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Research Paper

Pharmacological Evaluation of Anti-inflammatory Activity of *Nyctanthes arbor-tristis* in Experimental Animal Models

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ABSTRACT

Inflammation is a multifaceted physiological reaction driven by various chemical substances, including prostaglandins, cytokines, and nitric oxide [1,2]. While traditional anti-inflammatory medications are effective, their prolonged usage can lead to considerable adverse effects [3]. This study aimed to assess the anti-inflammatory properties of *Nyctanthes arbor-tristis* through experimental animal models. An ethanolic extract of the plant was obtained via Soxhlet extraction and underwent phytochemical analysis. The anti-inflammatory effects were evaluated using dextran-induced and formalin-induced paw edema models in mice. The extract was administered at dosages of 200 mg/kg and 400 mg/kg and was compared with the standard diclofenac at 10 mg/kg. Various parameters, including paw edema, locomotor activity, C-reactive protein levels, body weight, and feed intake, were measured. The findings indicated a significant dose-dependent decrease in inflammation, with the increased dosage demonstrating effects similar to those of the conventional medication. These results support the traditional application of *Nyctanthes arbor-tristis* and indicate its promise as a safer option for treating inflammatory conditions.

INTRODUCTION

Inflammation serves as the body's defense mechanism against harmful agents, including pathogens, damaged cells, or irritants. This response entails a series of intricate biochemical processes driven by inflammatory mediators like

prostaglandins, leukotrienes, cytokines, and nitric oxide [1]. Acute inflammation is marked by the widening of blood vessels, heightened permeability of blood vessels, and the infiltration of white blood cells, while chronic inflammation is associated with tissue damage and the body's repair processes [10].

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The activation of phospholipase A2 triggers the liberation of arachidonic acid, which is subsequently converted into prostaglandins and leukotrienes—essential mediators of inflammation. Nitric oxide (NO), generated by inducible nitric oxide synthase (iNOS), also significantly contributes to inflammatory processes.

Non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac function by blocking cyclooxygenase (COX) enzymes. Nonetheless, extended use of NSAIDs is linked to negative effects such as gastric ulcers, kidney damage, and cardiovascular issues. This has sparked a growing interest in plant-based alternatives that offer improved safety profiles.

Nyctanthes arbor-tristis, also known as Parijat, is a medicinal plant in the Oleaceae family. It is commonly employed in traditional medicine to treat inflammatory conditions like arthritis, fever, and joint pain [4,5]. Phytochemical analyses have identified the presence of iridoid glycosides, flavonoids, alkaloids, and tannins, which are recognized for their anti-inflammatory and antioxidant effects.

Previous research indicates that the plant extract can inhibit inflammatory mediators such as TNF- α , IL-1 β , and prostaglandins, as well as lower oxidative stress. Nonetheless, a thorough pharmacological assessment using standard experimental models is necessary to confirm its effectiveness. Therefore, the present study was designed to evaluate the anti-inflammatory activity of *Nyctanthes arbor-tristis* using dextran-induced and formalin-induced paw edema models in mice



Figure 1: *Nyctanthes arbor-tristis*

5. MATERIALS AND METHODS

5. 1. Experimental Animals

Healthy adult Male mice (20 – 25 g) were used for the study. The creatures were housed under controlled environmental conditions (temperature 25 ± 1 °C, relative moisture 55 – 65, and 12- hour light/ dark cycle). Standard pellet diet and water were handed ad libitum.

All experimental procedures were carried out in agreement with guidelines of the CPCSEA and approved by the Institutional Animal Ethics Committee (IAEC blessing No. 751/ PO/ Re/ S/ 03/ CPCSEA/2026/1-1) (6).

5. 2. Plant Material and Authentication

The flowers of *Nyctanthes arbor-tristis* were collected from original sources and authenticated by a good botanist from the institutional botany department. The factory material was gutted, shade- dried, and coarsely pulverized for further processing.

5. 3. Preparation of Extract

The dried powdered factory material was subjected to Soxhlet extraction using 90 ethanol as detergent. The extraction process was carried out for several cycles until complete extraction was achieved. The obtained extract was concentrated using a water bath and stored in a watertight vessel at room temperature for further use.

