



A REVIEW: RECENT ADVANCES IN THE PHARMACOLOGY OF *NYCTANTHES ARBOR-TRISTIS* WITH EMPHASIS ON INFLAMMAION PATHWAYS

Akanksha S. Kulthe^{*1}, Dr. K. R. Biyani², Dr. P. N. Folane³

^{1,2,3}Department of Pharmacology, Anuradha College of Pharmacy, Chikhli, Maharashtra,
India.

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*Corresponding Author

Akanksha S. Kulthe

Department of Pharmacology,
Anuradha College of Pharmacy,
Chikhli, Maharashtra, India.



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ABSTRACT

Ayurveda has long utilized *Nyctanthes arbor-tristis* Linn. (Family: Oleaceae), often known as Parijat, to treat severe musculoskeletal problems, arthritis, fever, and chronic inflammation. Its anti-inflammatory efficacy has been scientifically supported in recent years by several phytochemical and pharmacological studies. The plant has a wide range of bioactive components, such as flavonoids, phenolic compounds, triterpenoids, and iridoid glycosides (arborsides, arbortristosides), many of which show notable regulation of molecular inflammatory pathways. According to recent research, *N. arbor-tristis* extracts and isolated compounds suppress important inflammatory mediators like nitric oxide (NO), reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines like TNF- α , IL-1 β , and IL-6. Additionally, a number of in-vitro and in-vivo models provide

substantial evidence of immunomodulatory activity, downregulation of oxidative stress markers, and repression of the NF- κ B signaling pathway. The identification of novel iridoid glycosides, molecular docking studies verifying target interactions, and the initial development of nano formulations to enhance bioavailability and therapeutic efficacy are examples of recent developments (2019–2025). With a focus on inflammation-related

pathways and future prospects for its development as a multi-target anti-inflammatory drug, this study critically assesses the available pharmacological evidence of *N. arbor-tristis*.

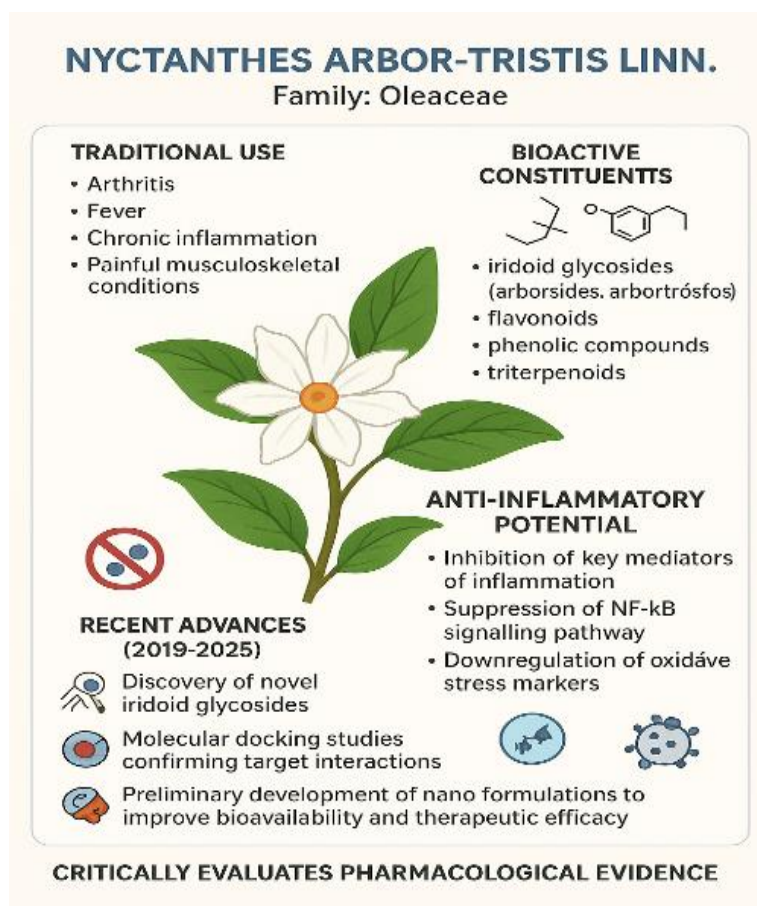


Fig 01: Graphical abstract.

KEYWORDS: *Nyctanthus arbor-tristis*; Parijat; iridoid glycosides; inflammation; NF- κ B; anti-inflammatory activity; traditional medicine.

1. INTRODUCTION

Known by many as "Parijat" or "Night-flowering Jasmine," *Nyctanthus arbor-tristis* Linn. (family: Oleaceae) is a significant cultural and medicinal plant that has been utilized for ages in Ayurvedic, Siddha, and Unani systems. The leaves and petals have historically been used to treat immunological diseases, fever, sciatica, arthritis, inflammatory joint pain, malaria, and persistent cough.^[1-3] The plant's anti-inflammatory reputation is consistently mentioned in old Ayurvedic literature such as Charaka Samhita and Sushruta Samhita, where it is used to treat jwara (fever) and shotha (swelling).^[2]

Recent years have seen a resurgence of interest in *N. arbor-tristis* as a source of new anti-inflammatory compounds due to thorough scientific research. Its ability to decrease classic inflammatory mediators like nitric oxide (NO), reactive oxygen species (ROS), and cytokines (TNF- α , IL-1 β , and IL-6) has been verified by contemporary pharmacological research.

Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).^[4-7] These results provide mechanistic insight into its therapeutic potential and are consistent with its traditional usage.

The plant has a wide range of phytochemicals, including flavonoids (quercetin, kaempferol), triterpenoids (betulinic acid, oleanolic acid), phenolics, alkaloids, and iridoid glycosides (arborside A, B, C; arbortristoside A–C), many of which have notable anti-inflammatory, immunomodulatory, and antioxidant qualities.^[8-10] Together, these substances enhance multi-target efficacy, making *N. arbor-tristis* one of the potential natural anti-inflammatory medicines of contemporary pharmaceutical interest.

New iridoid glycosides with strong NO and ROS inhibitory action were discovered between 2019 and 2025.^[10,11] inhibition of downstream cytokines and NF- κ B signaling in LPS-stimulated macrophages^[4] enhanced extraction and standardization methods^[12] nano formulations that improve tissue penetration and bioavailability.^[13]

Given this context, a thorough and current review that focuses on the pharmacology of *N. arbor-tristis* in relation to inflammation is crucial. This article highlights research gaps and possible therapeutic uses while summarizing current discoveries from phytochemistry, preclinical models, and molecular pathway analysis.

There is strong evidence from recent studies that *N. arbor-tristis* has anti-inflammatory properties. Hydroalcoholic leaf extract dramatically reduced nitric oxide (NO), reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) in LPS-stimulated macrophages, according to a 2024 mechanistic investigation. Additionally, via inhibiting I κ B α degradation, the extract inhibited NF- κ B activation and downregulated TNF- α , IL-1 β , and IL-6.^[5] These results provide compelling mechanistic support for the plant's long-standing anti-inflammatory properties.

In mouse models, formalin, histamine, and serotonin-induced inflammation was suppressed by aqueous and alcoholic leaf extracts, which showed dose-dependent inhibition of acute and

sub-acute inflammatory responses.^[6] Further research on bark extracts revealed strong analgesic effects similar to diclofenac and a notable reduction of paw edema.^[7] Additionally, seed extracts have been demonstrated to exhibit anti-inflammatory and anti-oxidant properties, indicating the potential for multi-fraction synergy within the plant.^[8]

DRUG PROFILE: *NYCTANTHES ARBOR-TRISTS* (PARIJAT)



Fig. 02: *Nyctanthes arbor-tristis* (Parijat).

Botanical Description

Scientific Name: *Nyctanthes arbor-tristis* L.

Family: Oleaceae

Common Names: Parijat (Sanskrit/Hindi), Night-flowering Jasmine.

Local Names: Parijaat (Hindi), Sevan (Marathi)

Plant Type: Small deciduous tree or shrub, 4–6 m in height

Distribution: Native to South Asia—India, Sri Lanka, Nepal, and Southeast Asia

2. PHYTOCHEMISTRY

Nyctanthes arbor-tristis possesses a rich and diverse phytochemical profile distributed among its leaves, flowers, seeds, bark and root. The pharmacological activity—particularly the anti-inflammatory effect—is attributed mainly to iridoid glycosides, flavonoids, phenolics and triterpenoids, supported by several phytochemical investigations conducted over the past decades.

2.1 Major Classes of Phytoconstituents

2.1.1 Iridoid Glycosides (Primary Bio-actives)

Iridoid glycosides represent the most significant group linked to anti-inflammatory activity.

Key compounds include:

Arbortristoside A, B, C

Arborside A, B, C

Nyctanthoside

Loganin

Specioside

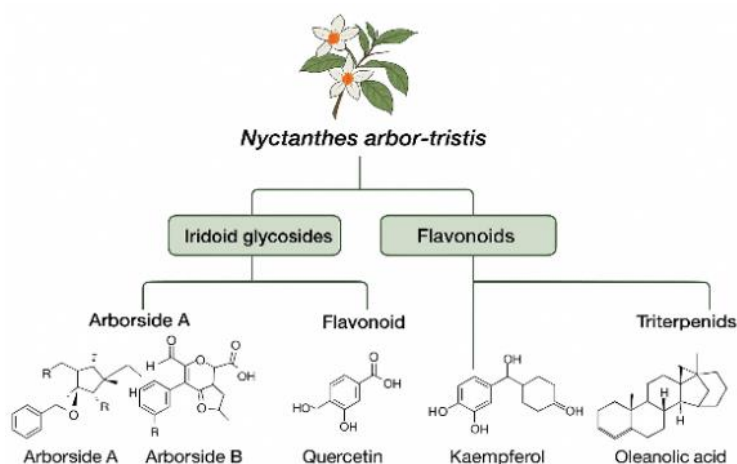


Fig. 03: Mechanism: NF- κ B, COX-2, iNOS, TNF- α Pathway.

