International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 18s, 2025
https://theaspd.com/index.php

In Vivo Evaluation Of Nyctanthes Arbor-Tristis Ethosomal Gel For Topical Anti-Inflammatory Efficacy Using Carrageenan-Induced Rat Paw Edema Model

Kamlesh Kumar Yadav[1], Ravindra B. Laware[2], Shubham N. Kanawade[3]
[1]Research Scholar Bhagwant University, Ajmer Rajasthan
[2]Professor and Principal College of Pharmaceutical Sciences, Pravara Institute of Medical Sciences, Loni.
[3]Assistant Professor College of Pharmaceutical Sciences, Pravara Institute of Medical Sciences, Loni.

Abstract
Nyctanthes arbor-tristis ethosomal gel (EG-NAT-12) was developed using an optimized ratio of ethanol and phospholipids with Carbopol 940 as a gelling agent to enhance topical delivery and bioadhesion. The formulation exhibited favorable physicochemical properties, including a pH of 5.72 ± 0.06, high drug content (97.1 ± 0.46%), satisfactory spreadability, and an entrapment efficiency of 91.8%. The anti-inflammatory efficacy of EG-NAT-12 was evaluated using the carrageenan-induced paw edema model in Wistar rats and compared with a standard diclofenac gel. Results showed that the ethosomal gel significantly reduced paw edema in a time-dependent manner, achieving 34.69% inhibition at 4 hours post-carrageenan administration, closely comparable to the 40.82% inhibition observed with diclofenac gel. Statistical analysis confirmed the significance of these findings ($p < 0.05$). The enhanced anti-inflammatory activity of EG-NAT-12 is attributed to the ethosomal system's ability to improve skin penetration and sustain drug release. This study demonstrates that ethosomal encapsulation of Nyctanthes arbor-tristis extract is a promising approach for developing effective herbal topical anti-inflammatory therapies, providing a potential alternative to conventional nonsteroidal anti-inflammatory drugs. Further investigations including long-term safety and clinical studies are recommended.

Keywords: Nyctanthes arbor-tristis, *ethosomal gel, anti-inflammatory, carrageenan, topical delivery, edema inhibition*

1. INTRODUCTION

Inflammation is a protective biological response to injury or infection, but chronic or excessive inflammation can lead to various pathological conditions including arthritis, dermatitis, and autoimmune diseases [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammation but often cause adverse effects like gastrointestinal irritation, cardiovascular risks, and skin sensitization [2]. Hence, plant-based anti-inflammatory agents are gaining attention due to their better safety profile. Nyctanthes arbor-tristis (family: Oleaceae), also known as night jasmine, is a traditionally used medicinal plant recognized for its anti-inflammatory, antipyretic, and analgesic activities [3]. Its leaves are rich in bioactive compounds like flavonoids, iridoid glycosides, and phenolic acids that contribute to its pharmacological effects [4]. Despite its therapeutic potential, the clinical use of Nyctanthes arbor-tristis leaf extract in topical formulations is limited due to poor dermal permeability and bioavailability of its phytoconstituents [5]. Conventional topical gels may not adequately deliver active compounds to deeper skin layers. Ethosomes—soft, phospholipid-based nanocarriers with high ethanol content—have shown promising results in improving the transdermal delivery of both lipophilic and hydrophilic drugs [6]. Recent studies have reported the use of ethosomal systems for enhancing the topical effectiveness of herbal extracts [7-8]. However, no comprehensive study has yet explored the formulation of Nyctanthes arbor-tristis extract into an ethosomal gel and its in vivo evaluation using a standard inflammatory model. The present study aims to evaluate the in vivo topical anti-inflammatory efficacy of Nyctanthes arbor-tristis ethosomal gel using the carrageenan-induced rat paw edema model. Specifically, this research seeks to determine whether the ethosomal gel formulation enhances the anti-inflammatory activity of NAT leaf extract compared to conventional topical preparations. This study will provide valuable insights into the application of NAT in novel drug delivery systems and its potential as a safe and effective topical anti-inflammatory agent.

Abbreviations:
NAT – Nyctanthes arbor-tristis; EG – Ethosomal Gel; CIPE – Carrageenan-Induced Paw Edema.