5. 4. Phytochemical Screening

Primary phytochemical webbing of the ethanolic excerpt was performed to descry the presence of colorful bioactive ingredients similar as flavonoids, alkaloids, tannins, phenolic composites, glycosides, and triterpenoids using standard qualitative tests. The presence of these ingredients was verified grounded on characteristic color changes and precipitate conformation.

5. 5. Acute Toxicity Study

Acute oral toxin study was conducted as per OECD guideline 425. The excerpt was set up to be safe at advanced boluses, and experimental boluses (200 mg/ kg and 400 mg/ kg) were named for pharmacological evaluation.

5. 6. Experimental Design

A total of 30 mice were divided into five groups (n = 6):

Table 1: Experimental design of animals

Group	Treatment
Group I	Normal control (0.5 mL normal saline)
Group II	Disease control
Group III	Standard (Diclofenac 10 mg/kg)
Group IV	Extract (200 mg/kg)
Group V	Extract (400 mg/kg)

5. 7. Induction of Inflammation

5. 7. A. Dextran-Induced Paw Edema

Acute inflammation was convinced by edging in 0.1 mL of 1 dextran result into the sub-plantar region of the right hind paw. Paw volume was measured at different time intervals (0,1, 2, and 3 hours). The increase in paw volume indicated the extent of inflammation (7,8).

5. 7. B. Formalin- Induced Paw Edema

Inflammation was convinced by edging in 0.1 mL of 2 formalin result into the paw. This model produces biphasic seditious response involving neurogenic and seditious intercessors. Paw edema was measured up to 4 hours post-induction (7,8).

5. 8. Evaluation Parameters

5. 8.1 Paw Edema Measurement

Paw volume was measured using a plethysmometer. The degree of inflammation was calculated as the increase in paw volume compared to birth.

% inhibition of edema was calculated using the formula:

$$\% \text{Inhibition} = [1 - (V_t - V_c) / (V_d - V_c)] \times 100$$

Where:

V_t = treated group

V_c = control group

V_d = disease group

5. 8.2 Locomotor exertion

Locomotor exertion was assessed using a Rota-Rod outfit at 25 rpm with a cut- off time of 180 seconds. Reduction in locomotor exertion indicates inflammation- convinced discomfort, while enhancement suggests remedial effect.

5. 8.3 C- Reactive Protein (CRP)

Blood samples were collected from the tail tone, and serum was separated by centrifugation. CRP situations were measured using ELISA accoutrements. Elevated CRP situations indicate systemic inflammation.

5. 8.4 Body Weight



Body weight was recorded ahead and after treatment. Reduction in body weight indicates complaint progression, while enhancement reflects remedial effect.

5.8.5 Feed Input

Feed input was measured daily. Reduced input is associated with inflammation, while normalization indicates recovery.

5.9. Statistical Analysis

All data were expressed as mean $\pm$ SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test. Values were considered statistically significant at * p < 0.05 and p < 0.01

6. Results

6.1 Phytochemical Screening

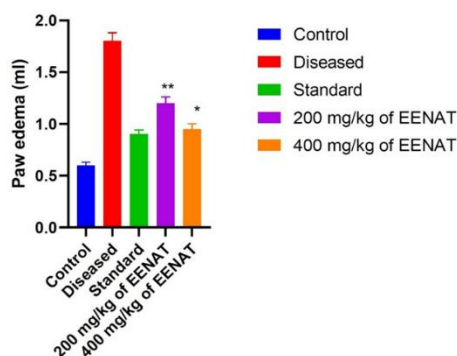
Table 02: Phytochemical constituents of ethanolic extract of *Nyctanthes arbor-tristis*

Phytochemical	Test	Observation	Inference
Flavonoids	Shinoda test	Pink/red color	Present
Flavonoids	Lead acetate	Yellow precipitate	Present
Alkaloids	Mayer's test	Cream precipitate	Present
Alkaloids	Dragendorff's test	Orange precipitate	Present
Phenols/Tannins	FeCl ₃ test	Blue-green color	Present
Glycosides	Keller–Killiani	Brown ring	Present

6.2 Dextran-Induced Paw Edema

Table 03: Effect on paw edema (Dextran model)