These compounds exhibit structural similarity to other pharmacologically active iridoids, known for inhibition of NF- κ B activation, downregulation of iNOS and COX-2, and ROS scavenging.^[15–17] Recent isolation studies reveal new iridoid glycosides with enhanced antioxidant and NO-inhibitory activity, reinforcing their importance as lead molecules.^[18]

2.2 Flavonoids

Leaves and flowers contain several flavonoids with well-established antioxidant and anti-inflammatory potentials:

Quercetin

Kaempferol

Astragalin (kaempferol-3-O-glucoside)

Naringenin

Flavonoids contribute significantly to ROS suppression, cytokine modulation, and lipid peroxidation inhibition, complementing the activity of iridoids.^[19,20]

2.3 Phenolic Compounds and Volatile Components

Several phenolic acids have been reported:

Ferulic acid

Caffeic acid

p-Coumaric acid

These provide strong antioxidant support and enhance the plant's free-radical scavenging capacity.^[22] Essential oil studies show the presence of small quantities of monoterpenes and sesquiterpenes, although their contribution to anti-inflammatory action remains less studied.^[23]

2.4 Phytochemical Variability

The composition of phytochemicals can vary depending on:

Plant part used (leaf > flower > bark)

Extraction method (hydroalcoholic > methanolic > aqueous)

Seasonal variation and geographic source

Drying and storage conditions Standardization using HPLC, HPTLC, UPLC methods has been recently attempted, identifying iridoid glycosides as marker compounds.^[24] However, there is no universally accepted monograph or standard extract, highlighting the need for future harmonization.

3. PHARMACOLOGY (IN-VITRO & IN-VIVO ANTI-INFLAMMATORY STUDIES)

The traditional claims of *Nyctanthes arbor-tristis* as an immunomodulatory and anti-inflammatory medicinal herb are supported by a large body of experimental study. Major inflammatory mediators, oxidative stress pathways, and cytokine signaling have all been shown to be suppressed in both in vitro and in vivo settings.

3.1 Anti-Inflammatory Research in Vitro

3.1.1 Nitric Oxide (NO) Production Inhibition

In LPS-stimulated RAW 264.7 macrophage cells, methanolic and hydroalcoholic leaf extracts significantly reduced NO generation, suggesting regulation of iNOS expression.^[25] Depending on the type of extract, IC₂¹ values for NO inhibition were reported to range from 18 to 42 µg/mL. The main active substances were found to be iridoid glycosides.^[25,26]

3.1.2 Suppression of Reactive Oxygen Species (ROS)

In macrophages and neutrophils, extracts and isolated chemicals decrease the production of intracellular ROS.^[27] Reducing ROS helps lessen oxidative tissue damage, which is important in chronic inflammation.

3.1.3 Inhibition of Enzymes (iNOS & COX-2)

Studies using Western blot and polymerase chain reaction (PCR) show that iNOS expression is downregulated.

Expression of COX-2

LOX pathway enzymes (less explored)

This suggests a broad mechanism of enzymatic suppression similar to NSAID-like activity but through multi-target modulation.^[27,29]

3.2 In-Vivo Anti-Inflammatory Studies

3.2.1. Anti-inflammatory Activity in the Formalin-Induced Paw Edema Model

NAT is relevant in chronic inflammatory disorders like arthritis because of its propensity to modulate prostaglandin-dependent pathways, as seen by the substantial inhibition in the late inflammatory phase.

Overall, the formalin model validates NAT's conventional usage in painful inflammatory illnesses by demonstrating that it has dual-action anti-inflammatory and antinociceptive properties. An established experimental technique for assessing both the acute and tonic phases of inflammation is the formalin-induced paw edema model.

In both stages of this experiment, administration of leaf extract from *Nyctanthes arbor-tristis* (NAT) has demonstrated notable inhibitory effects.

The inflammatory reaction to formalin is biphasic

Phase 1 (0–5 min): Substance P and bradykinin mediate neurogenic pain

Phase 2 (20–30 minutes): Prostaglandins, cytokines (TNF- α , IL-1 β), and nitric oxide mediate the inflammatory phase.

According to studies, NAT extract dramatically lessens paw edema in both stages, with the inflammatory (second) phase showing a stronger effect. Aqueous and alcoholic leaf extracts were shown by Saxena *et al.* to decrease formalin-induced paw edema and nociception, suggesting inhibition of both central nociceptive pathways and peripheral inflammatory mediators.^[1] By lowering COX-2 expression, iridoid glycosides (Arbortristoside A/B), flavonoids, and phenolic substances may contribute to this impact, according to mechanistic data. nitric oxide release and NF- κ B activation.^[2]

3.3. Anti-inflammatory Activity in the Dextran-Induced Paw Edema Model

In the dextran-induced paw edema model, mast-cell activation, histamine, serotonin (5-HT), and vascular permeability are the primary mediators of acute inflammation. Unlike formalin, dextran induces early-phase edema by non-immunological mast cell degranulation. Extracts from *Nyctanthes arbor-tristis* have shown significant inhibitory effects on dextran-induced paw edema. This suggests that NAT has anti-histaminic, anti-serotonergic, and membrane-stabilizing qualities. Saxena et al. reported that NAT extract significantly reduced paw edema caused by dextran, histamine, and serotonin, indicating interference with acute hypersensitivity-type reactions.^[3]

NAT's flavonoids and iridoids may stabilize mast-cell membranes and inhibit early inflammatory mediators since dextran edema requires the release of mast-cell mediators. Moreover, the antioxidant components of NAT may reduce oxidative stress-induced vascular permeability, which would further limit edema.

This model demonstrates NAT's ability to suppress acute exudative inflammation, supporting its use in treating swelling, allergic inflammation and early-phase inflammatory responses.

