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Original Article

Evaluation of *Neolamarckia cadamba* (Roxb.) Methanolic Extract Loaded Niosomal Gel for Anti- Inflammatory and Anti- Rheumatic Activity

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Abstract:

Neolamarckia cadamba is a medicinal plant known for its anti-inflammatory and therapeutic properties. The present study aimed to evaluate the anti-inflammatory and anti-rheumatic activities of *Neolamarckia cadamba* leaf extract and its niosomal formulation. The leaf extract was prepared by Soxhlet extraction and formulated into niosomes to enhance its therapeutic efficacy. Anti-inflammatory activity was assessed using protein denaturation inhibition and HRBC membrane stabilization assays, while anti-rheumatic activity was evaluated by protein denaturation and anti-collagenase inhibition methods. The results demonstrated concentration-dependent activity for both the extract and niosomal formulation. However, the niosomal formulation exhibited significantly higher inhibition and membrane stabilization compared to the crude extract in all assays. The enhanced activity may be attributed to improved stability, bioavailability, and sustained release of phytoconstituents. The findings support the traditional use of *Neolamarckia cadamba* and suggest that niosomal drug delivery systems can be a promising approach for the treatment of inflammatory and rheumatic disorders.

Keywords: *Neolamarckia cadamba*, Niosomes, Anti-inflammatory, Anti-rheumatic, Herbal drug delivery.

Introduction

Herbal Drug Technology is a specialized branch of pharmaceutical science that focuses on the development, standardization, and formulation of plant-based medicinal products. By integrating traditional herbal knowledge with modern scientific methodologies, it ensures the quality, safety, and therapeutic efficacy of herbal drugs. Furthermore, the application of advanced drug delivery systems, such as nanoparticles and niosomes, has significantly improved the stability, bioavailability, and clinical effectiveness of herbal formulations.[1] Niosomal gels are advanced vesicular drug delivery systems that combine the advantages of niosomes with topical gel formulations to enhance the therapeutic performance of herbal drugs. Niosomes are microscopic vesicles composed of non-ionic surfactants and cholesterol, capable of encapsulating both hydrophilic and lipophilic phytoconstituents. This encapsulation protects the active constituents from degradation while improving their stability, bioavailability, and skin permeation. Incorporation of niosomes into a gel matrix enables controlled drug release, prolonged therapeutic action, enhanced retention at the site of application, and improved patient compliance. Niosomal gels are particularly suitable for the topical delivery of herbal agents possessing anti-inflammatory, antioxidant, antimicrobial, wound-healing, and antirheumatic activities, as they facilitate enhanced skin penetration while minimizing systemic adverse effects [2]. A drug

delivery system is a pharmaceutical approach designed to transport therapeutic agents to their intended site of action at an appropriate rate and for a desired duration in order to achieve optimal therapeutic outcomes. Although conventional dosage forms, such as tablets, capsules, and injections, are widely employed in clinical practice, they often fail to maintain optimal drug concentrations at the target site. Consequently, novel drug delivery systems have been developed to improve drug bioavailability, stability, safety, and patient compliance, thereby enhancing overall therapeutic efficacy.[3]

Vesicular drug delivery systems are microscopic carrier systems designed to improve the stability, bioavailability, and targeted delivery of therapeutic agents. The major types include liposomes, niosomes, transfersomes, and ethosomes, each possessing distinct advantages for the encapsulation and delivery of both hydrophilic and lipophilic drugs. These vesicular systems enhance drug penetration, provide controlled and sustained drug release, reduce systemic side effects, and improve therapeutic efficacy, making them valuable tools in modern pharmaceutical formulations.[1]. Niosomes are vesicular drug delivery systems composed of non-ionic surfactants that are capable of encapsulating both hydrophilic and lipophilic therapeutic agents. Compared with conventional drug carriers, niosomes exhibit improved physicochemical stability, biocompatibility, and drug protection. They enhance drug bioavailability, facilitate controlled drug release, and enable targeted drug delivery, making them suitable for various routes of administration, including topical, oral, ocular, and transdermal delivery.[4,5].

