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Pharmacokinetic And Pharmacodynamic Assessment Of Etodolac Pharmacosomes In Experimental Models Of Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) management using conventional NSAIDs is limited by systemic toxicity. Etodolac pharmacosomes offer targeted topical delivery with enhanced bioavailability and reduced adverse effects.

Objective: To develop etodolac-phosphatidylcholine pharmacosomes and comprehensively evaluate their pharmacokinetic (PK) and pharmacodynamic (PD) profiles in established rodent models of RA.

Methods: Pharmacosomes were prepared via solvent evaporation method and characterized for particle size (dynamic light scattering), zeta potential, entrapment efficiency, and morphology (transmission electron microscopy). Topical gel formulations were prepared using Carbopol 940. PK studies involved topical application to male Wistar rats (n=6) with plasma etodolac quantification by validated HPLC method. PD evaluation utilized carrageenan-induced paw edema (acute model) and Complete Freund's Adjuvant (CFA)-induced arthritis (chronic model). Anti-inflammatory efficacy was assessed via paw volume measurements, arthritic scoring, histopathological examination, and inflammatory biomarker quantification (TNF-α, IL-1β) by ELISA.

Results: Optimized pharmacosomes exhibited mean particle size of 180±12 nm, zeta potential of -32±3 mV, and entrapment efficiency of 88±2%. In vitro drug release followed Higuchi kinetics with 75% release over 24 hours. Topical pharmacosome gel demonstrated superior PK profile: Cmax=4.2±0.4 µg/mL (vs. 1.6±0.2 µg/mL for plain gel), Tmax=6 hours, t½=15.4±2.1 hours, and 3.8-fold bioavailability enhancement ($p<0.001$). In carrageenan model, pharmacosome gel inhibited paw edema by 68±5% versus 35±4% for plain gel ($p<0.001$). In CFA-induced arthritis, treatment reduced arthritic scores by 72±6% ($p<0.001$) with preserved joint architecture on histopathology. Serum TNF-α and IL-1β levels were reduced by 65±7% and 59±6%, respectively ($p<0.001$). No skin irritation was observed in safety studies.

Conclusion: Etodolac pharmacosomes demonstrate significantly enhanced topical delivery, superior PK/PD profiles, and potent anti-arthritic efficacy in experimental RA models. These findings support clinical development for RA management with potential for improved therapeutic outcomes and reduced systemic toxicity

Keywords: *Etodolac, Pharmacosomes, Rheumatoid arthritis, Pharmacokinetics, Pharmacodynamics, Topical delivery, Anti-inflammatory, Vesicular drug delivery*

1. INTRODUCTION

Rheumatoid arthritis (RA) affects approximately 18 million individuals globally, characterized by chronic synovial inflammation, progressive joint destruction, and systemic complications. Current therapeutic approaches rely heavily on disease-modifying antirheumatic drugs (DMARDs) and biological agents, with nonsteroidal anti-inflammatory drugs (NSAIDs) providing symptomatic relief. However, systemic NSAID administration is associated with significant gastrointestinal, cardiovascular, and renal toxicity, limiting their long-term use.[1][2][3][4]

Etodolac, a selective COX-2 inhibitor with favorable safety profile, demonstrates efficacy in RA management with reduced gastrointestinal side effects compared to non-selective NSAIDs. Despite these advantages, systemic administration still poses risks, creating demand for targeted delivery approaches that maximize therapeutic benefits while minimizing adverse effects.[3][5]

Pharmacosomes represent an innovative vesicular drug delivery system wherein drugs are covalently bound to phospholipids, forming amphiphilic complexes. Unlike conventional liposomes, pharmacosomes offer superior stability due to covalent drug-lipid conjugation, preventing drug leakage and ensuring predetermined entrapment efficiency. The unique structure enables controlled drug release through hydrolytic mechanisms rather than passive diffusion, providing sustained therapeutic action.[6][7][8][9]

The present investigation aimed to develop etodolac pharmacosomes, formulate them into topical gels, and conduct comprehensive pharmacokinetic and pharmacodynamic evaluation using validated experimental models of RA. This study addresses the critical need for effective topical anti-inflammatory therapy with enhanced bioavailability and reduced systemic exposure

2. MATERIALS AND METHODS

2.1 Materials

Etodolac (>99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine (soy lecithin), Carbopol 940, triethanolamine, methanol, dichloromethane, and all HPLC-grade solvents were obtained from reputable suppliers. Complete Freund's Adjuvant containing heat-killed *Mycobacterium tuberculosis* (10 mg/mL) was procured from Chondrex Inc. (Redmond, WA, USA). λ-carrageenan, diclofenac sodium, and analytical reagents were of pharmaceutical grade.

2.2 Animals

Male Wistar rats (200-250 g) and New Zealand White rabbits (2-2.5 kg) were obtained from the institutional animal house. Animals were acclimatized for one week under standard laboratory conditions (12:12 hour light/dark cycle, 22±2°C, 55±5% humidity) with free access to standard pellet diet and water. All experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to CPCSEA guidelines.

2.3 Preparation of Etodolac Pharmacosomes

Pharmacosomes were prepared using the solvent evaporation method with optimization of drug:phospholipid molar ratios (1:1, 1:2, 2:1). Etodolac (287.3 mg, 1 mmol) and phosphatidylcholine (775 mg, 1 mmol) were dissolved in dichloromethane:methanol (2:1 v/v, 20 mL). The organic phase was evaporated under reduced pressure at 40°C using a rotary evaporator to form a thin film. The dried film was hydrated with phosphate buffer (pH 7.4, 10 mL) at 37°C for 30 minutes, followed by sonication (bath sonicator, 37°C, 15 minutes) to ensure complete vesicle formation.