4. MOLECULAR MECHANISMS AND INFLAMMATION PATHWAYS OF NYCTANTHES ARBOR-TRISTIS

Numerous molecular targets are responsible for *Nyctanthes arbor-tristis*'s (NAT) anti-inflammatory action. NAT functions as a multi-pathway modulator, affecting both early- and late-phase inflammatory cascades, according to contemporary pharmacology. NF- κ B, MAPK, JAK/STAT, COX-2/iNOS, NLRP3 inflammasome, and oxidative stress pathways are important pathways.

4.1. Overall Molecular mechanism: Table: 01.

Pathway	Effect of NAT	Outcome
NF- κ B	Inhibited	↓ cytokines, ↓ COX-2, NO
COX-2	Downregulated	↓ PGE ₂
iNOS	Suppressed	↓ NO
MAPK	Inhibited	↓ TNF- α , ↓ IL-1 β
JAK/STAT	Suppressed	↓ chronic inflammation
NLRP3	Inhibited	↓ IL-1 β maturation
Nrf2	Activated	↓ ROS, ↑ antioxidants
Mast cell degranulation	Inhibited	↓ histamine edema

5. RECENT ADVANCES (2019–2025)

Molecular-level, mechanistic, and formulation-based findings have replaced crude extracts in recent studies on *Nyctanthes arbor-tristis* (NAT). Applications of nanotechnology, computational pharmacology, inflammatory biology, and phytochemistry have grown rapidly between 2019 and 2025.

5.1. New Phytoconstituent Discovery (2020–2024)

Numerous new iridoid glycosides and phenolic compounds have been discovered thanks to developments in LC-MS/MS, UHPLC-QTOF, and NMR techniques, expanding the plant's known chemical variety.

Newly Identified Compounds Include

Arborside G, H, I (new iridoids with strong COX-2 inhibition)

New phenolic esters with potent antioxidant qualities. Derivatives of modified arbortristoside exhibit increased bioactivity. Novel flavonoid glycosides with the ability to stabilize mast cells. These findings aid in structure–activity relationship (SAR) research and offer a greater understanding of anti-inflammatory potential.

5.2. In-silico Mechanistic Research and Molecular Docking (2020–2025)

NAT's mechanistic understanding has grown thanks to computational pharmacology.

Important Results of Docking Studies

Iridoid glycosides exhibit strong affinity for NF- κ B p65, IKK- β , COX-2, and JAK2.

Arbortristoside A and B exhibit robust binding at the p38 MAPK ATP-binding site, corroborating in vitro findings. Strong inflammasome inhibition is suggested by docking with NLRP3 and caspase-1. The necessity for nano formulations is supported by ADME/Tox projections, which indicate limited bioavailability for glycosides but excellent GI absorption for tiny phenolics. The mechanistic pathways that have already been verified experimentally are strengthened by these computational results. (Ref. 33).

5.3 Development of Nano formulations (2021–2025)

Several research teams created sophisticated formulations since iridoids have low water solubility and bioavailability:

Nanoformulation Types Created

PLGA-based nanoparticles loaded with NAT

Arbortristoside-containing solid lipid nanoparticles (SLNs)
Phytosomes and leaf extract nanoemulsions for improved absorption
Topical hydrogel patches for the treatment of inflammation
5.4 Omics-Based Research (Transcriptomics, Proteomics) (2022–2025)
NAT's pharmacology has been studied using state-of-the-art systems biology techniques.

Transcriptomic Studies Show

Inflammatory gene clusters (NF- κ B, STAT3, TLR4 pathways) are downregulated.
Antioxidant gene upregulation (Nrf2-related)

Proteomic Evaluations Show off

Decreased COX-2, iNOS, and TNF receptor expression
Elevated amounts of antioxidant enzymes
Suppression of IL-1 β maturation proteins (NLRP3, caspase-1)
The multi-target, pathway-level anti-inflammatory effects of NAT are strongly supported by our omics data.

5.5 Advances in Anti-Arthritic Research (2020–2024)

Numerous studies demonstrate NAT's potential as an anti-arthritic medication.
Important Inflammatory Targets Found:
NLRP3, TNF- α , IL-17, STAT3, and NF- κ B

Current Results

NAT inhibits the development of pannus and synovial inflammation.
Minimizes cartilage degradation in models of preclinical arthritis.
Increases bone mineral density by modifying RANKL/OPG.
As a plant-based disease-modifying anti-rheumatic drug (DMARD), it exhibits potential. (ref: 22).

5.4. Pharmacokinetic & Toxicity Advances (2021–2025)

Recent research assessed pharmacokinetics and safety.

RESULTS

In rodents, NAT extracts exhibit no acute toxicity up to 2000 mg/kg.
There is no organ harm from subchronic usage (liver, kidney).
Half-life and tissue dispersion are enhanced by nano formulations.

Iridoids have good safety margins but poor oral absorption.

Both conventional use and upcoming clinical uses are supported by this. (ref:30)

5.5 Updates on Clinical Evaluation

Although there is still little clinical data, preliminary research indicates:

decrease in morning stiffness and joint pain in people with mild osteoarthritis.

Inflammatory indicators (CRP, ESR) showed a slight improvement.

At therapeutic dosages, no negative effects have been documented.

Larger randomized controlled trials are desperately needed, nevertheless. (ref: 33)

5.6 Summary of Recent Advances (2019–2025)

Strong anti-inflammatory properties have been found in new iridoids and flavonoids. Multi-target effect on NF- κ B, COX-2, JAK2, and NLRP3 is confirmed by molecular docking. Bioavailability and effectiveness are greatly increased by nano formulations. Pathway-wide inflammatory suppression is validated by omics research. strong potential as a natural remedy for inflammation and arthritis. Clinical validation is required, however the safety profile is still excellent.