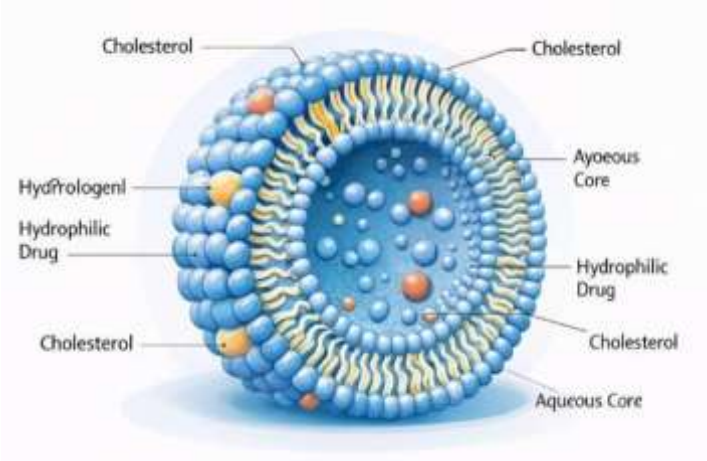

Fig. No. 01: Structure of Niosome

Neolamarckia cadamba (Roxb.), commonly known as Kadam, is a medicinal tree belonging to the family Rubiaceae and is widely distributed throughout the tropical regions of Asia. Various parts of the plant, including the bark, leaves, roots, flowers, and fruits, have been traditionally used for the treatment of fever, inflammation, wounds, skin disorders, and gastrointestinal ailments. The plant contains a wide range of bioactive phytoconstituents and has demonstrated significant antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective activities. Owing to its extensive ethnomedicinal applications and promising pharmacological potential, *N. cadamba* has emerged as an important source for herbal drug development and therapeutic research.[6]

Inflammation is a complex protective biological response initiated by tissue injury, infection, or other harmful stimuli and is mediated through the

release of various inflammatory mediators. Although inflammation plays a vital role in host defense and tissue repair, persistent or uncontrolled inflammatory responses contribute to the pathogenesis of numerous chronic diseases. Anti-inflammatory agents exert their therapeutic effects by modulating inflammatory pathways and suppressing the production or activity of inflammatory mediators. Herbal anti-inflammatory compounds have gained considerable scientific interest because of their therapeutic efficacy, multiple mechanisms of action, and comparatively lower incidence of adverse effects than conventional synthetic drugs.[7,8]

Rheumatic disorders comprise a group of diseases characterized by inflammation, pain, stiffness, and impaired mobility affecting joints, muscles, and connective tissues. Rheumatoid arthritis, one of the most prevalent autoimmune rheumatic diseases, is associated with chronic synovial inflammation, progressive joint destruction, and functional disability. Antirheumatic agents are employed to suppress inflammation, preserve joint integrity, and improve functional outcomes. Herbal antirheumatic agents have attracted increasing research interest due to their immunomodulatory properties, efficacy in the management of chronic inflammatory conditions, and favorable safety profile.[9]

The present study was undertaken to evaluate the anti-inflammatory and antirheumatic potential of *Neolamarckia cadamba* leaf extract. The leaves were collected, authenticated, and extracted using the Soxhlet extraction method, followed by preliminary phytochemical screening. The obtained extract was subsequently formulated into a niosomal drug delivery system to enhance its stability, bioavailability, and therapeutic efficacy. The developed formulation was characterized using various physicochemical parameters and compared with the plain extract to assess improvements in its pharmacological activity. Furthermore, the study aimed to provide scientific validation for the traditional medicinal use of *Neolamarckia cadamba* and to develop a safe, effective, and novel herbal formulation for the management of inflammatory disorders.

Plant Authentication

The plant material used in the present investigation was authenticated by the Department of Botany, Government College Khimlasa, Sagar (M.P.), India. The collected specimen was identified as Neolamarckia cadamba (Roxb.) Bosser belonging to the family Rubiaceae. The authenticated plant parts included leaves and bark, and the authentication was performed through detailed macroscopic, organoleptic, and microscopic examinations with reference to standard botanical literature. An authentication certificate (Ref. No. AC/054/26) dated 13 January 2026 was issued by Dr Jagrati Tripathi, Assistant Professor, Department of Botany, Government College Khimlasa, Sagar, confirming the botanical identity of the plant material. The authenticated specimen was subsequently used for all phytochemical, pharmacological, and formulation studies to ensure the accuracy, reproducibility, and reliability of the research findings.

Materials and Methods

Materials

Fresh *Neolamarckia cadamba* leaves were used as the plant material for extraction and formulation development. Analytical-grade solvents, surfactants, cholesterol, and reagents were used throughout the study. Diclofenac sodium was employed as the standard anti-inflammatory and antirheumatic drug for comparative evaluation.

