

EVALUATION OF ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF NYCTANTHES ARBOR-TRISTIS ON EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

BACKGROUND

Inflammation is characterised by pain, redness, swelling and tenderness. Usually, conventional anti-inflammatory drugs are used to treat inflammation. But these drugs have side effects like acceleration of damage and erosion of joint spaces and advance osteoporosis. Traditionally, *Nyctanthes arbor-tristis* has been used to treat various diseases. So, the present study is undertaken to evaluate the anti-inflammatory and anti-arthritis activities of *Nyctanthes arbor-tristis*.

Aim- The present study was designed to evaluate the anti-inflammatory and anti-arthritis activity of the ethanolic extracts of leaves of *Nyctanthes arbor-tristis* (EENA). The extract was prepared by percolation method and acute oral toxicity test was performed as per OECD guidelines.

Objectives-

1. To evaluate the anti-inflammatory activity of *Nyctanthes arbor-tristis* against acute inflammation by Carrageenan induced rat paw oedema method.
2. To evaluate the anti-arthritis activity of *Nyctanthes arbor-tristis* against chronic inflammation by adjuvant arthritis method.

MATERIALS AND METHODS

Acute inflammation was studied by Carrageenan induced rat paw oedema method and paw volumes were measured at various intervals. Activity against chronic inflammation was studied by Freud's complete adjuvant induced arthritis method. Paw volumes were measured on 1st day, 5th day (injected paws) and on 21st day. Aspirin was taken as the standard drug for all the experiments and a control group was maintained in all the models.

RESULTS

EENA showed highly significant ($p < 0.05$) anti-inflammatory activity against carrageenan induced acute inflammation at the end of 4th hr. It also showed significant reduction ($p < 0.05$) of exudates formation in all the doses. In Freud's complete adjuvant induced arthritis model, EENA significantly reduced primary and secondary lesions when compared to the control. Both 200 mg/kg and 400 mg/kg of EENA significantly ($p < 0.05$) down-regulated the index of arthritis and they reduced ($p < 0.05$) the decrease in weight in the adjuvant arthritis rats as compared to the control group on the 21st day of adjuvant injection. In chronic inflammation, significant inhibitions of paw oedema and weight reduction were found.

CONCLUSION

Based on the findings, we can conclude that the ethanolic extract of leaves of *Nyctanthes arbor-tristis* contains anti-inflammatory and anti-arthritis activity.

KEYWORDS

Anti-Inflammatory, Anti-Arthritis, *Nyctanthes Arbor-Tristis*, Aspirin, Flavonoids.

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BACKGROUND

Inflammation is a complex reaction to injurious agents such as microbes and damaged usually necrotic cell that consists of vascular responses, migration and activation of leukocytes and systemic reactions. It is part of the host's defence, but when the response becomes too great it may be far worse

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than the disease state which is counteracted and in extreme cases it may be fatal. The characteristics of inflammation are numerous, reddening if it is visible, swelling (oedema), soreness and the corresponding histological changes.^[1]

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder involving multiple systems.^[2] It mainly affects joints in a polyarticular manner. It is characterised by progressive joint destruction, deformity, disability and premature death. Currently, the main stream of treatment of RA is the non-steroidal anti-inflammatory drugs along with steroids.^[3,4] Long term treatment with NSAIDs and steroids is associated with lots of complications.^[5]

Nyctanthes arbor-tristis (sewali, sephalika) is a large deciduous shrub or small tree with quadrangular branches, rough all over with an uneven epidermis and stiff white hairs. 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. It is mainly 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.^[6,7]

Objectives

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2. To evaluate the anti-arthritic activity of *Nyctanthes arbor-tristis* against chronic inflammation by adjuvant arthritis method.

MATERIALS AND METHODS

Plant Materials

The leaves of *Nyctanthes arbor-tristis* were collected in the month of May from different parts of Dibrugarh district and were authenticated by Dr. LR 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 arbor-tristis* 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.^[8] 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.