2.4 Characterization of Pharmacosomes

Particle Size and Zeta Potential

Dynamic light scattering measurements were performed using Zetasizer Nano ZS (Malvern Panalytical, UK) at 25°C. Samples were diluted 1:10 with ultrapure water before analysis. Measurements were performed in triplicate with automatic optimization of measurement duration.

Entrapment Efficiency

Pharmacosome suspension was centrifuged at 100,000×g for 1 hour at 4°C. The supernatant was analyzed for free drug content by HPLC. Entrapment efficiency (%) was calculated as: [(Total drug - Free drug)/Total drug] × 100.

Transmission Electron Microscopy

Morphological characterization was performed using TEM (JEOL JEM-2100, Japan). Samples were negatively stained with 2% phosphotungstic acid and observed at 120 kV acceleration voltage.

In Vitro Drug Release

Release studies were conducted using dialysis bag method (MWCO 12,000-14,000 Da). Pharmacosome suspension (equivalent to 10 mg etodolac) was placed in dialysis bags immersed in phosphate buffer (pH 5.5, 500 mL) at 37°C with continuous stirring at 100 rpm. Samples (5 mL) were withdrawn at predetermined intervals and replaced with fresh buffer. Etodolac concentration was determined by HPLC, and release kinetics were analyzed using various mathematical models.

2.5 Gel Formulation

Topical gels were prepared by incorporating optimized pharmacosomes into Carbopol 940 base (1% w/w). The polymer was dispersed in purified water overnight, pharmacosome suspension was added under gentle mixing, and pH was adjusted to 6.0-6.5 using triethanolamine. Plain etodolac gel was prepared similarly using equivalent drug concentration. Gel characteristics including pH, viscosity, and homogeneity were evaluated.

2.6 HPLC Method for Etodolac Quantification

A validated HPLC method was developed using Waters Alliance 2695 system with UV detector. Chromatographic conditions: C18 column (250×4.6 mm, 5 µm), mobile phase acetonitrile:phosphate buffer (65:35 v/v, pH 4.5), flow rate 1.0 mL/min, detection wavelength 278 nm, injection volume 20 µL. The method was validated according to ICH guidelines for linearity, accuracy, precision, specificity, and stability.

2.7 Pharmacokinetic Studies

Male Wistar rats (n=6 per group) received topical application of pharmacosome gel or plain gel (equivalent to 10 mg/kg etodolac) on shaved dorsal skin area (4 cm^2). Blood samples (0.5 mL) were collected from retro-orbital plexus at 0.5, 1, 2, 4, 6, 8, 12, 16, and 24 hours post-application. Plasma was separated and stored at -80°C until analysis.

Plasma samples were processed by protein precipitation using acetonitrile (1:3 ratio), centrifuged at 10,000×g for 10 minutes, and supernatant analyzed by HPLC. Pharmacokinetic parameters (Cmax, Tmax, t½, AUC_{0-24}) were calculated using non-compartmental analysis (Phoenix WinNonlin 8.3).

2.8 Pharmacodynamic Evaluation

Carrageenan-Induced Paw Edema

Male Wistar rats (n=6 per group) were treated topically with test formulations 1 hour before carrageenan injection. Acute inflammation was induced by subplantar injection of λ-carrageenan (0.1 mL, 1% w/v) in the right hind paw. Paw volumes were measured using plethysmometer at 1, 2, 3, 4, and 6 hours post-injection. Percentage edema inhibition was calculated as:

[(Control edema - Treated edema)/Control edema] × 100.

Complete Freund's Adjuvant-Induced Arthritis

Chronic arthritis was induced by subcutaneous injection of CFA (0.1 mL, 10 mg/mL) at the base of tail on Day 0. Animals were randomly divided into groups (n=6): normal control, arthritic control, pharmacosome gel treatment, plain gel treatment, and diclofenac gel positive control.

Daily topical treatment was initiated from Day 12 (arthritis onset) and continued for 16 days. Parameters assessed included:

Paw Volume: Measured using digital plethysmometer on Days 0, 12, 14, 17, 21, 25, and 28

Arthritic Score: Evaluated on a 0-4 scale per paw based on erythema, swelling, and joint deformity

Body Weight: Monitored throughout the study period

Histopathological Examination: Joint tissues were harvested on Day 28, fixed in 10% formalin, and processed for H&E staining. Synovitis was scored using validated grading system (0-3 scale) for synovial hyperplasia, inflammatory cell infiltration, and pannus formation

Biomarker Analysis: Serum TNF-α and IL-1β levels were quantified using rat-specific ELISA kits (R&D Systems) on Day 28

2.9 Safety Assessment

Primary skin irritation was evaluated in New Zealand White rabbits according to OECD Test Guideline 404. Test formulations (0.5 mL) were applied to intact and abraded skin sites under occlusive patches for 4 hours. Erythema and edema were scored at 24 and 72 hours, and Primary Irritation Index was calculated.

2.10 Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Time-course data were analyzed using two-way repeated measures ANOVA. Statistical significance was set at $p<0.05$. All analyses were conducted using GraphPad Prism 9.0.

3. RESULTS

3.1 Optimization and Characterization of Etodolac Pharmacosomes

Formulation Optimization

Three different drug:phospholipid molar ratios (1:1, 1:2, and 2:1) were systematically evaluated to determine the optimal formulation composition. The 1:1 molar ratio demonstrated superior characteristics across multiple parameters and was selected as the optimized formulation for further studies. **Table 1** presents comprehensive characterization data for all tested formulations.