Fresh leaves of Neolamarckia cadamba were collected from the local region and used as the plant material. Analytical reagent (AR) grade chemicals, including ethanol/methanol, chloroform, ferric chloride, sodium hydroxide, cholesterol, phosphate buffer salts, Mayer's reagent, and Dragendorff's reagent, were procured from Merck, Himedia, and Sigma-Aldrich. Distilled water was obtained from the laboratory supply. Diclofenac sodium (pharmaceutical grade, Cipla Ltd.) was used as the standard drug for pharmacological studies.

Fig. No.02: Leaves

The materials used included 1% w/v bovine serum albumin (BSA), phosphate buffer (pH 6.4), phosphate-buffered saline (pH 7.4), hypotonic saline (0.25% NaCl), distilled water, fresh human blood, Alsever's solution, collagenase enzyme (*Clostridium histolyticum*), tricine buffer (pH 7.5), gelatin substrate (1 mg/mL), *Neolamarckia cadamba* leaf extract or *Neolamarckia cadamba* extract, the extract-loaded niosomal gel formulation, and diclofenac sodium gel as the standard topical anti-inflammatory formulation or standard reference, depending on the assay performed.

Instruments

The study employed standard laboratory instruments required for plant extraction, vesicular formulation, and in vitro evaluation, including Soxhlet apparatus, rotary evaporator, UV–Visible spectrophotometer, centrifuge, pH meter, and optical microscope. These instruments were used for extraction, solvent removal, vesicle observation, and assay analysis.

Various laboratory instruments were used throughout the study, including a Soxhlet apparatus for extraction, a heating mantle for continuous heating, and a rotary evaporator for solvent evaporation. A hot air oven was employed for drying the samples, while an analytical balance was used for accurate weighing. Absorbance measurements were carried out using a UV–Visible spectrophotometer. A magnetic stirrer facilitated mixing during formulation preparation, and a centrifuge was used for separation processes. The pH of formulations was determined using a pH meter, and vesicle morphology was examined with an optical microscope.

Extraction of Plant Material

Fresh and healthy leaves of *Neolamarckia cadamba* were collected, washed thoroughly, and shade-dried at room temperature for 10–15 days in order to preserve thermolabile phytoconstituents. The dried leaves were then pulverized into coarse powder and subjected to Soxhlet extraction using ethanol or methanol as the extraction solvent. Solvent selection was based on its ability to extract polar and semi-polar phytoconstituents such as flavonoids, phenolics,

and alkaloids. After extraction, the solvent was removed under reduced pressure using a rotary evaporator to obtain a concentrated extract suitable for formulation and pharmacological evaluation. [10,11].

Table No. 01 Parameter used in Plant Extraction

Parameter	Common value
Plant material	50–100 g
Solvent	500–1000 mL ethanol
Plant: solvent ratio	1:10 (w/v)
Ethanol concentration	95% (or 70% depending on study)
Extraction time	6–8 hours
Temperature	Near ethanol's boiling point (~78 °C)

A weighed quantity (100 g) of the shade-dried and coarsely powdered leaves of *Neolamarckia cadamba* was packed into a cellulose thimble and placed in the Soxhlet apparatus. Ethanol (95%, 1000 mL) was used as the extraction solvent because of its ability to extract a broad spectrum of polar and semi-polar phytoconstituents. The extraction was carried out for 6–8 hours under continuous reflux until the solvent in the siphon tube became colorless, indicating exhaustive extraction. The ethanolic extract was then concentrated under reduced pressure using a rotary evaporator and further dried to obtain a semisolid mass. The dried extract was weighed to calculate the percentage yield and stored in airtight containers at 4°C until further use.

Prepartion of Neosomal Gel

Step 1: Preparation of Niosomes (Thin Film Hydration Method) – The required quantities of Span 60 (200 mg) and cholesterol (100 mg) were accurately weighed and dissolved in 30 mL of chloroform:methanol (2:1, v/v) in a round-bottom flask The organic solvent was evaporated using a rotary evaporator under reduced pressure to form a thin, uniform lipid film on the inner wall of the flask. The dried film was hydrated with phosphate buffer (pH 7.4) containing Neolamarckia cadamba extract and gently rotated to form multilamellar niosomal vesicles. The dispersion was further sonicated to reduce vesicle size and obtain a uniform niosomal suspension.

Step 2: Preparation of Gel Base - Carbopol 934 was dispersed in distilled water with continuous stirring and allowed to hydrate completely for 24 hours. The dispersion was neutralized using triethanolamine to obtain a clear and smooth gel base of suitable consistency.