Animals used in the Study

Healthy albino rats of the species *Rattus norvegicus* of either sex weighing 150 - 200 gm and healthy albino mice of the species *Mus musculus* of either sex weighing 20 - 30 gm 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 Regd. No. 351/CPCSEA-3/1/2001). They were allowed to acclimatise to the laboratory environment for one week. They were housed in light-controlled room (12:12 hrs.) and at constant temperature (22 ± 2°C) conditions and fed with balanced laboratory diet and water ad libitum.

Acute Toxicity Study

Acute toxicity test was done for the ethanolic extract of *Nyctanthes arbor-tristis* following OECD^[9] 425 guidelines. As the LD50 of EENA is found to be more than 2000 mg/kg, 100, 200 and 400 mg/kg were selected for the study.^[10]

Experimental Design

For each experiment, animals are divided into five groups with 6 animals in each group. Experimental design-

Group A (Control): Normal saline 10 ml/kg p.o.

Group B (Test): EENA 100 mg/kg p.o.

Group C (Test): EENA 200 mg/kg p.o.

Group D (Test): EENA 400 mg/kg p.o.

Group E (Standard)

Aspirin 100 mg/kg p.o.

Anti-Inflammatory Study against Acute Inflammation

Groups of rats were pre-treated with normal saline, test drug and standard drug per orally one hour before carrageenan injection. Freshly prepared carrageenan 1% in 0.9% NaCl solution was injected in a volume of 0.1 mL into sub-plantar region of the right hind paw of the rats.^[11] The foot volume was measured by plethysmometer just before 1st hr, 2nd hr, 3rd hr and 4th hr after carrageenan injection. The volume of oedema was recorded as the difference between the paw volume at 0 hr and at the end of each hour.^[12] The percentage of anti-inflammatory activity was calculated.^[13]

$$\text{Percentage Inhibition} = \frac{(\text{Control mean} - \text{Treated mean})}{\text{Control mean}} \times 100$$

Anti-Inflammatory Study against Chronic Inflammation

The anti-inflammatory activity of ethanolic extract of EENA against chronic inflammation was tested by Freund's adjuvant-induced arthritis method in rats.^[14]

On day 1, the arthritic syndrome is induced by the intradermal injection of 0.05 mL of a fine emulsion of dead tubercle bacilli (5 mg/mL) into the plantar surface of right hind paw of the rats. Treatment with the test compounds and the standards to the respective groups was started on the same day and continued for 12 days. The paw volumes of both sides and the body weights were recorded on day 0. The paw volumes were measured plethysmographically.

On day 5 the volume of the injected paw was measured again, indicating the primary lesion and the influence of the therapeutic agent on this phase. The severity of the induced adjuvant disease was followed by measurement of the non-injected paw (secondary lesions) with a plethysmometer. Purposely, from day 13 to 21, the animals were not dosed with the test compound or the standard. The following parameters are noted in the control and the treated groups throughout the study period-

- a. Paw oedema.
- b. The severity of the secondary lesions.
- c. Body weight changes.

On day 21, the non-injected paw volume and the body weight were determined again^[15] and the polyarthritis severity were graded on a scale of 0 - 4.

- 0= no swelling;
- 1= isolated phalanx joint involvement;
- 2= involvement of phalanx joint and digits;
- 3= involvement of the entire region down to the ankle;
- 4= involvement of entire paw including ankle.

The Maximum Joint Score was 12 including 3 Secondary Arthritis Paws for each Rat

1. For primary lesions: The percentage inhibition of paw volume of the injected right paw over control was measured at day 5.
2. For secondary lesions: The percentage inhibition of paw volume of non-injected left paw over control was measured at day 21.

- An Arthritis index was calculated as the sum of the scores as indicated above for each animal.

Statistical Analysis

Statistical analysis will be done using one-way ANOVA followed by Dunnett’s multiple comparison tests.^[16] P value <0.05 will be considered significant. The results were calculated with the use of GraphPad Prism software version 5.0.

RESULTS

Carrageenan induced rat paw oedema test

Results were given in Table I. The EENA in doses 200 and 400 mg/kg showed significant acute anti-inflammatory activity as compared to control (p < 0.01) as evidenced by significant decrease in paw oedema at the end of 4th hr in carrageenan induced rat paw oedema test.