2. MATERIALS AND METHODS

2.1. Materials

Plant Material and Extract Preparation:
The bark and leaves of Nyctanthes arbor-tristis L. were collected from Ahmednagar district, Maharashtra, India, in January 2023. Authentication was performed by Mrs. Madhuri Pawar, Botanist at the Botanical Survey of India, Pune. A voucher specimen (voucher number: KKY 01) was deposited at the Botanical Survey of India Herbarium (Letter No. BSI/WRC/IDEN.CER/2023/127, dated 11 January 2023). The leaves were shade-dried and powdered for extraction.

Chemicals and Reagents:
Ethanol (analytical grade), soy phosphatidylcholine (soya lecithin), cholesterol, and carrageenan were procured from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Diclofenac gel (1% w/w) was obtained commercially and used as the standard anti-inflammatory agent.

Animals:
Male Wistar albino rats (6–8 weeks old, weighing 150–250 g) were used. Animals were housed under controlled temperature (22 ± 2°C) and humidity (55 ± 5%) with a 12 h light/dark cycle and free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC approval no. BU/IAEC/2024/07) and conducted as per CPCSEA guidelines [9].

2.2. Preparation of Nyctanthes arbor-tristis Ethosomal Gel

Preparation of Leaf Extract:
The powdered leaves were extracted using 70% ethanol in a Soxhlet apparatus for 6–8 hours. The extract was filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until use [10].

Ethosome Preparation:
Ethosomal vesicles were prepared by a modified cold method combined with probe sonication to obtain nanosized vesicles suitable for topical delivery. A 3^3 factorial design optimized the concentrations of ethanol (20–40% v/v), soya lecithin (1–3% w/v), and cholesterol (0.3–0.8% w/v). Soya lecithin and cholesterol were dissolved in ethanol and heated to approximately 60°C under stirring. The N. arbor-tristis extract (10 mg) was added, followed by gradual addition of distilled water to 100 mL. The mixture was probe-sonicated at 20% amplitude for 10 minutes to reduce particle size and enhance stability. Formulations were coded EG-NAT-12 [11].

Gel Formulation and Characterization [11-12]:
The ethosomal dispersions were incorporated into a carbopol-based gel. The gel was characterized for

- **Appearance, Grittiness & Homogeneity:** Assessed visually and by touch to confirm smooth, lump-free consistency and uniform dispersion.
- **pH:** Measured using a digital pH meter after dispersing 1 g of gel in 10 mL of distilled water. Values reported as mean ± SD (n=3).
- **Spreadability:** Determined by the slip and drag method using glass slides and a 500 g weight. Spreadability (gm·cm/sec) calculated and averaged.
- **Drug Content:** 1 g of gel was dissolved in ethanol, filtered, and analyzed spectrophotometrically at λmax 274 nm. Calculated using a standard calibration curve.
- **Viscosity:** Measured using a Brookfield viscometer with spindle 64 at 25 ± 1°C and 50 rpm. Reported in centipoise (cP).
- **Entrapment Efficiency (%):** Determined by ultracentrifugation (15,000 rpm, 30 min). Supernatant analyzed to estimate unentrapped drug. EE% calculated as:

$$\% EE = [Qt\text{-}Qs/Qt] X100$$

Where, EE is the entrapment efficiency, Qt is amount of extract added, Qs is amount detected in the supernatant.

2.3. In Vivo Experimental Design

Animal Grouping and Treatment Regimen [13].:

Animals were randomly divided into four groups (n = 6):

- Group I: Negative control (saline solution 0.9% w/v, no carrageenan)
- Group II: Positive control (carrageenan injection without treatment)
- Group III: Test group (topical application of N. arbor-tristis ethosomal gel)
- Group IV: Standard group (topical diclofenac gel 1% w/w)

Dose and Application:

Test and standard gels were applied topically on the right hind paw 30 minutes prior to carrageenan injection.

Induction of Inflammation:

Acute inflammation was induced by injecting 0.1 mL of 1% w/v lambda carrageenan solution into the subplantar region of the right hind paw [14].