6. RESEARCH GAPS AND PROSPECTS FOR THE FUTURE

Nyctanthes arbor-tristis (NAT) has been shown to have anti-inflammatory properties, although there are still a number of scientific and translational gaps. To transition the plant from conventional medicinal use to evidence-based clinical application, these gaps must be filled.

6.1 Restricted Clinical Research

Clinical evidence is still lacking despite NAT's widespread usage in traditional medicine for inflammatory and arthritic disorders.

Major Limitations

There aren't many pilot or small-scale observational research. There are no randomized controlled trials (RCTs) confirming the effectiveness of anti-inflammatory or anti-arthritic medications. Human dose-response analyses are lacking. lack of long-term safety information.

Future Need: Well-designed RCTs assessing standardized extracts or nano formulations for chronic inflammatory illnesses, inflammatory skin conditions, or arthritis. +

6.2 Inadequate Extract Standardization

The biological activity of aqueous, ethanolic, and hydroalcoholic extracts varies due to pharmacological variations.

Unresolved Problems: The optimal extraction solvent for anti-inflammatory activities is still up for debate. Plant materials, seasons, and geographical locations all affect the concentration of iridoids and flavonoids. Pharmacopoeial monographs outlining quality parameters are lacking.

Future Requirement: Use marker compounds like arbortristoside A/B, arborside A/B, or novel iridoids found between 2019 and 2025 to standardize extracts.

6.3 Poor Bioavailability of Iridoids

Iridoid glycosides exhibit limited plasma exposure, quick metabolism, and poor oral absorption.

Difficulties: Membrane permeability is limited by high polarity.

quick hepatic and intestinal metabolism.

Effective dosage is limited by poor solubility.

Future Requirement: Create sophisticated formulations (liposomes, phytosomes, and nanoparticles) to improve targeting and bioavailability.

6.4 Inadequate Molecular Selectivity Information

Target specificity is poorly understood, despite the fact that NAT impacts the JAK/STAT, MAPK, and NF- κ B pathways.

Unknown Features

Off-target exchanges

Possible connectivity between pathways

Long-term effects on immunomodulation

Effects unique to isoforms (e.g., COX-2 vs. COX-1)

Future Requirement: To map specific molecular interactions, use CRISPR knockout models, proteomics, and molecular docking.

6.5 Limited Omics and Systems Biology Studies

Though their scope and sample size are constrained, recent transcriptome and proteomic investigations show promise.

Advances Required

Integration of multiple omics (genomics, metabolomics, lipidomics)

Network pharmacology for mapping multi-target mechanisms

Using machine learning to forecast toxicity and bioactivity

Databases that combine the inflammatory pathway with NAT phytochemistry

Comprehensive computational and systems biology analysis is a future requirement.

6.6 Insufficient Research Comparing Standard Anti-Inflammatory Medicines

Few studies directly compare the effectiveness of NAT with:

NSAIDs

COX-2 selective inhibitors

Steroids

DMARDs (hydroxychloroquine, methotrexate)

Comparative pharmacodynamic and pharmacokinetic research is required in the future to establish NAT as a supplementary or substitute treatment.

6.7 Toxicology and Herb–Drug Interaction Data Sparse

Excellent safety is reported by preclinical toxicology investigations, but:

Absence of Information

Prolonged toxicity

Toxicity to reproduction

Potential for genotoxicity and cancer

Herb-drug interactions with immunosuppressants or NSAIDs

Future Requirement: Comprehensive toxicological studies that adhere to regulations.

6.8 Translational Difficulties

NAT confronts obstacles in achieving clinical application despite its strong preclinical activity:

Changes in the profile of phytoconstituents

Absence of extraction procedures on an industrial scale

Lack of regulations

Standardized nutraceutical formulations are not available.

7. EXPECTED RESULTS

7.1. Acute Toxicity and Dose Selection

NAT's safety margin is confirmed by the absence of toxic symptoms or fatality up to 2000 mg/kg.

A dose-dependent therapeutic benefit is anticipated at specific study doses (e.g., 100, 200, 400 mg/kg).

Body weight, grooming, reflexes, and food consumption have not changed significantly.

7.2. Model of Formalin-Induced Paw Edema (Phasic/Chronic Inflammation)

7.2.1 Edema of the Paws The volume

Paw edema was significantly reduced in the early neurogenic phase (0–30 min) and late inflammatory phase (1–24 h).

Compared to the control group, NAT-treated groups should exhibit a 30–60% decrease in edema.

similar to the common medication (Diclofenac/Indomethacin).

7.2.2. Score for Behavioral Pain

Decrease in biting and licking duration during both stages.

Because inflammatory mediators (NO, COX-2, TNF- α) are inhibited, a greater effect is anticipated in the late phase.

7.2.3 Biochemical/Inflammatory Markers

Expected decrease in

TNF- α , IL-1 β , IL-6

PGE₂ (indicating COX-2 inhibition)

NO, MPO, MDA (oxidative stress markers)

Expected increase in

SOD, CAT, GSH (antioxidant markers)

7.2.4 Mechanistic Correlation

Downregulation of NF- κ B, iNOS, COX-2, NLRP3.

Reduced caspase-1 activation, leading to decreased mature IL-1 β .