Drugs	Dose/Route	Mean Increase in Paw Oedema (Mean ± SEM) in mL			
		1 st hr	2 nd hr	3 rd hr	4 th hr
A (Control)	2 mL po	0.40 ± 0.01 (-)	0.49 ± 0.008 (-)	0.51 ± 0.008 (-)	0.54 ± 0.007 (-)
B (EENA)	100 mg/kg po	0.32 ± 0.01 (14.2%)	0.35 ± 0.007 (22.4%)	0.36 ± 0.007 (23.1%)	0.34 ± 0.01 (22.6%)
C (EENA)	200 mg/kg po	0.26 ± 0.007 (27.5%)	0.29 ± 0.009 (31.4%)	0.29 ± 0.01 (41.6%)	0.28 ± 0.01 (49.8%)
D (EENA)	400 mg/kg po	0.23 ± 0.004 (36.1%)	0.26 ± 0.01 (39.3%)	0.22 ± 0.008 (48.1%)	0.20 ± 0.008 (57.2%)
E (EENA)	100 mg/kg po	0.20 ± 0.01 (43.1%)	0.22 ± 0.008 (44.9%)	0.18 ± 0.01 (53.6%)	0.16 ± 0.008 (64.1%)
One-Way ANOVA	F	69.09	115.9	192.2	270.8
	df	4, 25	4, 25	4, 25	4, 25
	P	< 0.05	< 0.05	< 0.05	< 0.05

Table I. Anti-Inflammatory Activity of Ethanolic Extract of Nyctanthes Arbor-Tristis on Carrageenan-Induced Rat Paw Oedema at the End of 1st, 2nd, 3rd and 4th hour

N= 6 in each group; p< 0.05 vs. control.

Adjuvant-Induced Arthritis in Rats

Results were given in Table II. Significant and arthritic action was also observed with EENA in doses 200 and 400 mg/kg and aspirin as compared to control (p < 0.05) as evidenced by % inhibition in parentheses.

Group	Drug Dose	Mean Increase in Paw Volume (mean ± SEM) in mL (% Inhibition in Parentheses)				Weight Change on 21 st Day (% Change in Parentheses)	Arthritis Index
		Injected Paw (mL)		Non-Injected Paw (mL)			
		On 0 Day	On 5 th Day	On 0 Day	On 21 st Day		
A (Control)	2 mL PO	0.63 ± 0.007	1.20 ± 0.011 (-)	0.40 ± 0.01	1.26 ± 0.01 (-)	-15.6 ± 0.62	10.02 ± 0.25
B (EENA)	100 mg/kg PO	0.67 ± 0.003	1.12 ± 0.02 (20.6%)	0.35 ± 0.01	1.24 ± 0.01 (59.2%)	-10.3 ± 0.33	8.31 ± 0.03
C (EENA)	200 mg/kg PO	0.73 ± 0.009	0.79 ± 0.002 (29.7%)	0.24 ± 0.01	0.97 ± 0.01 (67.4%)	-6.4 ± 0.22	5.64 ± 0.02
D (EENA)	400 mg/kg PO	0.79 ± 0.003	0.66 ± 0.01 (37.9%)	0.23 ± 0.006	1.07 ± 0.02 (73.9%)	-4.5 ± 0.11	4.05 ± 0.15
E (EENA)	100 mg/kg PO	0.56 ± 0.006	0.36 ± 0.006 (42.3%)	0.19 ± 0.001	0.48 ± 0.001 (81.4%)	-2.6 ± 0.12	3.32 ± 0.05
One-Way ANOVA	F	178.6	950.5	64.34	423.3	234.1	440.2
	df	4, 25	4, 25	4, 25	4, 25	4, 25	4, 25
	P	>0.05	<0.05	>0.05	<0.05	<0.05	<0.05

Table II. Anti-Inflammatory Activity of Ethanolic Extract of Nyctanthes Arbor-Tristis on Chronic Inflammation by Adjuvant-Induced Arthritis in Rats

N= 6 in each group; p < 0.05 vs. control.

