral administration of AENA with the highest dose 2000 mg/kg and had no toxic effect on normal behavior of mice and rats.

Acetic Acid Induced Writhing Method:

The results of the analgesic effect of the AENA on Acetic acid induced writhing test in Mice are presented in table No. 1. In vehicle treated mice 38.67± 0.33 writhing were observed in observation period of 20 min. Aspirin (20 mg/kg p.o.) reduced the number of writhing induced by acetic acid to 19 ± 0.57. In the test groups, the AENA decreased the number of writhing dose dependently and the differences in writhing were statistically significant at $p<0.05$. The observations are given in Graph1.

Graph 1. Effect of varying doses of AENA on Acetic acid induced writhing test in Mice.

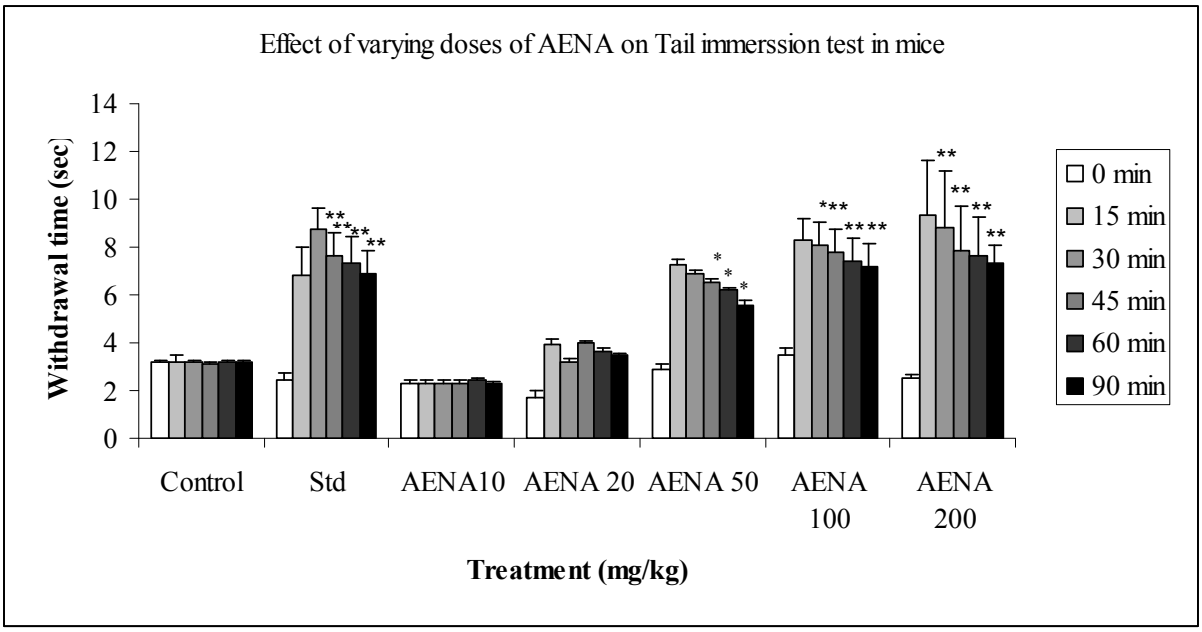


The observations are mean ± SEM. *p< 0.05, ** p<0.01 as compared to control (ANOVA followed by Dunnett's test).

Tail Immersion Method:

The effect of AENA on latency to flick tail in tail immersion in mice is presented in table No. 2. In vehicle treated mice, latency to flick tail in tail immersion test was 3.20 ± 0.05 sec. Aspirin (20 mg/kg i.p.) increased the latency to flick tail to 4.81 ± 0.83. The AENA dose dependently showed a significant increase in the latency to flick tail at 15, 30, 60, 90, and 120 min. The observations are given in Graph 2.

Graph 2. Effect of varying doses of AENA on tail immersion method in mice.