Step 3: Incorporation of Niosomes into Gel - The prepared niosomal suspension was slowly incorporated into the Carbopol gel base with gentle stirring to obtain a homogeneous extract-loaded niosomal gel. The final formulation was allowed to equilibrate and stored in a well-closed container for further evaluation.

Table No. 02: Composition of *Neolamarckia cadamba* Extract-Loaded Niosomal Gel Formulations.

Formulation	Span 60 (mg)	Cholesterol (mg)	Extract (mg)	Span 60:Cholesterol Ratio
F1	100	25	100	4:1
F2	100	50	100	2:1
F3	100	75	100	1.33:1
F4	150	50	100	3:1
F5	200	50	100	4:1

Methods

The ethanolic extract of *Neolamarckia cadamba* leaves was prepared by Soxhlet extraction. The concentrated extract was dried under reduced pressure and weighed. The percentage yield of the extract was 12.5% (w/w), calculated based on the initial weight of the dried leaf powder.

In Vitro Anti-Inflammatory Activity Protein Denaturation Inhibition Assay.

Test samples of Neolamarckia cadamba extract and extract-loaded niosomal gel were prepared at concentrations of 50, 100, 200, and 400 µg/mL. The standard group consisted of diclofenac sodium gel, suitably diluted to an equivalent concentration for comparison.

HRBC Membrane Stabilization Assay

HRBC suspension was mixed with hypotonic saline and test samples (50–400 µg/mL).After incubation at 37 °C for 30 min, samples were centrifuged and absorbance was measured at 560 nm.

In Vitro Anti-Rheumatic Activity

Protein Denaturation Assay (Antirheumatic Model)

Same procedure as anti-inflammatory assay with concentrations 100–500 µg/mL.Absorbance was recorded at 660 nm.

Anti-Collagenase Inhibition Assay

Collagenase was incubated with test samples (50–400 µg/mL), followed by gelatin substrate.Reaction was stopped with EDTA and absorbance was measured at 340 nm.

Result

In Vitro Anti-Inflammatory Activity Protein Denaturation Inhibition Assay

The anti-inflammatory potential of *Neolamarckia cadamba* extract and its niosomal formulation was evaluated using the heat-induced bovine serum albumin protein denaturation model. This assay is commonly used to assess anti-inflammatory potential because denatured proteins may trigger inflammatory and autoimmune responses. The assay involved incubation of BSA with the test sample followed by heat-induced denaturation and spectrophotometric analysis at 660 nm. The inhibitory activity of the extract and formulation was compared with diclofenac sodium as the standard.[12,13]

Table No. 03 Effect of *Neolamarckia cadamba* on Protein Denaturation

Concentration (µg/mL)	%Inhibition (Extract)	%Inhibition (Niosomes)	%Inhibition (Diclofenac)
50	28.4	35.6	42.3
100	36.9	45.8	55.6
200	48.2	58.7	69.8
400	61.5	72.9	83.4

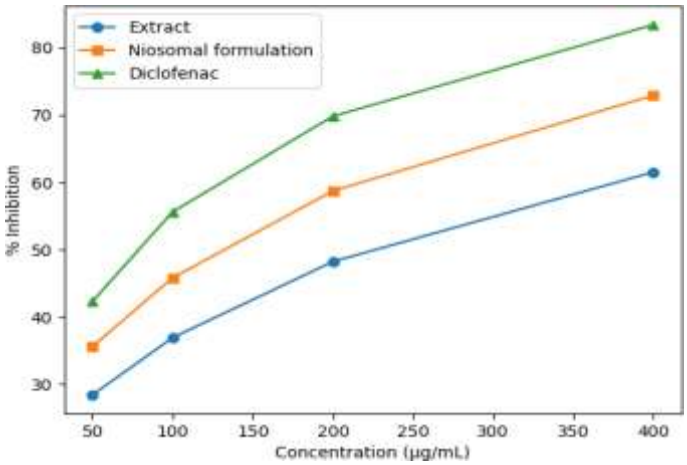

Fig. No. 03 Effect of *Neolamarckia cadamba* extract and niosomal formulation on inhibition of protein denaturation.

The results demonstrated concentration-dependent inhibition of protein denaturation by both the crude extract and the niosomal formulation. However, the niosomal formulation showed consistently higher inhibition than the plain extract at all tested concentrations, indicating improved anti-inflammatory performance. This enhanced activity may be attributed to improved solubility, better dispersion, and sustained release of phytoconstituents encapsulated within the vesicular system. The anti-inflammatory effect may also be linked to the flavonoids, phenolic compounds, and alkaloids present in *Neolamarckia cadamba*, which are known to stabilize proteins and reduce oxidative damage.