DISCUSSION

Carrageenan induced hind paw oedema in rats is the standard model of acute inflammation. The result of the present study suggests that the ethanolic extract of leaves of Nyctanthes arbor-tristis in doses 100, 200 and 400 mg/kg significantly suppressed carrageenan induced paw oedema in rats when compared with the control group. The maximum effects of EENA are seen at the dose of 200 and 400 mg/kg at

the end of 3rd and 4th hrs. The test drug at a dose of 400 mg/kg was found almost as effective as the standard in inhibiting oedema by carrageenan.

Prostaglandins (PGs) play a significant role in different phases of inflammatory reactions. Moreover, PGs especially PGE1 was reported to act on cell membrane during inflammatory conditions leading to changes in lipoprotein structure of cell membrane. This causes destabilisation of cell membrane furthering to degenerative cellular changes.^[17] As

the process of carrageenan induced paw oedema is considered to involve a biphasic response; the first phase is attributed to the release of histamine, 5-HT and kinins in the first hour, while the second phase is related to the release of prostaglandins like substances in 2 - 3 hours.^[18] It appears that the alcoholic extract of dried leaves of *Nyctanthes arbor-tristis* inhibit these mediators to account for its anti-inflammatory activity.

The model of adjuvant-induced arthritis in rats is a useful tool to study the pathophysiology of rheumatoid arthritis, especially because the experimental model and the human disease share various signs and symptoms.^[19] Administration of Freund's complete adjuvant to the rat induces acute inflammation in the injected paw and chronic inflammation and arthritic lesions in the uninjected paw, between 10 and 14 days. The chronic phase is accompanied by splenomegaly and lymphocyte-mediated events.^[20]

In our present study, it was seen that there was a gradual increase in the inhibitory activity by *Nyctanthes arbor-tristis* from 5th day to 21st day. However, aspirin exhibited increase in the inhibitory activity from 6th day onwards. Comparing the efficacy of EENA and aspirin, EENA was found to be equally potent as aspirin.

Chronic inflammation involves the release of inflammatory mediators such as cytokines (IL-1B and TNF- α), interferon and Platelet-derived growth factors (PDGF). These mediators are mainly responsible for the pain, destruction of bone cartilage which can cause severe disability. The present investigations established the anti-arthritic potential of *Nyctanthes arbor-tristis* extracts using adjuvant induced arthritis model rats because rats develop a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It is very close to human arthritis disease.^[21]

Phytochemical studies done on different parts of *Nyctanthes arbor-tristis* revealed the presence of alkaloids, tannins, triterpenoids, glycosides and flavonoids as well as the significant antioxidant properties of phenolic compounds of this plant.^[22,23] In previous phytochemical studies, phytoconstituents like steroids, flavonoids, alkaloids, terpenoids and tannins have been shown to possess anti-inflammatory and analgesic activity.^[24,25] Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase.^[26] Some flavonoids are predominant inhibitors of either cyclooxygenase or lipoxygenase.^[27,28] Isolated arbortristoside-A from ethanolic extracts of seeds of *N. arbor-tristis* is proved to possess significant and dose dependent anti-inflammatory activity.^[29] Since prostaglandins are involved in the pain perception and are inhibited by flavonoids, it could be suggested that the anti-inflammatory effect of *Nyctanthes arbor-tristis* might be due to its inhibitory action on PG biosynthesis.

It was seen that EENA have significant anti-inflammatory and anti-arthritic activity. So it can be used as a potential natural source of inflammation disorders by preventing or slowing the progress of symptoms of arthritis. The study should be further extended to identify and characterise the most active bioactive fractions and phytoconstituents, which are responsible for the observed significant anti-inflammatory and anti-arthritic activity and to understand the mechanism of action against adjuvant-induced arthritis in rats.

CONCLUSION

From the above results, it was found that the leaves of *Nyctanthes arbor-tristis* have significant anti-inflammatory and anti-arthritic activity against both acute and chronic models of inflammation. This establishes its traditional uses in acute inflammatory conditions. So, these can be used as a potential natural source of inflammatory disorders by preventing or slowing the process of symptoms of inflammation. The study can be further extended to identify and establish the most bioactive fractions and the phytoconstituents and to understand the mechanism of action against the inflammation and its clinical uses.

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