2.4. Assessment of Anti-Inflammatory Activity

Measurement of Paw Edema:

Paw volume was measured using a plethysmometer at 0 (baseline), 1, 2, 3, and 4 hours after carrageenan injection to evaluate the progression of inflammation [14].

Calculation of Percentage Inhibition of Edema

Percentage Inhibition of Edema is calculated using the formula:

Percentage Inhibition = ((Vc - Vt) / Vc) × 100

Where,

Vc = Mean paw volume of the positive control group

Vt = Mean paw volume of the treated group

3.0 RESULT AND DISCUSSION:

3.1 Physicochemical Characterization of Optimized Ethosomal Gel:

Nyctanthes arbor-tristis ethosomal gel formulations were prepared using the optimized ratio of ethanol and phospholipids to enhance the ethosomal carrier system. Carbopol 940 was utilized as the gelling agent due to its hydrophilic nature and bio-adhesive properties, contributing to prolonged residence time at the site of application through interaction with mucosa. The prepared gel formulations were systematically evaluated for appearance, pH, viscosity, drug content, and in-vitro drug diffusion characteristics.

Formulation Code	Color	Grittiness	Homogeneity
EG-NAT-12	Light Brown	Non-gritty	Homogeneous

Table 3.1: Physical Evaluation

Formulation Code	pH	Spreadability (gm. cm/sec)	Drug content %	Viscosity (cps)	Entrapment Efficiency (%)
EG-NAT-12	5.72±0.06	12.6±0.4	97.1 ±0.46	14842±0.39	91.8

Values are expressed in mean±SD (n = 3)

Table 3.2: pH determination, spreadability, and viscosity results for Formulation & EG-NAT-12.

3.2 Anti-inflammatory Activity Study

Ethosomal Gel and Diclofenac Gel on Carrageenan-Induced Paw Edema in Rats Observation table:

Time (hours)	Group I (Control)	Group II (Carrageenan-only)	Group III (Ethosomal Gel-EG-NAT-12)	Group IV (Diclofenac Gel)
30 min	0.26 ± 0.02	0.53 ± 0.02	0.51 ± 0.01	0.52 ± 0.01
1	0.26 ± 0.03	0.52 ± 0.04	0.45 ± 0.03	0.48 ± 0.02
2	0.27 ± 0.02	0.51 ± 0.05	0.37 ± 0.04	0.33 ± 0.03
3	0.26 ± 0.03	0.50 ± 0.06	0.34 ± 0.04	0.31 ± 0.03
4	0.27± 0.03	0.49 ± 0.06	0.32 ± 0.04	0.29 ± 0.03

Table 3.3: Effect of Ethosomal Gel and Diclofenac Gel on Carrageenan-Induced Paw Edema in Rats (Mean ± SD) Data are expressed as mean ± standard deviation (SD). Statistical comparisons were

performed using one-way ANOVA followed by Tukey's multiple comparison test. A p-value < 0.05 was considered statistically significant.