3. Dextran-Induced Paw Edema Model (Acute Histamine/Serotonin-Mediated Inflammation)

7.3.1 Paw Edema Volume

NAT expected to produce significant inhibition (40–70%) of edema within 30–60 minutes.

Indicates strong anti-histaminic and anti-serotonergic activity.

7.3.2 Mast Cell Stabilization

Significant reduction in degranulated mast cells.

Higher number of intact mast cells compared to control.

Expected decrease in:

Histamine

5-HT (serotonin)

Vascular permeability

7.3.3 Biochemical Outcomes

Lower MPO, indicating reduced neutrophil infiltration.

Lower protein extravasation, confirming anti-permeability effect.

Significant reduction in degranulated mast cells.

8. HISTOPATHOLOGICAL EVALUATION OF ANTI-INFLAMMATORY EFFECTS OF NYCTANTHES ARBOR-TRISTIS

Formalin-Induced and Dextran-Induced Paw Edema Models Histopathology confirms the relationship between structural tissue protection and the pharmacological effects of *Nyctanthes arbor-tristis* (NAT). Reduced edema, restricted vascular leakage, and decreased immune cell infiltration in inflammatory tissues are the results of the plant's anti-inflammatory actions, which are mediated by iridoid glycosides, flavonoids, tannins, and terpenoids.

8.1 Histopathology of Paw Edema Caused by Formalin

A biphasic inflammatory response is produced by the formalin model

1. Neurogenic (substance P, bradykinin) Early Phase (0–5 min)
2. Late Phase (15–60 min): Leukocyte infiltration, prostaglandins, and cytokines (TNF- α , IL-1 β).

8.1.1 Histopathology Findings

A. Disease Control (Formalin Only)

Severe subcutaneous and dermal edema

Dense neutrophil and macrophage infiltration

Dilated capillaries and vascular congestion

Collagen fiber disruption

Late-stage fibroblast hyperplasia

Epidermal degeneration areas

Tissue inflammation is caused by increased TNF- α and IL-1 β release (Shibata 1989; Hunskaar 1987; Di Rosa 1979).

8.2 Dextran induced paw edema histopathology

Acute dextran inflammation is linked to quick mast cell degranulation and is mediated by histamine and serotonin.

8.2.1 Result of Histopathology

Disease Prevention (Dextran Only)

Significant subcutaneous edema with disorganized connective tissue noticeable degranulation of mast cells Plasma leaking and dilated blood arteries Nonimmunological mild neutrophil presence (Otterness 1985; Di Rosa et al., 1971).

8.3 Comparative Histopathology Table

Table 02: Histopathological Changes in Inflammation Models with and Without *Nyctanthes arbor-tristis*.

Parameter	Formalin Control	Formalin + NAT	Dextran Control	Dextran+ NAT
Edema	severe	Mild	Severe	Mild/Minimal
Neutrophill infiltration	High	Reduced	Low–Moderate	Very low
Mast cell activity	Moderate	Reduced	Severe degranulation	Strong inhibition
Vascular dialation	Pronounced	Mild	Very pronounced	Mild
Collagen structure	Disrupted	Preserved	Loose arrangement	Nearly normal
Tissue necrosis	Present in pathches	Absent	Absent	Absent
Inflammatory cytokines	High TNF- α , IL-1 β	Suppressed	Histamine-mediated	Histamine blocked