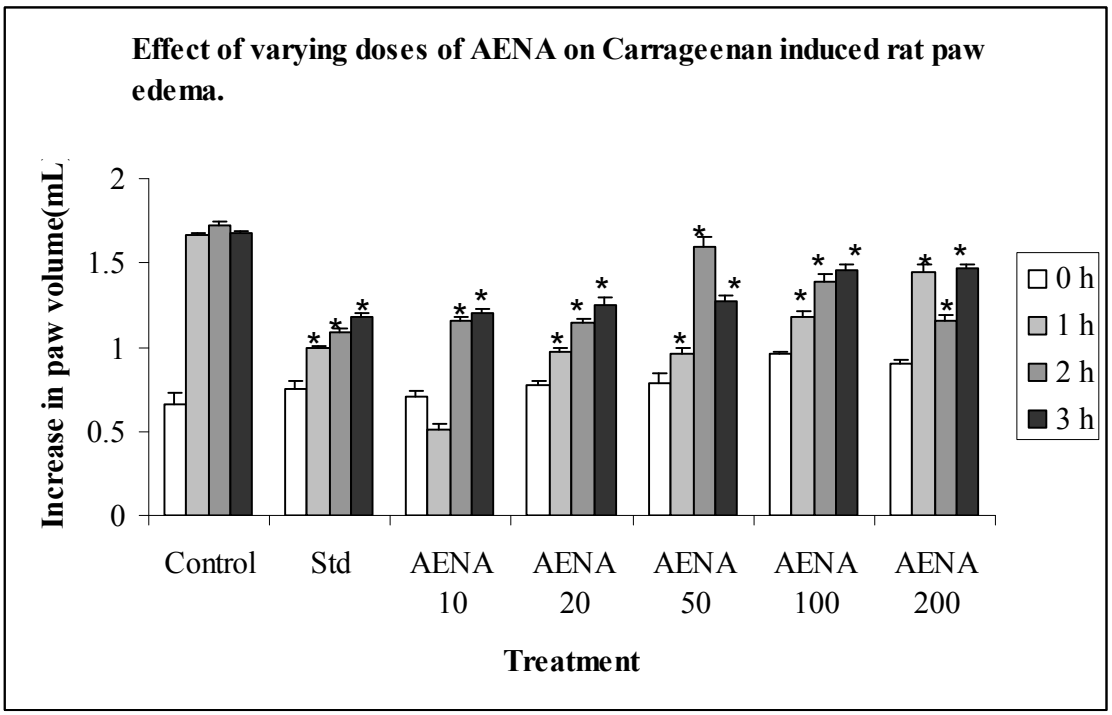


The observations are mean ± SEM. *p< 0.05, ** p<0.01 as compared to control (ANOVA followed by Dunnett's test).

Carrageenan Induced Rat Paw Edema

The results of the anti-inflammatory effect of the AENA on carrageenan induced edema in rat's right hind paw are presented in table No.3. In vehicle treated rats maximum increase of paw volume at 3 h was 1. 68 ± 0.01 mL. The corresponding mean paw volume in aspirin (20 mg/kg p.o.) was 1.18 ± 0.02 mL. The AENA dose dependently showed a significant p<0.05 inhibition of paw volume at 1sth, 2nd h, and 3rd h. The observations are given in Graph 3.

Graph 3. Effect of varying doses of AENA on Carrageenan induced rat paw edema.