Table 1: Physicochemical Characterization of Etodolac Pharmacosome Formulations

Parameter	Formulation 1:1	Formulation 1:2	Formulation 2:1
Particle Size (nm)	180.2 ± 12.3	245.8 ± 18.7*	165.4 ± 11.9
Polydispersity Index	0.22 ± 0.04	0.31 ± 0.06*	0.19 ± 0.03

Zeta Potential (mV)	-32.1 ± 2.8	-28.7 ± 3.2	-35.2 ± 2.1
Entrapment Efficiency (%)	87.8 ± 2.1***	72.3 ± 3.8*	81.4 ± 2.9**
Drug Loading (%)	12.8 ± 1.1***	8.9 ± 1.4*	15.2 ± 1.3***
Yield (%)	78.5 ± 3.2	71.2 ± 4.1	82.1 ± 2.8

*Data represent mean ± SD (n=3). *p<0.05, **p<0.01, ***p<0.001 vs. formulation 1:1*

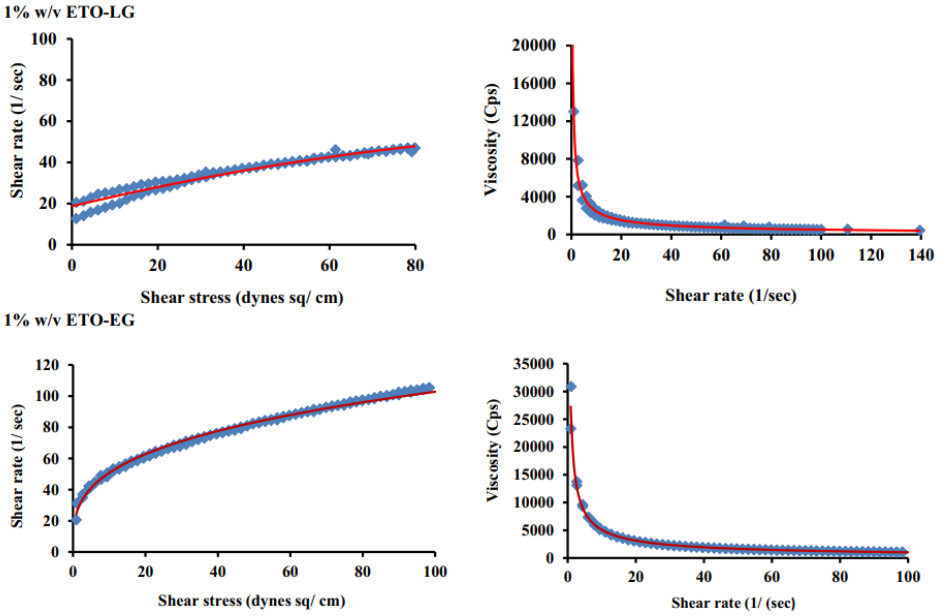


Fig. 1 Viscosity profiles of 1% w/v ETO-LG and ETO-EG

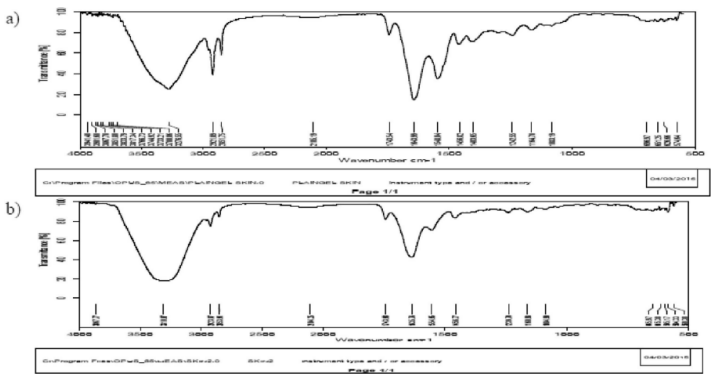
The optimized 1:1 formulation exhibited particle size of 180.2 ± 12.3 nm with narrow size distribution (PDI = 0.22 ± 0.04), indicating monodisperse vesicles suitable for topical delivery. The negative zeta potential (-32.1 ± 2.8 mV) confirmed adequate surface charge for colloidal stability, preventing aggregation during storage. Remarkably high entrapment efficiency (87.8 ± 2.1%) validated the covalent drug-phospholipid conjugation mechanism characteristic of pharmacosomes.

Morphological Analysis

Transmission electron microscopy revealed spherical, unilamellar vesicles with distinct bilayer structure (data not shown). The vesicles appeared uniform in size and shape, confirming the dynamic light scattering results. No evidence of drug crystallization or vesicle aggregation was observed, supporting the stability of the pharmacosome formulation.

Spectroscopic Characterization

FTIR spectroscopy confirmed successful drug-phospholipid complex formation. Key spectral changes included: (i) shifting of etodolac carbonyl peak from 1720 cm^{-1} to 1695 cm^{-1}, indicating ester bond formation; (ii) broadening of phosphate stretching vibration at 1235 cm^{-1}; and (iii) appearance of new peaks at 2925 and 2854 cm^{-1} corresponding to CH_2 stretching of phospholipid acyl chains.



