

Topical Polyherbal Ethosomal Gel of *Salvia officinalis* and *Sarcostemma brevistigma* Therapeutic Evaluation in Complete Freund's Adjuvant-Induced Rheumatoid Arthritis

Shweta Rawal* & Sachin Kumar Jain

*Research Scholar, Faculty of Pharmacy, Oriental University, Indore (M.P.)

Faculty of Pharmacy, Oriental University, Indore (M.P.)

Corresponding author email: shetu.rwl@gmail.com

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by persistent synovial inflammation, joint swelling, cartilage destruction, and progressive functional impairment. The present study was undertaken to develop and evaluate a topical polyherbal ethosomal gel containing *Salvia officinalis* leaf extract and *Sarcostemma brevistigma* stem extract for enhanced anti-inflammatory and anti-arthritic activity. The ethanolic extracts were incorporated into ethosomal vesicles using soya phosphatidylcholine, ethanol, and cholesterol and subsequently formulated into a Carbopol 940 gel. Five formulations (PEG1–PEG5) were prepared by varying phosphatidylcholine and ethanol concentrations and evaluated for physicochemical and vesicular characteristics. Among the formulations, PEG3 was selected as the optimized formulation based on its superior properties. PEG3 exhibited a skin-compatible pH of 6.42 ± 0.03 , viscosity of $28,760 \pm 240$ cps, spreadability of 22.6 ± 0.5 g·cm/s, drug content of $98.6 \pm 0.8\%$, particle size of 198.3 ± 6.4 nm, PDI of 0.216 ± 0.01 , zeta potential of -27.8 ± 0.9 mV, and entrapment efficiency of $86.7 \pm 1.0\%$. The formulation showed $92.8 \pm 1.2\%$ drug release at 8 h. In vitro anti-inflammatory evaluation demonstrated $82.36 \pm 1.05\%$ HRBC membrane stabilization and $86.38 \pm 1.05\%$ inhibition of BSA protein denaturation. PEG3 was further investigated in Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis in Wistar albino rats. Topical treatment significantly reduced paw edema and arthritic score, improved body weight, and decreased inflammatory biomarkers including TNF- α , IL-1 β , IL-6, CRP, and ESR compared with the arthritic control group. Radiographic examination demonstrated reduced soft-tissue swelling, preservation of joint space, and decreased bone erosion. Overall, the optimized polyherbal ethosomal gel PEG3 demonstrated promising anti-arthritic activity comparable to diclofenac sodium and may represent a potential topical herbal delivery system for the management of rheumatoid arthritis.

Keywords: Rheumatoid arthritis; *Salvia officinalis*; *Sarcostemma brevistigma*; Polyherbal formulation; Ethosomal gel; Carbopol 940; Anti-inflammatory activity; Complete Freund's Adjuvant; Anti-arthritic activity; Topical drug delivery.

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune inflammatory disorder primarily characterized by persistent inflammation of synovial joints, progressive cartilage destruction, bone erosion, and irreversible functional impairment. It affects approximately 0.5–1% of the global population and represents one of the most prevalent autoimmune diseases associated with significant morbidity and reduced quality of life. The pathogenesis of RA involves complex interactions between genetic susceptibility, immune dysregulation, inflammatory cytokines, oxidative stress, and activation of cellular pathways responsible for synovial hyperplasia and joint destruction [1]. Inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), prostaglandins, and reactive oxygen species play crucial roles in the initiation and progression of rheumatoid inflammation. Persistent activation of these pathways results in pannus formation, destruction

of articular cartilage, and subsequent bone deformities.

Current pharmacological approaches for the management of rheumatoid arthritis include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs), and biological agents. Although these therapies provide significant symptomatic relief and delay disease progression, their long-term application is frequently associated with adverse effects. NSAIDs, including diclofenac sodium, are widely used for reducing inflammation and pain; however, prolonged use may cause gastrointestinal irritation, renal impairment, and cardiovascular complications. Corticosteroids are effective anti-inflammatory agents but are associated with serious complications such as osteoporosis, metabolic disturbances, immunosuppression, and hormonal imbalance upon chronic administration. Similarly, biological therapies targeting inflammatory cytokines are expensive and may increase susceptibility to

infections. Therefore, the development of safer, effective, and patient-friendly therapeutic alternatives with improved delivery and reduced toxicity remains an important area of pharmaceutical research [2].

Medicinal plants have received increasing attention as potential sources of anti-inflammatory agents due to their rich content of biologically active phytoconstituents, including flavonoids, phenolic acids, terpenoids, alkaloids, and glycosides. These natural compounds exhibit anti-inflammatory and antioxidant activities through multiple mechanisms, including inhibition of cyclooxygenase and lipoxygenase pathways, suppression of inflammatory cytokine production, reduction of oxidative stress, and stabilization of cellular membranes. Polyherbal formulations have gained considerable interest because the combination of different plant extracts may produce synergistic therapeutic effects by targeting multiple inflammatory pathways simultaneously [3].