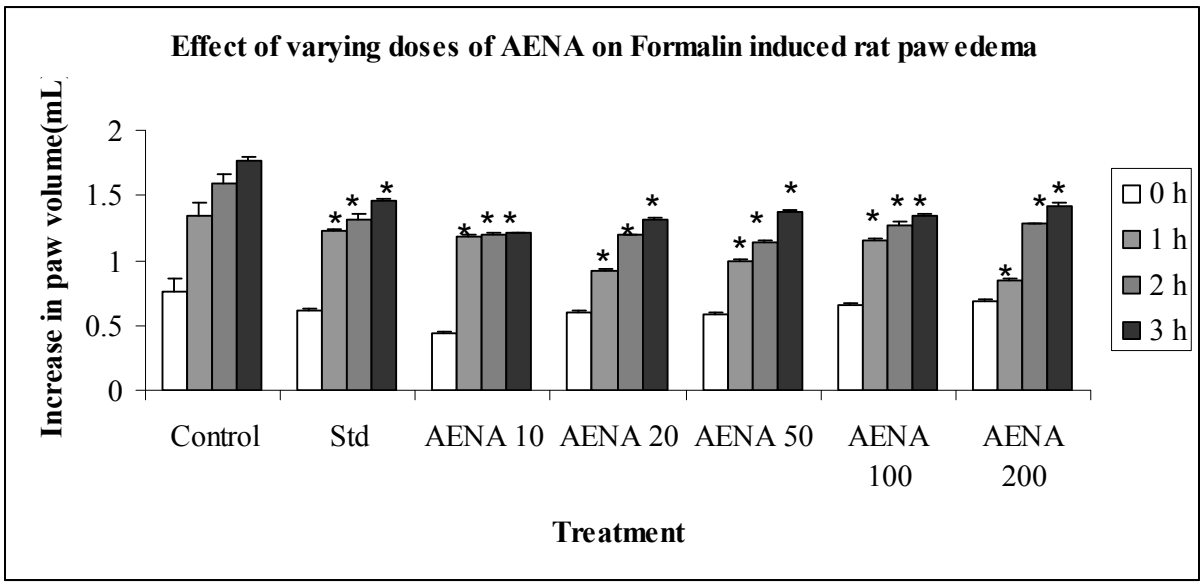


The observations are mean ± SEM. *p< 0.05, ** p<0.01 as compared to control (ANOVA followed by Dunnett's test).

Formalin Induced Rat Paw Edema

The anti-inflammatory effects of AENA on formalin induced rat paw edema are presented in table No. 4. In vehicle treated rats maximum increase of paw volume at 3 h was 1.76 ± 0.03 mL. The corresponding mean paw volume in aspirin (20 mg/kg p.o.) was 1.36 ± 0.02 mL. The AENA dose dependently showed a significant p<0.05 inhibition of paw volume at 1h, 2 h, and 3 h. The observations are given in Graph 4.

Graph 4. Effect of varying doses of AENA on Formalin induced rat paw edema.



The observations are mean ± SEM. *p< 0.05, ** p<0.01 as compared to control (ANOVA followed by Dunnett's test).

Discussion

It has become imperative to scrutinize herbal products for evaluating their acclaimed properties, as recently numbers of herbs are being introduced in the market. Keeping this view, we have attempted to study the AENA for its analgesic and anti-inflammatory activity in acute models.

A significant (p<0.05) analgesic and anti-inflammatory activity was observed for AENA in acetic acid induced writhing, tail immersion method, Carrageenan, formalin induced rat paw edema models.

The preliminary phytochemical screening of AENA showed the presence of alkaloids, steroids, and tannins (11), have been found to be active anti-inflammatory agents at lower doses (18).

Acetic acid induced writhing is a sensitive method for screening peripheral analgesic effect of compounds. The stimulation of peritoneal nociceptors is indirect and occurs through the release of endogenous substances, which stimulate nerve endings (19).

A great increase occurs in concentration of PGE2 and PGF2α in the peritoneal fluid after acetic acid injection, and the analgesic effect of substances similar to aspirin could be due to the blockade of prostaglandin synthesis (20, 21). In our study, AENA (10, 20, 50, 100, and 200, mg/kg, p.o.) significantly ($p<0.05$) reduced the number of writhing induced by acetic acid. Tail immersion method originally described by Woolfe and Mac Donald, 1994 has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics. It involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level (22). The nociceptors seem to be sensitized by sensory nerves. In our study, AENA (10, 20, 50, 100, and 200, mg/kg, p.o.) significantly ($p<0.05$) increased latency to flick tail in tail immersion method.

Carrageenan induced rat paw edema has been a popular inflammatory model to investigate nonsteroidal anti-inflammatory effect of compounds (23). Serotonin, histamine, bradykinins and prostaglandins have been identified as mediators for carrageenan induced rat paw edema (24). The first phase is due to release of histamine and serotonin (5-HT) (1 h), plateau phase is maintained by kinin like substance (2 h) and second accelerating phase of swelling is attributed to PG release (3 h.) (25 26). In our study, edema produced by carrageenan was significantly ($p<0.05$) inhibited by AENA (10, 20, 50, 100, and 200, mg/kg, p.o.). Formalin induced edema also shows a biphasic response and originate mainly from neurogenic inflammation followed by participation of kinin and leukocytes with their pro-inflammatory factors including PGs (27). According to Yuh-Fung 1995 (28) acute inflammation induced by formalin results from cell damage which provides the production of endogenous mediators. Edema produced by formalin was significantly ($p<0.05$) inhibited by AENA (10, 20, 50, 100, and 200, mg/kg, p.o.). Thus it can concluded that AENA posses analgesic and anti-inflammatory properties which are probably mediated *via* inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may have a potential benefit for the management of pain and inflammatory disorders.

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