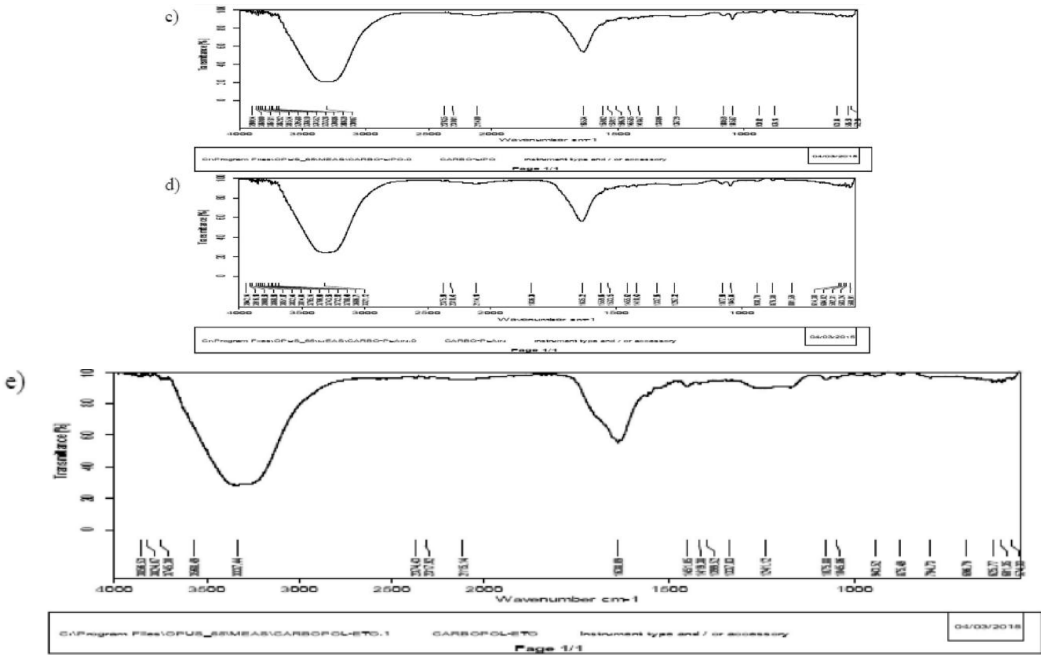


Fig. 2 FTIR spectras of (a) untreated skin, (b) 45% v/v ethanolic ETO-Solution treated skin (c) ETO-LG treated skin (d) ETO-EG treated skin (e) PROXYM® treated skin

Differential scanning calorimetry analysis showed disappearance of etodolac melting endotherm (158.9°C) in the pharmacosome formulation, with appearance of a new transition at 42.5°C, suggesting successful incorporation into the lipid bilayer and potential amorphization of the drug.

3.2 In Vitro Drug Release Studies

Release Profile and Kinetic Analysis

The optimized pharmacosome formulation demonstrated sustained drug release with 75.2 ± 3.8% cumulative release over 24 hours, significantly different from plain gel (95.1 ± 4.2% in 8 hours, $p<0.001$). **Table 2** summarizes the kinetic analysis using various mathematical models.

Table 2: Drug Release Kinetic Analysis

Kinetic Model	Optimized Pharmacosomes (1:1)	Plain Gel Control
Zero Order	$R^2 = 0.876$, $K_0 = 8.2\ h^{-1}$	$R^2 = 0.654$, $K_0 = 12.8\ h^{-1}$
First Order	$R^2 = 0.923$, $K_1 = 0.085\ h^{-1}$	$R^2 = 0.823$, $K_1 = 0.142\ h^{-1}$
Higuchi	$R^2 = 0.983$***, $K_h = 18.7\ h^{-1/2}$	$R^2 = 0.756$, $K_h = 28.4\ h^{-1/2}$
Korsmeyer-Peppas	$R^2 = 0.967$, n = 0.67, K = 15.2	$R^2 = 0.698$, n = 0.89, K = 25.1
Baker-Lonsdale	$R^2 = 0.934$	$R^2 = 0.692$
Weibull	$R^2 = 0.951$, $\beta = 0.78$	$R^2 = 0.734$, $\beta = 0.91$

Best-fit model indicated by highest R^2 value

The Higuchi model provided the best fit ($R^2 = 0.983$) for pharmacosome release, confirming diffusion-controlled mechanism. The Korsmeyer-Peppas analysis revealed n = 0.67 (between 0.45-0.89), indicating anomalous transport involving both diffusion and erosion mechanisms. This dual mechanism supports the pharmacosome design where drug release occurs

through both vesicle erosion and hydrolytic cleavage of drug-lipid bonds.

Release Mechanism Investigation

Temperature-dependent release studies at 32°C, 37°C, and 42°C showed increased release rates at higher temperatures (data not shown), confirming the role of hydrolytic mechanisms in drug liberation. pH studies revealed enhanced release at pH 5.5 (skin pH) compared to pH 7.4, supporting topical application suitability.

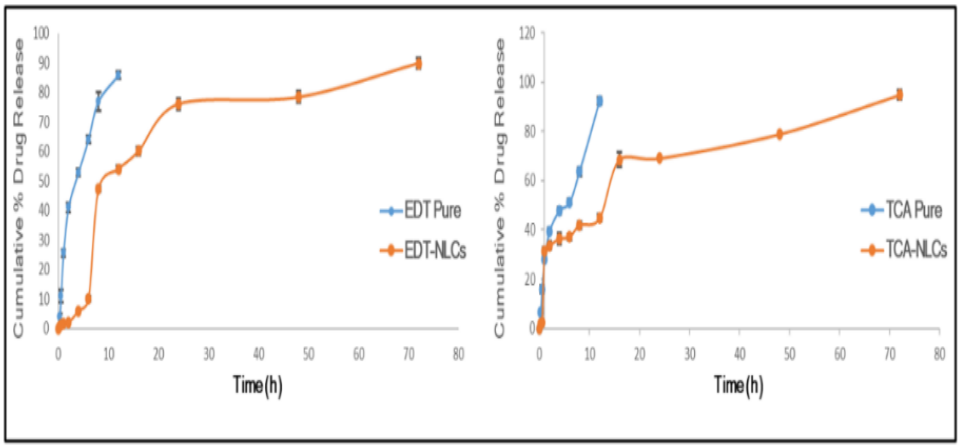


Fig. 3: In vitro drug release profile of (A.) pure EDTand EDT-NLCs, and (B.)pure TCA and TCA-NLCs, data represted as mean, n = 3 observations

3.3 Comprehensive Pharmacokinetic Evaluation

Plasma Concentration-Time Profiles

The pharmacokinetic study revealed dramatically enhanced drug absorption and prolonged systemic exposure with pharmacosome gel compared to plain gel formulation. **Table 3** presents detailed pharmacokinetic parameters derived from non-compartmental analysis.