Group	Paw Edema (ml) Mean $\pm$ SEM	% Inhibition
Control	0.60 $\pm$ 0.03	—
Disease	1.80 $\pm$ 0.08	0%
Standard (Diclofenac)	0.90 $\pm$ 0.04	50%
200 mg/kg	1.20 $\pm$ 0.06**	33%
400 mg/kg	0.95 $\pm$ 0.05*	47%



Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at **p < 0.01 and *p < 0.05

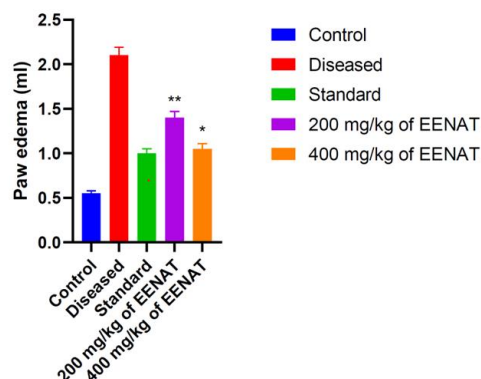
Figure 02: Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* on dextran induced paw edema in mice.



6.3 Formalin-Induced Paw Edema

Table 04: Effect on paw edema (Formalin model)

Group	Paw Edema (ml) Mean $\pm$ SEM	% Inhibition
Control	0.55 $\pm$ 0.03	—
Disease	2.10 $\pm$ 0.09	0%
Standard	1.00 $\pm$ 0.05	52%
200 mg/kg	1.40 $\pm$ 0.07**	33%
400 mg/kg	1.05 $\pm$ 0.06*	50%



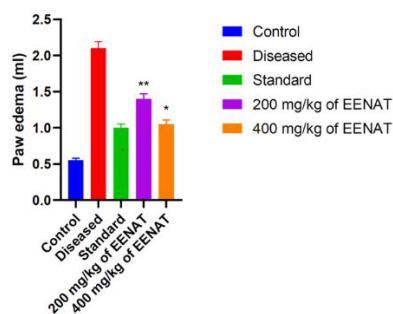
Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statistically significant at **p < 0.01 and *p < 0.05

Figure 03- Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on formalin-induced paw edema in mice.

6.4 Locomotor Activity

Table 05: Locomotor activity (Dextran model)

Group	Locomotor Activity (sec)
Control	180 $\pm$ 0
Disease	111 $\pm$ 7
Standard	165 $\pm$ 3
200 mg/kg	151 $\pm$ 4**
400 mg/kg	161 $\pm$ 5*



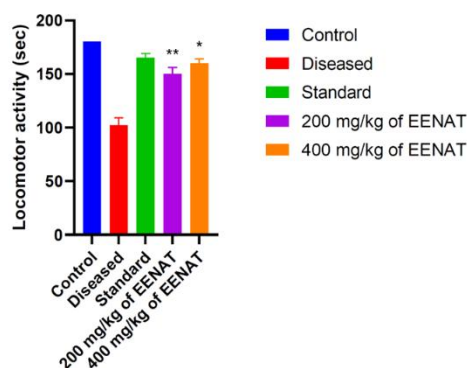
Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Fig 04. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Locomotor activity in Dextran-induced mice.

6.5 Locomotor Activity

Table 06: Locomotor activity (Formalin model)

Group	Locomotor Activity (sec)
Control	180 $\pm$ 0
Disease	102 $\pm$ 7
Standard	165 $\pm$ 4
200 mg/kg	150 $\pm$ 6**
400 mg/kg	160 $\pm$ 4*



Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered stastically significant at **p < 0.01 and *p < 0.05

Fig 05. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Locomotor activity in Formalin-induced mice.