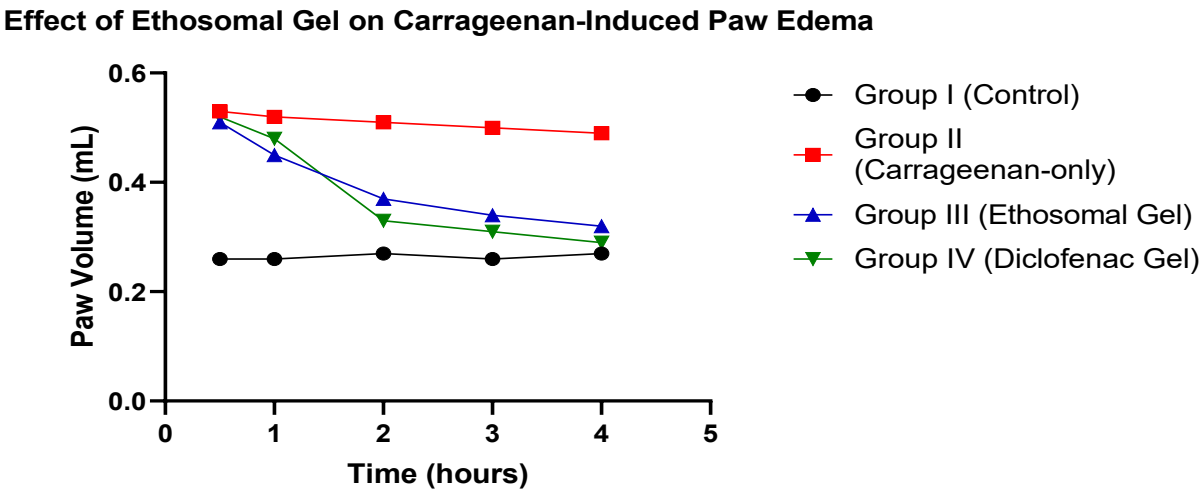

Figure 3.1: Effect of Ethosomal Gel on Carrageenan-Induced Paw Edema

The table presents the effect of ethosomal gel and diclofenac gel on carrageenan-induced paw edema in rats. At 30 minutes, Groups III (Ethosomal Gel- EG-NAT-12) and IV (Diclofenac Gel) exhibit similar paw volumes (0.51 ± 0.01 and 0.52 ± 0.01 mL, respectively), slightly lower than Group II (Carrageenan-only) at 0.53 ± 0.02 mL. Over time, Group III shows a steady reduction in paw volume, achieving 0.32 ± 0.04 mL at 4 hours. Group IV demonstrates the most significant reduction, reaching 0.29 ± 0.03 mL at 4 hours. Group I (Control) shows no substantial change, maintaining a stable volume around 0.26 ± 0.02 mL. The percentage inhibition of edema highlights the anti-inflammatory potential of ethosomal gel, which closely approximates that of diclofenac gel.

4 DISCUSSION:

The anti-inflammatory potential of the developed Nyctanthes arbor-tristis ethosomal gel (EG-NAT-12) was assessed in comparison to a standard diclofenac gel using the carrageenan-induced paw edema model in Wistar rats, a well-established model for evaluating acute inflammation. The findings are summarized in Table 3.1 and Figure 3.1. Following carrageenan administration, Group II (Carrageenan-only) exhibited a marked increase in paw edema, peaking at 0.53 ± 0.02 mL at 30 minutes and tapering slightly to 0.49 ± 0.06 mL by 4 hours. This pattern is indicative of the classical biphasic inflammatory response, wherein the initial phase (0–1 h) is mediated by histamine and serotonin, followed by a delayed phase (1–4 h) involving prostaglandins and leukotrienes. In contrast, both Group III (Ethosomal Gel EG-NAT-12) and Group IV (Diclofenac Gel) demonstrated a progressive and time-dependent reduction in paw edema volume, reflecting significant anti-inflammatory activity. Group III showed a notable decrease in inflammation beginning at the 2-hour mark, with paw volume reducing from 0.45 ± 0.03 mL at 1 hour to 0.32 ± 0.04 mL at 4 hours. The calculated edema inhibition at 4 hours was 34.69%, which was comparable to that of the diclofenac gel (40.82%), indicating the effectiveness of the ethosomal delivery system. The consistent performance of the standard Group IV (Diclofenac Gel) confirms the validity of the model and highlights the encouraging performance of the ethosomal gel. The improved efficacy of EG-NAT-12 may be attributed to enhanced skin penetration and retention provided by the ethosomal system, which consists of phospholipids and ethanol that disrupt the stratum corneum and facilitate deeper dermal absorption. As expected, Group I (Control) animals showed no significant variation in paw volume throughout the study duration, maintaining a baseline measurement of around 0.26 ± 0.02 mL, confirming the absence of inflammatory stimuli. Statistical analysis using one-way ANOVA followed by Tukey's multiple comparison test demonstrated that the reductions in paw volume in Groups III and IV were statistically significant ($p < 0.05$) compared to Group II, affirming the anti-inflammatory efficacy of both formulations. In summary, the results substantiate that the Nyctanthes arbor-tristis ethosomal gel exhibits potent anti-inflammatory activity, closely paralleling the performance of standard diclofenac gel.

The data further support the potential of ethosomal carriers in enhancing the topical delivery and therapeutic efficacy of herbal bioactives, offering a promising alternative to conventional synthetic treatments.