HRBC Membrane Stabilization Assay

The HRBC membrane stabilization assay was employed as a secondary in vitro anti-inflammatory model based on the structural similarity between erythrocyte membranes and lysosomal membranes. Stabilization of the HRBC membrane suggests inhibition of inflammatory mediator release and membrane lysis during inflammatory stress. The method involved incubation of HRBC suspension with hypotonic saline and the test samples, followed by centrifugation and absorbance measurement at 560 nm. [14,15]

Table No. 04 HRBC Membrane Stabilization Activity

Concentration (µg/mL)	**% Protection (Extract)**	**% Protection (Niosomes)**	**%Protection Diclofenac**
50	31.2	39.8	46.7
100	42.5	52.3	60.8
200	55.7	66.4	75.2
400	67.8	78.9	86.5

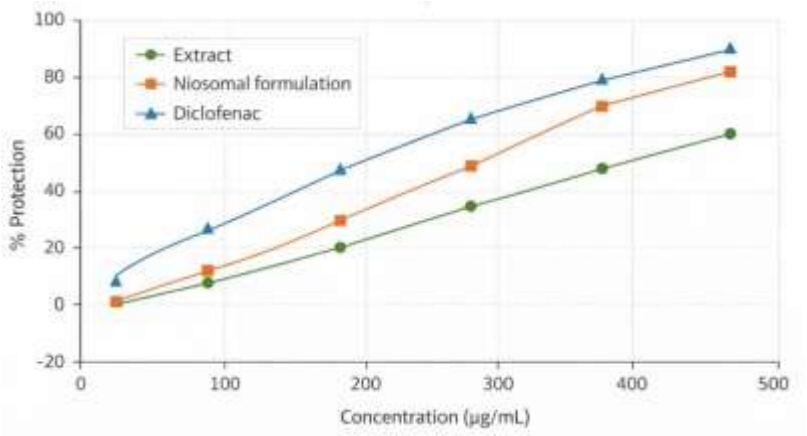

Fig. No. 04 HRBC membrane stabilization of *Neolamarckia Cadamba* extract niosomal

The membrane stabilization assay further supported the anti-inflammatory potential of *Neolamarckia cadamba*. According to your thesis discussion, the niosomal formulation exhibited better membrane-protective action than the crude extract, likely due to improved interaction of phytoconstituents with membrane lipids and proteins. This observation is consistent with the known membrane-stabilizing properties of flavonoids and tannins.

In Vitro Anti-Rheumatic Activity

Protein Denaturation Assay (Antirheumatic Model)

The anti-rheumatic activity of the extract and niosomal formulation was assessed using protein denaturation as a model of rheumatoid arthritis. Inhibition of protein denaturation is considered relevant in rheumatic disease because denatured proteins may act as auto-antigens and trigger immune-mediated joint inflammation. The thesis used the same assay principle as the anti-inflammatory model but with a higher concentration range of 100–500 µg/mL. [16]

Table No. 05 Antirheumatic Activity by Protein Denaturation Method

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Niosomes)	% Inhibition (Diclofenac)
100	34.6	42.8	50.5
200	46.9	56.7	65.9
300	58.4	68.3	78.6
500	69.7	81.2	90.4

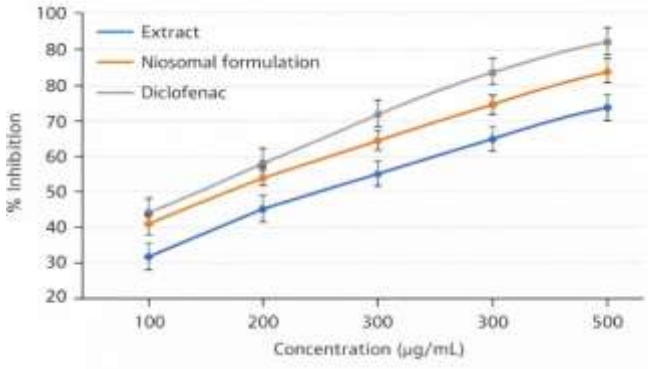

Fig. No. 05 Effect of *Neolamarckia cadamba* extract and niosomal formulation on Protein Denaturation Assay (Antirheumatic Model)

The results revealed that both the extract and the niosomal formulation possessed significant anti-rheumatic potential, with the niosomal system showing superior inhibition at all tested concentrations. This suggests that vesicular encapsulation enhanced stabilization and functional availability of the plant-derived phytoconstituents. Since protein denaturation is linked with autoimmune inflammatory pathways, the findings support the use of *Neolamarckia cadamba* in rheumatic conditions.