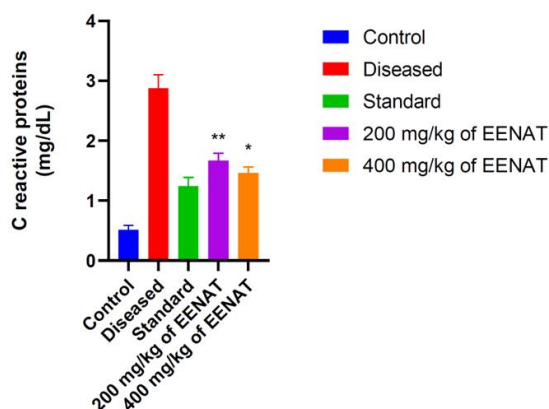
6.6 C-Reactive Protein (CRP)

Table 07: CRP levels (Dextran model)

Group	CRP (mg/dL)
Control	0.51 $\pm$ 0.08
Disease	2.87 $\pm$ 0.23
Standard	1.24 $\pm$ 0.15



Group	CRP (mg/dL)
200 mg/kg	$1.67 \pm 0.12^{**}$
400 mg/kg	$1.46 \pm 0.10^{*}$



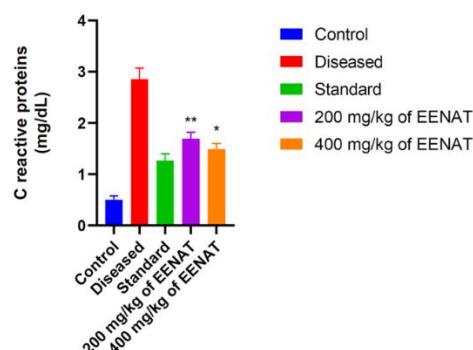
Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at $^{**}p < 0.01$ and $^{*}p < 0.05$

Figure 06: Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on C Reactive Proteins in Dextran-induced mice

6.7 C-Reactive Protein (CRP)

Table 08: CRP levels (Formaline model)

Group	Treatment	C reactive proteins (mg/dL)
Control Group	No treatment	0.50 ± 0.08
Diseased Group	Formalin induced inflammation	2.85 ± 0.22
Standard	10 mg/kg Diclofenac	1.26 ± 0.14
200 mg/kg of EENAT	200mg/kg ethanolic extract of <i>Nyctanthes arbor-tristis</i>	$1.69 \pm 0.13^{**}$
400 mg/kg of EENAT	400mg/kg ethanolic extract of <i>Nyctanthes arbor-tristis</i>	$1.49 \pm 0.11^{*}$



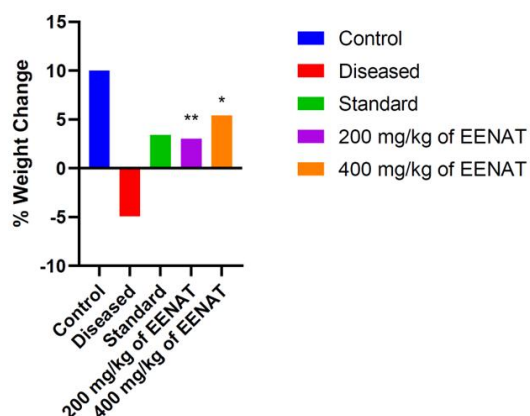
Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at $^{**}p < 0.01$ and $^{*}p < 0.05$

Fig 07. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on C Reactive Proteins in Formalin-induced mice.

6.8 Body Weight

Table 09: Body weight (Dextran model)

Group	Initial	Final	% Change
Control	200 ± 10	219 ± 12	+10%
Disease	204 ± 8	196 ± 9	-4.9%
Standard	203 ± 7	211 ± 11	+3.4%
200 mg/kg	201 ± 9	209 ± 10	+3.0%
400 mg/kg	203 ± 8	216 ± 10	+5.4%



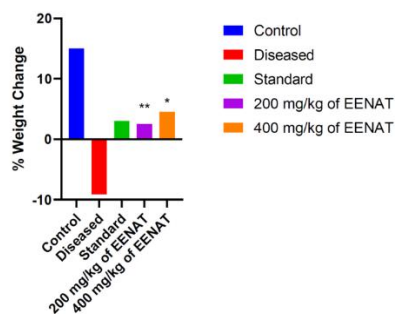
Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at **p < 0.01 and *p < 0.05

Fig 08: Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Body Weight in Dextran-induced mice.