5. CONCLUSION:

The present study successfully demonstrated that the ethosomal gel formulation of Nyctanthes arbor-tristis (EG-NAT-12) possesses significant anti-inflammatory activity in the carrageenan-induced paw edema model in rats. The ethosomal gel achieved a 34.69% inhibition of paw edema at 4 hours, which was comparable to the standard diclofenac gel (40.82%). The enhanced performance of the ethosomal gel can be attributed to improved dermal penetration and sustained drug release offered by the ethosomal carrier system.

These findings suggest that ethosomal encapsulation is an effective strategy to improve the topical delivery of herbal extracts like Nyctanthes arbor-tristis, making it a viable herbal alternative to conventional NSAIDs for managing inflammatory conditions. Further studies involving long-term safety, histopathological evaluation, and clinical validation are warranted to establish its therapeutic potential for topical anti-inflammatory therapy.

6. Acknowledgements

The authors sincerely acknowledge Bhagwant University, Ajmer, for providing the necessary facilities and support to carry out this research. Special thanks are extended to Dr. Ravindra B. Laware for his valuable guidance and encouragement throughout the study. We also acknowledge the technical assistance provided by the Central Research Laboratory staff.

7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

8. REFERENCES: (Vancouver referencing style)

1. Zhang F, Mears JR, Shakib L, Beynor JI, Shanaj S, Korsunsky I, Nathan A, Accelerating Medicines Partnership Rheumatoid Arthritis and Systemic Lupus Erythematosus (AMP RA/SLE) Consortium, Donlin LT, Raychaudhuri S. IFN-γ and TNF-α drive a CXCL10+ CCL2+ macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases with tissue inflammation. Genome Medicine. 2021 Dec;13:1-7.
2. Schjerning AM, McGettigan P, Gislason G. Cardiovascular effects and safety of (non-aspirin) NSAIDs. Nature Reviews Cardiology. 2020 Sep;17(9):574-84.
3. Shukla R, Kumar A, Singh G, et al. Therapeutic potential of Nyctanthes arbor-tristis Linn.: a comprehensive review. Phytotherapy Research. 2021;35(8):4250–4264. https://doi.org/10.1002/ptr.7101
4. Gupta V, Verma S, Tiwari S. Evaluation of anti-inflammatory potential of Nyctanthes arbor-tristis leaf extract. Journal of Ethnopharmacology. 2020;261:113131. https://doi.org/10.1016/j.jep.2020.113131
5. Arora S, Rathore C. Potential role of herbal nanoformulations for skin disorders: a review. Therapeutic Delivery. 2023 Aug 1;14(8):511-25.
6. Pandey M, Choudhury H, Gorain B, Tiong SQ, Wong GY, Chan KX, They X, Chieu WS. Site-specific vesicular drug delivery system for skin cancer: a novel approach for targeting. Gels. 2021 Nov 16;7(4):218.
7. Sharma T, Thakur S, Kaur M, Singh A, Jain SK. Novel Hyaluronic Acid ethosomes based gel formulation for topical use with reduced toxicity, better skin permeation, deposition, and improved pharmacodynamics. Journal of Liposome Research. 2023 Apr 3;33(2):129-43.
8. Boggione DM, Santos IJ, de Souza SM, Mendonça RC. Preparation of polyvinyl alcohol hydrogel containing bacteriophage and its evaluation for potential use in the healing of skin wounds. Journal of Drug Delivery Science and Technology. 2021 Jun 1;63:102484.
9. CPCSEA guidelines for laboratory animal use and care. Committee for the Purpose of Control and Supervision of Experiments on Animals, India; 2020.
10. Kumar R, Saini S. Extraction and standardization of herbal drugs: Soxhlet technique. J Pharm Res. 2020;9(2):80–85.
11. Sharma P, et al. Formulation optimization of ethosomal gel using factorial design approach. Pharm Dev Technol. 2022;27(7):833–841.
12. Singh S, et al. Characterization techniques for topical gels: particle size, zeta potential, pH, and viscosity measurements. Int J Pharm Sci. 2021;13(3):123–131.
13. Tripathi KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers; 2019.
14. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111(3):544–547.