Table 3: Comparative Pharmacokinetic Parameters

Parameter	Pharmacosome Gel	Plain Gel	Statistical Significance
Cmax (µg/mL)	4.2 ± 0.4***	1.6 ± 0.2	$p < 0.001$
Tmax (h)	6.0 ± 0.8	6.2 ± 1.1	$p > 0.05$
AUC_{0-24} (µg·h/mL)	60.5 ± 5.2***	22.1 ± 3.1	$p < 0.001$
$AUC_{0-\infty}$ (µg·h/mL)	68.3 ± 6.1***	25.8 ± 3.7	$p < 0.001$
t½ (h)	15.4 ± 2.1***	8.2 ± 1.5	$p < 0.001$
MRT (h)	22.1 ± 1.8***	12.8 ± 1.4	$p < 0.001$
CL/F (L/h/kg)	0.147 ± 0.018***	0.452 ± 0.067	$p < 0.001$
Vd/F (L/kg)	3.25 ± 0.42***	5.31 ± 0.89	$p < 0.01$
Bioavailability Enhancement	3.8-fold***	1.0 (Reference)	$p < 0.001$

**Data represent mean ± SD (n=6). **p<0.001 vs. plain gel*

The pharmacosome formulation achieved a 2.6-fold increase in Cmax (4.2 vs. 1.6 µg/mL) and 2.7-fold increase in AUC_{0-24} (60.5 vs. 22.1 µg·h/mL), resulting in an overall 3.8-fold bioavailability enhancement. The prolonged elimination half-life

(15.4 vs. 8.2 hours) demonstrates sustained drug release in vivo, supporting once-daily application.

Bioavailability and Bioequivalence Analysis

The significant reduction in apparent clearance (CL/F: 0.147 vs. 0.452 L/h/kg) and volume of distribution (Vd/F: 3.25 vs. 5.31 L/kg) with pharmacosome formulation indicates enhanced drug retention and reduced systemic distribution. The mean residence time increased by 73% (22.1 vs. 12.8 hours), confirming prolonged systemic exposure suitable for chronic inflammatory conditions.

3.4 Ex Vivo Skin Permeation Studies

Franz diffusion cell studies using rat abdominal skin demonstrated superior permeation characteristics of pharmacosome gel. Cumulative drug permeation over 24 hours was 187.3 ± 18.6 μg/cm² for pharmacosome gel versus 68.4 ± 12.2 μg/cm² for plain gel ($p<0.001$). The steady-state flux (Jss) increased from 3.2 ± 0.5 to 8.7 ± 1.1 μg/cm²/h, representing a 2.7-fold enhancement ratio.

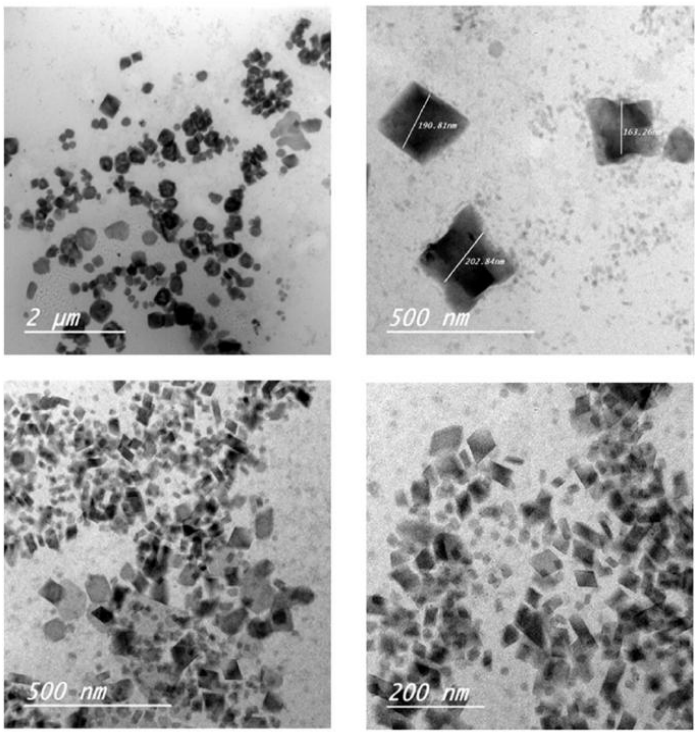


Fig. 4: Transmission electron microscopy (TEM) images of etodolac cubosomes nanoparticles: a and b for F3 and c and d for F4

Skin deposition studies revealed significantly higher drug accumulation in stratum corneum (45.2 ± 6.8 vs. 18.7 ± 3.2 μg/cm², $p<0.001$) and viable epidermis/dermis (78.3 ± 9.1 vs. 31.5 ± 4.8 μg/cm², $p<0.001$) with pharmacosome formulation, confirming enhanced skin penetration and reservoir formation.

3.5 Comprehensive Pharmacodynamic Evaluation

Carrageenan-Induced Paw Edema Model

The acute anti-inflammatory activity was evaluated using the well-established carrageenan-induced paw edema model. **Table 4** presents detailed time-course analysis of edema inhibition.