6.9 Body Weight

Table 10: Body weight (Formaline model)

Group	Initial Weight	Final Weight	% Weight Change
Control Group	200 ± 8	229 ± 11	+15%
Diseased Group	197 ± 7	181 ± 9	-9.1%
Standard	198 ± 6	204 ± 9	+3.0%
200 mg/kg of EENAT	196 ± 5	201 ± 8**	+2.5%**
400 mg/kg of EENAT	200 ± 7	209 ± 10*	+4.5%*



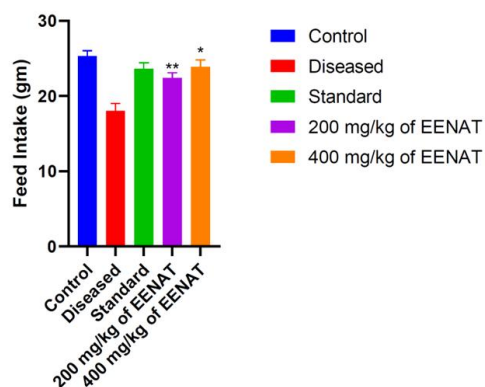
Values were expressed as mean ± SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at **p < 0.01 and *p < 0.05

Fig09. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Body Weight in Formalin-induced mice.

6.10. Feed Intake

Table 12: Feed intake (Dextran model)

Group	Feed Intake (g)
Control	25.3 ± 0.7
Disease	18 ± 1.0
Standard	23.6 ± 0.8
200 mg/kg	22.4 ± 0.7
400 mg/kg	23.9 ± 0.9



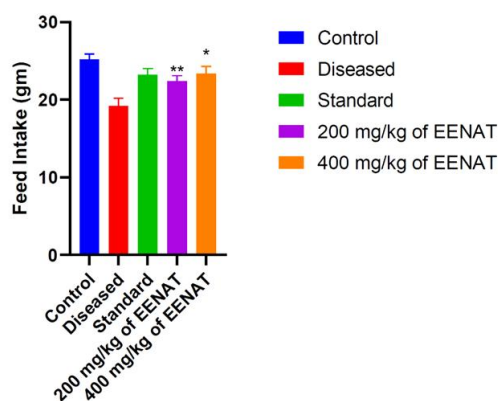
Values were expressed as mean ± SEM of six replicates and subjected to one way ANOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statistically significant at **p < 0.01 and *p < 0.05

Fig 10. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Feed Intake in Dextran-induced mice.

6.11. Feed Intake

Table 13: Feed intake (Formaline model)

Group	Treatment	Feed Intake (gm)
Control Group	No treatment	25.2 ± 0.7
Diseased Group	Formalin induced inflammation	19.2 ± 1.0
Standard	10 mg/kg Diclofenac	23.2 ± 0.8
200 mg/kg of EENAT	200mg/kg ethanolic extract of <i>Nyctanthes arbor-tristis</i>	22.4 ± 0.7**
400 mg/kg of EENAT	400mg/kg ethanolic extract of <i>Nyctanthes arbor-tristis</i>	23.4 ± 0.9*



Values were expressed as mean $\pm$ SEM of six replicates and subjected to one way ANNOVA followed by Duncan's multiple range test to determine significant differences in all parameters. Values are considered statically significant at **p < 0.01 and *p < 0.05

Fig 11. Effect of oral administration of ethanolic extract of *Nyctanthes arbor-tristis* (EENAT) on Feed Intake in Formalin-induced mice.

DISCUSSION

The present study demonstrates significant anti-inflammatory exertion of *Nyctanthes arbor-tristis* in experimental models. The reduction in paw edema indicates inhibition of inflammatory mediators similar as prostaglandins and cytokines (2).

The observed exertion may be attributed to the presence of flavonoids and iridoid glycosides, which are known to inhibit COX and LOX pathways (9). Also, antioxidant properties of the extract may contribute to reduced oxidative stress and inflammation.

The drop in CRP levels further supports the anti-inflammatory potential of the extract. Enhancement in locomotor activity indicates reduction in pain and inflammation (5).

The results are similar with standard medicine diclofenac, suggesting that the extract may serve as an alternative option with smaller side effects.

CONCLUSION

The study confirms that *Nyctanthes arbor-tristis* possesses significant anti-inflammatory exertion in experimental models. The findings support its

traditional use and highlight its potential as a natural remedial agent for inflammatory diseases. Further studies including clinical trials are recommended.

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