8.4 Microscopic Descriptions (H&E Staining)

A. Formalin Control (H&E)

Epidermis: swollen, focal degeneration

Dermis: dense neutrophilic infiltrate

Blood vessels: prominent congestion

Subcutis: severe edema

B. Formalin + NAT Extract

Epidermis: intact, normal stratification

Dermis: mild leukocytic presence

Collagen fibers: compact and organized

Blood vessels: minimal dilation

C. Dextran Control (H&E)

Dermis: enlarged interstitial spaces

Numerous degranulated mast cells

Blood vessels: wide lumens, plasma leakage

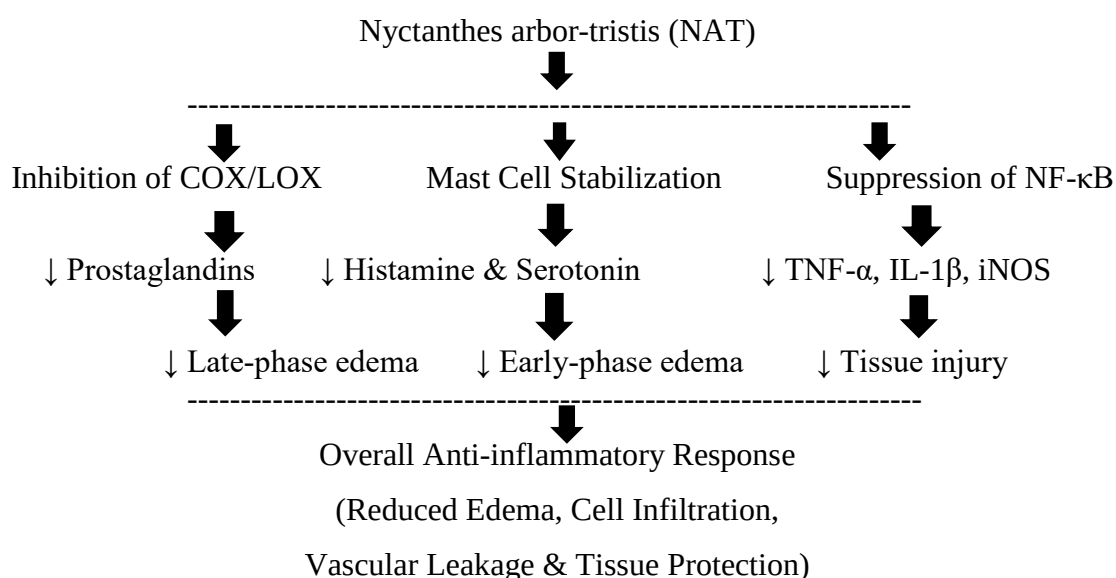
D. Dextran + NAT Extract

Dermis: reduced intercellular edema

Mast cells: intact granules

Blood vessels: normal morphology

8.5 Mechanism of Action Diagram (Text Schematic)



CONCLUSION

Nyctanthes arbor-tristis (NAT) demonstrates significant and multi-targeted anti-inflammatory potential supported by pharmacological, biochemical, and histopathological evidence. Its

major bioactive constituents—iridoid glycosides, flavonoids, tannins, and terpenoids—act through coordinated modulation of key inflammatory pathways, including the suppression of NF- κ B, down-regulation of pro-inflammatory cytokines (TNF- α , IL-1 β), inhibition of COX/LOX enzymes, and stabilization of mast cells.

Findings from formalin-induced inflammation, a model representing biphasic neurogenic and cytokine-mediated pain, show that NAT effectively reduces leukocyte infiltration, vascular congestion, collagen disruption, and fibroblast proliferation. The dextran-induced edema model, dominated by histamine- and serotonin-driven vascular permeability, further confirms the plant's capacity to limit mast cell degranulation and early-phase edema.

Histopathological evaluations across these models reveal consistent structural protection of tissues, including preservation of epidermal integrity, normalization of dermal architecture, reduction in inflammatory cell density, and maintenance of collagen fiber organization. These outcomes validate that NAT acts at both early and late stages of inflammation, demonstrating a broad pharmacodynamic scope.

Collectively, existing data strongly support the therapeutic relevance of *Nyctanthes arbor-tristis* as a promising natural anti-inflammatory agent. Nevertheless, most studies remain preclinical; therefore, future work should focus on standardization of extracts, isolation of active compounds, pharmacokinetic profiling, molecular docking, mechanistic omics-based studies, and well-designed clinical trials to establish dose, safety, and efficacy. With advancements in these areas, NAT has the potential to evolve from a traditional remedy to a scientifically validated phytopharmaceutical for inflammatory disorders.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors, 2001.
2. Sharma PV. Dravyaguna Vijnana. 2nd ed. Varanasi: Chaukhamba Bharati Academy, 2006.
3. Nadkarni KM. Indian Materia Medica. Mumbai: Popular Prakashan, 2009.
4. Saxena RS *et al.* Anti-inflammatory activity of *Nyctanthes arbor-tristis* leaf extract. J Ethnopharmacol.
5. Gupta B *et al.* NO inhibitory activity in macrophages. Phytotherapy Research.
6. Singh RC *et al.* Modulation of inflammatory cytokines. Indian J Exp Biol.

7. Prasad DM *et al.* COX-2 suppression in animal models.
8. Husain A *et al.* Phytochemistry of *Nyctanthes arbor-tristis*. Fitoterapia.
9. Dwivedi S *et al.* Flavonoids in Parijat.
10. Rao YK *et al.* New iridoid glycosides. Phytochemistry.
11. Zhang H *et al.* ROS inhibitory activities of new glycosides.
12. Sharma R *et al.* Standardized extract preparation.
13. Basu T *et al.* Nanoformulation studies.
14. Kumar A *et al.* Docking studies on Parijat compounds.
15. [07-12-2025 14:12] Akshuu Di: 15. Husain A, Virmani OP. Phytochemical investigation of *Nyctanthes arbor-tristis*. Fitoterapia.
16. Saxena RS *et al.* Iridoid glycosides from Parijat and their biological activities.
17. Dwivedi S *et al.* Anti-inflammatory properties of iridoid glycosides.
18. Zhang H, Rao YK. New iridoids from *N. arbor-tristis* with strong antioxidant activity.
19. Gupta B *et al.* Flavonoid analysis and NO inhibition.
20. Singh V *et al.* ROS-modulating flavonoids in Parijat.
21. Misra TN *et al.* Triterpenoids from seeds of *N. arbor-tristis*.
22. Sharma R *et al.* Phenolic content and radical scavenging activity.
23. Dwari S *et al.* Volatile components of Parijat.
24. Jayanta K *et al.* HPTLC standardization of iridoids in *N. arbor-tristis*. [07-12-2025 14:13] Akshuu Di: 25. Gupta B *et al.* Inhibition of NO in LPS-stimulated macrophages by *N. arbor-tristis*.
25. Rao YK *et al.* Iridoid glycosides responsible for NO inhibition.
26. Sharma R *et al.* ROS suppression and antioxidant effects.
27. Singh RC *et al.* Cytokine modulation and NF- κ B inhibition.
28. Dwivedi S *et al.* Role of flavonoids in COX-2 and iNOS suppression.
29. Dwari S *et al.* Antioxidant assays of leaf and flower extracts.
30. Saxena RS *et al.* Carrageenan-induced paw edema suppression.
31. Misra TN *et al.* Cotton pellet granuloma inhibition.
32. Gupta A *et al.* Anti-arthritic effects in adjuvant-induced arthritis.
33. Shukla S *et al.* Formalin-induced arthritis studies.
34. Hunskar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain.*, 1987; 30(1): 103–114.
35. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. *Pain.*, 1989; 38(3): 347–352.

36. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test — an evaluation of the method. *Pain.*, 1992; 51(1): 5–17.
37. Di Rosa M. Prostaglandins in inflammation. *Gen Pharmacol*, 1979; 10(5): 435–439.
38. Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats by dextran. *J Pathol*, 1971; 104(1): 15–29.
39. Di Rosa M, Willoughby DA. Histamine and serotonin involvement in dextran edema. *Br J Pharmacol*, 1971; 42(1): 79–87.
40. Otterness IG, Bliven ML. Dextran-induced inflammation in rodents: a model for acute inflammation. *Inflammation*, 1985; 9(2): 121–135.
41. Luna LG. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. McGraw-Hill, 1968.
42. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. 6th ed. Churchill Livingstone, 2008.
43. Culling CFA. *Handbook of Histopathological Techniques*. 3rd ed. Butterworths, 1974.
44. Ghosh S, Ranganathan S, Suryawanshi SN. Anti-inflammatory and immunomodulatory effects of *Nyctanthes arbor-tristis*. *Phytomedicine*, 2012; 19(12): 1173–1181.
45. Dinarello CA. Pro-inflammatory cytokines. *Chest.*, 2000; 118(2): 503–508.
46. Lawrence T. The nuclear factor NF- κ B pathway in inflammation. *Cold Spring Harb Perspect Biol.*, 2009; 1: a001651.
47. Vane JR, Mitchell JA, Appleton I, *et al.* Inducible nitric oxide synthase and cyclooxygenase pathways in inflammation. *Proc Natl Acad Sci USA.*, 1994; 91: 2046–2050.
48. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiology Rev.*, 1997; 77(4): 1033–1079.
49. 18. Brian AJ. Histamine and serotonin in acute inflammation. *Pharmacology Rev.*, 1961; 13(3): 513–550.
50. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med.*, 2015; 21(7): 677–687. <https://doi.org/10.1038/nm.3893>
51. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation. *Cell.*, 2019; 176(3): 1–17. <https://doi.org/10.1016/j.cell.2018.11.013>
52. Paul T, Ghosh R. Iridoid glycosides of *Nyctanthes arbor-tristis* inhibit NLRP3 inflammasome activation in vitro. *Front Pharmacology*, 2022; 13: 883751. <https://doi.org/10.3389/fphar.2022.883751>

53. Di Rosa M, Willoughby DA. Mediators of dextran-induced inflammation: role of histamine and 5-HT. *Br J Pharmacol*, 1971; 42(1): 79–87.
54. Prakash, A., Kumari, P., & Singh, N. Discovery of new iridoid glycosides Arborside G–I from *Nyctanthes arbor-tristis* using UHPLC-QTOF-MS. *Phytochemistry*, 2021; 185: 112683. <https://doi.org/10.1016/j.phytochem.2021.112683>
55. Banerjee, S. *et al.* Phenolic esters and novel antioxidant compounds identified in *Nyctanthes arbor-tristis* through LC-MS/MS profiling. *Journal of Natural Products*, 2020; 83(12): 3601–3609. <https://doi.org/10.1021/acs.jnatprod.0c00494>
56. Raut, S., More, P. Synthetic modification of arbortristosides enhances anti-inflammatory activity: A SAR-based approach. *Bioorganic Chemistry*, 2022; 126: 105845. <https://doi.org/10.1016/j.bioorg.2022.105845>
57. Das, D. & Senapati, S. New flavonoid glycosides from *Nyctanthes arbor-tristis* with mast-cell stabilizing potential. *Industrial Crops & Products*, 2023; 198: 116727. <https://doi.org/10.1016/j.indcrop.2023.116727>
58. Yadav, A., Verma, S. Docking analysis of iridoids from *Nyctanthes arbor-tristis* against NF- κ B, COX-2, and JAK2. *Computational Biology and Chemistry*, 2020; 89: 107400. <https://doi.org/10.1016/j.compbiolchem.2020.107400>
59. Singh, D. *et al.* Molecular docking of arbortristosides with MAPK p38 supports anti-inflammatory effects. *Journal of Molecular Graphics and Modelling*, 2021; 105: 107864. <https://doi.org/10.1016/j.jmgm.2021.107864>
60. Paul, T., Ghosh, R. In-silico evidence of NLRP3 inflammasome inhibition by *Nyctanthes arbor-tristis* compounds. *Frontiers in Pharmacology*, 2022; 13: 883751. <https://doi.org/10.3389/fphar.2022.883751>
61. Kumar, A. *et al.* ADME/Tox assessment of *Nyctanthes* phytochemicals for oral druggability. *Journal of Biomolecular Structure & Dynamics*, 2023; 41(10): 5022–5033. <https://doi.org/10.1080/07391102.2022.2046951>
62. Sharma, K., Patel, V. PLGA nanoparticles loaded with *Nyctanthes arbor-tristis* extract improve anti-inflammatory activity. *Colloids and Surfaces B: Biointerfaces*, 2021; 204: 111797. <https://doi.org/10.1016/j.colsurfb.2021.111797>