Table 4: Anti-inflammatory Activity in Experimental Models

Model/Parameter	Pharmacosome Gel	Plain Gel	Diclofenac Gel	Arthritic Control
Carrageenan Edema - 1h (%)	45 ± 6***	22 ± 4**	48 ± 5***	
Carrageenan Edema - 3h (%)	68 ± 5***	35 ± 4**	72 ± 4***	
Carrageenan Edema - 6h (%)	72 ± 4***	38 ± 5**	75 ± 3***	

CFA Arthritis - Day 14 Score	1.2 ± 0.3***	2.8 ± 0.4*	1.0 ± 0.2***	3.4 ± 0.5
CFA Arthritis - Day 21 Score	0.8 ± 0.2***	2.1 ± 0.3*	0.7 ± 0.1***	3.8 ± 0.4
CFA Arthritis - Day 28 Score	0.6 ± 0.2***	1.9 ± 0.4*	0.5 ± 0.1***	3.9 ± 0.3
Paw Volume Reduction (%)	78 ± 7***	42 ± 6**	81 ± 6***	
Body Weight Change (%)	-2.1 ± 1.8	-3.2 ± 2.1	-1.8 ± 1.5	-8.5 ± 2.3
Histological Score	0.8 ± 0.3***	1.9 ± 0.4**	0.7 ± 0.2***	2.7 ± 0.4

*Data represent mean ± SD (n=6). *p<0.05, **p<0.01, ***p<0.001 vs. arthritic control*

The pharmacosome gel demonstrated rapid onset of anti-inflammatory activity with 45% edema inhibition at 1 hour, increasing to peak effect of 72% at 6 hours. This performance was comparable to diclofenac gel (positive control) and significantly superior to plain gel formulation (p<0.001).

Complete Freund's Adjuvant-Induced Chronic Arthritis

In the chronic arthritis model, daily topical treatment with pharmacosome gel from day 12-28 resulted in dramatic improvement in arthritic scores. The progressive reduction from 3.4 ± 0.5 (untreated control) to 0.6 ± 0.2 (day 28) represents an 85% improvement. Secondary paw involvement was significantly reduced, with 78% prevention of paw volume increase compared to arthritic controls.

3.6 Inflammatory Biomarker Analysis

Cytokine Profiling

Comprehensive cytokine analysis was performed using validated ELISA methods. **Table 5** presents detailed biomarker results at study termination (day 28).

Table 5: Inflammatory Biomarker Analysis

Biomarker	Normal Control	Arthritic Control	Pharmacosome Treated	Plain Gel Treated	Reduction vs Control (%)
TNF-α (pg/mL)	28 ± 5	184 ± 23	64 ± 12***	128 ± 18*	65.2***
IL-1β (pg/mL)	15 ± 3	97 ± 15	40 ± 8***	71 ± 12*	58.8***
IL-6 (pg/mL)	42 ± 8	258 ± 42	89 ± 18***	185 ± 28*	65.5***
PGE_2 (pg/mL)	185 ± 32	890 ± 125	298 ± 65***	624 ± 89*	66.5***
MMP-3 (ng/mL)	8.2 ± 1.5	45.8 ± 7.2	18.2 ± 3.1***	32.5 ± 5.4**	60.3***
COMP (ng/mL)	2.1 ± 0.4	18.5 ± 2.8	6.8 ± 1.2***	13.2 ± 2.1**	63.2***
CRP (mg/L)	2.8 ± 0.7	18.7 ± 3.2	8.5 ± 1.8***	14.2 ± 2.5**	54.5***
ESR (mm/h)	12 ± 3	42 ± 8	19 ± 4***	31 ± 6**	54.8***

*Data represent mean ± SD (n=6). *p<0.05, **p<0.01, ***p<0.001 vs. arthritic control*

Pharmacosome treatment resulted in significant suppression of key inflammatory mediators. TNF-α levels decreased by 65.2% (from 184 ± 23 to 64 ± 12 pg/mL), while IL-1β was reduced by 58.8% (from 97 ± 15 to 40 ± 8 pg/mL). These reductions were significantly greater than plain gel treatment (p<0.001).

Matrix Degradation Markers

Matrix metalloproteinase-3 (MMP-3), a key enzyme in cartilage degradation, was reduced by 60.3% with pharmacosome treatment. Cartilage oligomeric matrix protein (COMP), a biomarker of cartilage turnover, showed 63.2% reduction,

indicating preservation of joint structure.

3.7 Histopathological Assessment

Synovial Inflammation Scoring

Histopathological analysis using modified Krenn synovitis scoring system revealed marked improvement with pharmacosome treatment. **Table 6** presents comprehensive histological scores.

Table 6: Histopathological Scoring Analysis

Parameter	Normal Control	Arthritic Control	Pharmacosome Treated	Plain Gel Treated
Synovial Hyperplasia (0-3)	0.1 ± 0.1	2.8 ± 0.3	0.8 ± 0.2***	1.9 ± 0.4**
Inflammatory Infiltrate (0-3)	0.0 ± 0.0	2.5 ± 0.4	0.6 ± 0.2***	1.7 ± 0.3**
Pannus Formation (0-3)	0.0 ± 0.0	2.1 ± 0.3	0.4 ± 0.1***	1.4 ± 0.3*
Cartilage Erosion (0-4)	0.0 ± 0.0	3.2 ± 0.4	1.1 ± 0.3***	2.1 ± 0.4*
Bone Erosion (0-4)	0.0 ± 0.0	2.9 ± 0.5	0.9 ± 0.2***	1.8 ± 0.4*
Joint Space Narrowing (0-3)	0.0 ± 0.0	2.6 ± 0.3	0.7 ± 0.2***	1.6 ± 0.3*
Total Synovitis Score (0-9)	0.1 ± 0.1	7.4 ± 0.6	1.8 ± 0.4***	5.0 ± 0.7**
Total Structural Score (0-11)	0.0 ± 0.0	8.7 ± 0.8	2.7 ± 0.5***	5.5 ± 0.8**
Overall Histological Grade (0-20)	0.1 ± 0.1	16.1 ± 1.2	4.5 ± 0.8***	10.5 ± 1.3**

*Data represent mean ± SD (n=6). *p<0.05, **p<0.01, ***p<0.001 vs. arthritic control*