Anti-Collagenase Inhibition Assay

The anti-collagenase inhibition assay was used to evaluate cartilage-protective and anti-rheumatic potential. Collagenase is a key enzyme involved in the degradation of cartilage collagen during rheumatoid arthritis, and inhibition of this enzyme is considered a relevant marker of anti-rheumatic activity. In your thesis, collagenase from *Clostridium histolyticum*, tricine buffer, gelatin substrate, and diclofenac sodium were used for the assay. The absorbance was measured at 340 nm after incubation and termination of the EDTA-mediated reaction.[17]

Table No. 06 Anti-Collagenase Activity of *Neolamarckia cadamba*

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Niosomes)	% Inhibition (Diclofenac)
50	29.5	36.7	44.2
100	41.8	52.6	60.9
200	55.3	66.9	75.8
400	67.4	79.2	88.3

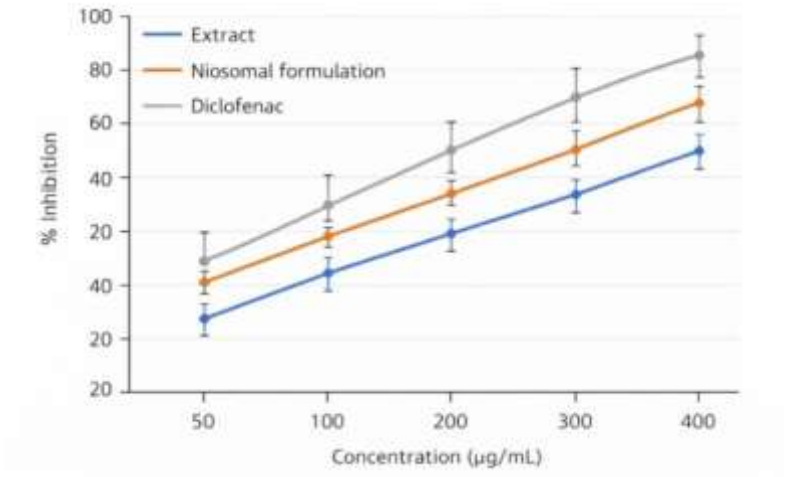

Fig. No. 06 Effect of *Neolamarckia cadamba* extract and niosomal formulation on Anti-CollagenaseInhibition Assay

Discussion

The present study demonstrated that *Neolamarckia cadamba* leaf extract possesses significant anti-inflammatory and anti-rheumatic activities, which were markedly enhanced after formulation into niosomes. In the protein denaturation inhibition assay, both the extract and niosomal formulation showed concentration-dependent activity; however, the niosomal formulation exhibited higher inhibition at all tested concentrations, indicating improved anti-inflammatory efficacy. Similar results were observed in the HRBC membrane stabilization assay, where the niosomal formulation provided greater protection against membrane lysis than the crude extract. This suggests enhanced stabilization of lysosomal membranes and

reduced release of inflammatory mediators. The anti-rheumatic studies further supported these findings, as the niosomal formulation showed superior inhibition of protein denaturation and collagenase activity compared to the plain extract. Since protein denaturation and collagenase-mediated cartilage degradation play important roles in the pathogenesis of rheumatoid arthritis, inhibition of these processes indicates promising anti-rheumatic potential. The enhanced activity of the niosomal formulation may be attributed to improved stability, bioavailability, sustained release, and better penetration of phytoconstituents such as flavonoids, phenolics, and alkaloids. Overall, the results suggest that niosomal encapsulation significantly improves the therapeutic effectiveness of Neolamarckia cadamba, supporting its potential use as a novel herbal formulation for the management of inflammatory and rheumatic disorders.

Conclusion

The present study demonstrated that *Neolamarckia cadamba* leaf extract possesses significant anti-inflammatory and antirheumatic activities. Formulation of the extract into a niosomal vesicular system enhanced its therapeutic performance compared to the plain extract, as evidenced by improved inhibition of protein denaturation, better membrane stabilization, and higher anti-collagenase activity. The enhanced efficacy may be attributed to improved stability, bioavailability, and sustained release of phytoconstituents from the niosomal formulation. These findings provide scientific support for the traditional use of Neolamarckia cadamba in inflammatory and rheumatic disorders and suggest that niosomal delivery systems can serve as a promising approach for developing effective herbal therapeutics.

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