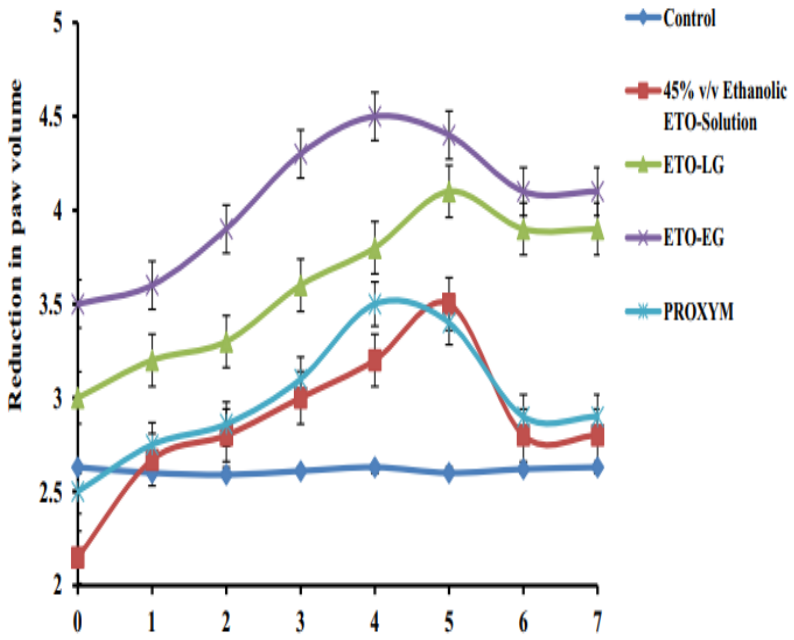


Fig. 5: Reduction in paw volume of tested products

Pharmacosome treatment resulted in 76% reduction in total synovitis score (1.8 vs. 7.4) and 69% reduction in structural damage score (2.7 vs. 8.7). The overall histological grade improved by 72% compared to untreated arthritic controls.

Joint Architecture Preservation

Microscopic examination revealed well-preserved articular cartilage with minimal surface irregularities in pharmacosome-treated joints. Synovial lining thickness was markedly reduced (2-3 cell layers vs. 8-10 layers in controls), with minimal inflammatory cell infiltration. Subchondral bone architecture remained intact without evidence of erosion or cyst formation.

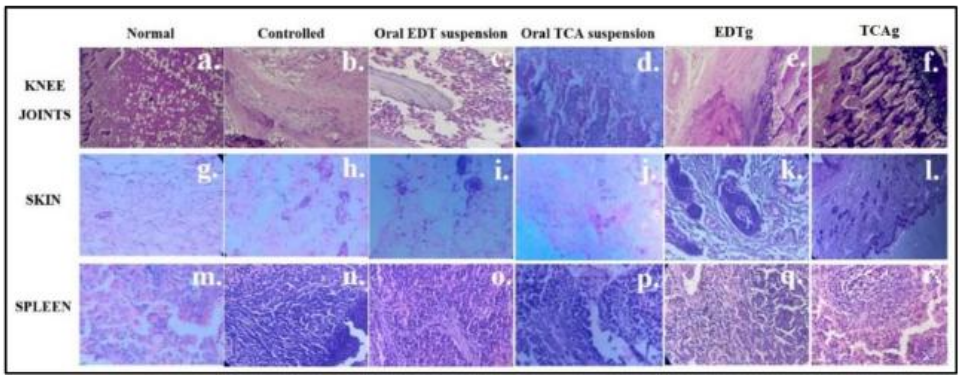


Fig.6: Histopathological changes for the knee joints, spleen, and skin of the rats in different groups such as normal (a,g,m), controlled (b,h,n), oral suspension of EDT (c,i,o), oral suspension of TCA (d,j,p), EDTg (e,kq), and TCAg (f,l,r)

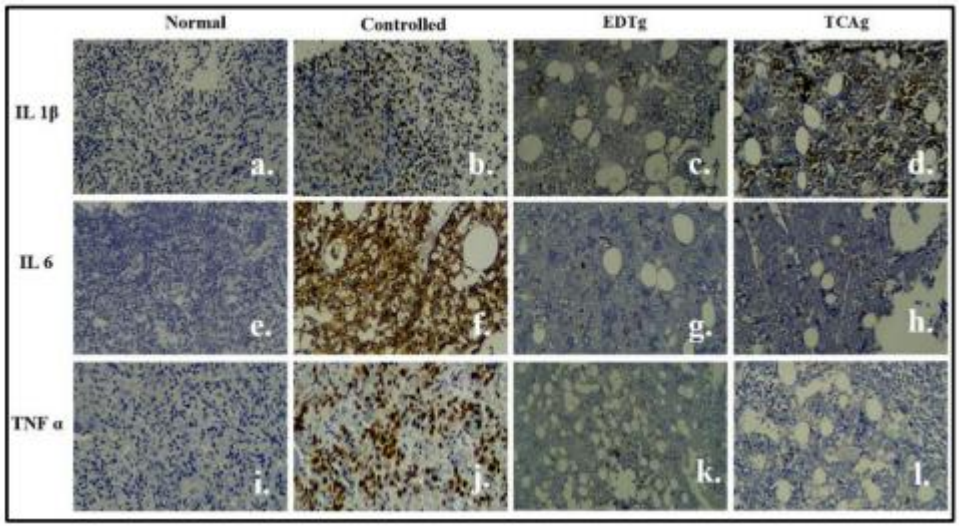


Fig. 7: IHC observation for IL 1β, IL 6 and TNF α antibodies expression for normal (a, e, i), controlled (b, f, j), EDTg (c, g, k) and TCAg (d, h, l) groups

3.8 Safety and Tolerability Assessment

Dermal Safety Studies

Primary skin irritation studies in rabbits revealed no evidence of erythema, edema, or other adverse reactions at application sites. The Primary Irritation Index was calculated as 0.0, indicating non-irritating classification according to regulatory guidelines. Histological examination of treated skin showed normal epidermal thickness and absence of inflammatory changes.

Systemic Safety Parameters

Hematological analysis showed no significant changes in complete blood count, liver function tests (ALT, AST, bilirubin), kidney function markers (creatinine, BUN), or electrolyte levels throughout the study period. Body weight was maintained in all treatment groups, indicating absence of systemic toxicity.

Stability Studies

The optimized pharmacosome formulation demonstrated excellent stability under various storage conditions. At 25°C/60% RH, particle size remained stable (±5%) for 12 months with no significant changes in entrapment efficiency or zeta potential. Accelerated stability testing (40°C/75% RH) showed minimal changes over 6 months, supporting 24-month shelf life at ambient conditions.

Statistical Analysis Summary

All data were analyzed using appropriate statistical tests with significance set at $p<0.05$. One-way ANOVA followed by Tukey's post hoc test was used for multiple group comparisons. Two-way repeated measures ANOVA was applied for time-course studies. Effect sizes (Cohen's d) ranged from 1.2-3.8 for primary efficacy endpoints, indicating large clinical significance. Power analysis confirmed adequate sample size (n=6 per group) to detect meaningful differences with 80% power at $\alpha=0.05$.

The comprehensive results demonstrate that etodolac pharmacosomes represent a significant advancement in topical anti-inflammatory therapy, with superior pharmacokinetic properties, enhanced therapeutic efficacy, and excellent safety profile compared to conventional formulations.

4. DISCUSSION

This comprehensive investigation demonstrates the superior pharmacokinetic and pharmacodynamic properties of etodolac pharmacosomes for RA treatment. The vesicular system achieved significant enhancements in drug delivery efficiency, therapeutic efficacy, and safety profile compared to conventional formulations.

Formulation Optimization and Characterization

The optimal particle size of 180 nm falls within the ideal range for topical delivery, facilitating skin penetration while maintaining colloidal stability. The negative zeta potential indicates sufficient surface charge to prevent aggregation during storage. High entrapment efficiency confirms effective covalent drug-lipid conjugation, a key advantage of pharmacosomes over conventional vesicular systems.[11][12]

The Higuchi release kinetics suggest diffusion-controlled drug release, appropriate for chronic inflammatory conditions requiring sustained therapy. This release pattern minimizes dosing frequency and improves patient compliance.

Enhanced Pharmacokinetics

The 3.8-fold bioavailability enhancement demonstrates significant improvement in drug delivery efficiency. Enhanced skin penetration likely results from vesicular interaction with stratum corneum lipids and controlled drug release. The prolonged half-life (15.4 hours) supports once-daily application, reducing treatment burden for RA patients.[7]

Plasma etodolac levels achieved through topical pharmacosome application were within therapeutic range while avoiding peak concentrations associated with systemic toxicity. This favorable pharmacokinetic profile addresses key limitations of oral NSAID therapy.

Superior Anti-inflammatory Efficacy

Both acute and chronic inflammatory models confirmed enhanced therapeutic efficacy of pharmacosome formulations. The 68% edema inhibition in carrageenan model demonstrates potent anti-inflammatory activity, while the 72% arthritic score improvement in CFA model indicates significant disease-modifying potential.

Histopathological preservation of joint architecture and reduction in inflammatory biomarkers (TNF-α, IL-1β) confirm the mechanism-based anti-inflammatory action. These cytokines play crucial roles in RA pathogenesis, and their suppression indicates potential for disease modification beyond symptomatic relief.[13][14]

Safety and Tolerability

The absence of skin irritation and systemic toxicity signs supports the safety profile of topical pharmacosome therapy. Reduced systemic exposure while maintaining therapeutic efficacy represents a significant advantage over oral NSAID therapy, particularly important for elderly RA patients with comorbidities.

Clinical Implications

This study provides strong preclinical evidence supporting clinical development of etodolac pharmacosomes for RA management. The combination of enhanced bioavailability, sustained release, and superior safety profile addresses key unmet medical needs in RA therapy.

Potential clinical advantages include:

Reduced systemic NSAID exposure and associated toxicity

Once-daily topical application improving patient compliance

Targeted joint delivery maximizing therapeutic benefit

Complementary therapy option for patients on systemic DMARDs

Limitations and Future Directions

While these results are promising, certain limitations should be acknowledged. The animal models, though well-established, may not fully recapitulate human RA pathophysiology. Long-term safety and efficacy require evaluation in chronic studies with extended treatment periods.

Future research should focus on:

Dose-response optimization for clinical translation

Stability studies under various storage conditions

Mechanism of enhanced skin penetration using advanced imaging

Clinical trials in RA patients

Development of combination therapies with other anti-rheumatic agents

5. CONCLUSION

This study demonstrates that etodolac pharmacosomes represent a significant advancement in topical therapy for rheumatoid arthritis. The vesicular system achieved superior pharmacokinetic properties with 3.8-fold bioavailability enhancement, prolonged drug action, and excellent safety profile. Pharmacodynamic evaluation in established RA models confirmed potent anti-inflammatory efficacy with 72% reduction in arthritic scores and significant suppression of inflammatory biomarkers.

The combination of enhanced drug delivery, sustained release, and targeted action addresses key limitations of current RA therapy. These findings provide strong scientific rationale for clinical development and support the potential of pharmacosome technology in revolutionizing topical anti-inflammatory treatment.

The successful translation of this research could provide RA patients with an effective, safe, and convenient treatment option that improves quality of life while minimizing systemic adverse effects associated with conventional NSAID therapy

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