Salvia officinalis L. (sage), belonging to the family Lamiaceae, is a medicinal plant traditionally used for inflammatory disorders due to its antioxidant, antimicrobial, and anti-inflammatory properties. The plant contains several pharmacologically important constituents, including rosmarinic acid, carnolic acid, carnosol, flavonoids, and diterpenoids. These compounds have been reported to inhibit inflammatory mediators, suppress oxidative damage, and regulate immune responses. Rosmarinic acid, a major phenolic constituent of *Salvia officinalis*, exhibits strong free radical scavenging activity and inhibits the production of pro-inflammatory cytokines, thereby contributing to its protective effects against inflammatory conditions [4].

Sarcostemma brevistigma is a medicinal plant belonging to the family Apocynaceae and has been traditionally used for various therapeutic applications. The plant contains bioactive secondary metabolites such as steroidal glycosides, flavonoids, phenolic compounds, and other phytoconstituents responsible for antioxidant and anti-inflammatory activities. Previous pharmacological investigations have demonstrated the potential of *Sarcostemma* species in reducing inflammatory responses and oxidative stress. The combination of *Salvia officinalis* and *Sarcostemma brevistigma* extracts may provide complementary therapeutic benefits by enhancing anti-inflammatory activity through synergistic interactions among their phytochemical constituents [5].

Despite the promising therapeutic potential of herbal extracts, their clinical effectiveness is often restricted due to poor aqueous solubility, limited permeability, rapid degradation, and inadequate bioavailability at the site of action. Conventional topical formulations may not efficiently transport

phytoconstituents across the skin barrier because the stratum corneum acts as a strong barrier against penetration. Therefore, advanced drug delivery systems are required to enhance dermal permeation and improve therapeutic availability of herbal compounds [6].

Ethosomes are advanced lipid-based vesicular delivery systems composed mainly of phospholipids, ethanol, and water. They possess unique characteristics compared with conventional liposomes due to the presence of high ethanol concentration, which increases vesicle flexibility and deformability. Ethanol disrupts the ordered lipid arrangement of the stratum corneum and enhances vesicle penetration into deeper skin layers. The highly deformable nature of ethosomes facilitates efficient transport of encapsulated molecules across the skin barrier, making them suitable carriers for topical and transdermal drug delivery. Additionally, ethosomes improve drug solubilization, protect unstable phytoconstituents, provide controlled release, and enhance therapeutic efficacy.

The incorporation of ethosomal vesicles into a suitable gel matrix further improves topical application characteristics. Carbopol 940 is extensively used as a gel-forming polymer due to its excellent viscosity, bioadhesive properties, stability, and compatibility with skin. Ethosomal gels combine the advantages of vesicular carriers and semisolid dosage forms by providing improved residence time, controlled drug release, ease of application, and enhanced patient compliance. The optimization of formulation variables, particularly phosphatidylcholine and ethanol concentration, is essential to achieve desirable vesicle characteristics and therapeutic performance [7].

In the present study, a polyherbal ethosomal gel containing extracts of *Salvia officinalis* leaves and *Sarcostemma brevistigma* stems was developed and evaluated for potential anti-inflammatory activity against rheumatoid arthritis. The formulation was optimized by varying soya phosphatidylcholine and ethanol concentrations while maintaining other formulation components constant. The prepared formulations were evaluated for critical physicochemical parameters including appearance, homogeneity, pH, viscosity, spreadability, extrudability, and drug content to determine their suitability for topical application [8].

The vesicular characteristics of the optimized formulations were assessed by evaluating particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. Particle size and PDI provide important information regarding vesicle uniformity, stability, and skin penetration capability, whereas zeta potential indicates electrostatic stability and resistance against vesicle aggregation. Entrapment efficiency determines the capacity of ethosomes to incorporate and retain

bioactive phytoconstituents within the vesicular structure. The optimized formulation was further evaluated for in vitro drug release behavior to understand the release pattern and diffusion characteristics of encapsulated phytoconstituents. The safety profile of the developed formulation was established through skin irritation studies to ensure dermal compatibility and absence of adverse reactions such as erythema or edema. Stability studies were conducted according to International Council for Harmonisation (ICH) guidelines under different storage conditions to evaluate changes in physical appearance, pH, viscosity, and drug content during storage. Statistical analysis was performed using appropriate statistical tools to determine significant differences among formulations and validate the experimental findings [9].

Furthermore, the anti-inflammatory potential of the developed polyherbal ethosomal gel was evaluated using in vitro models, including Human Red Blood Cell (HRBC) membrane stabilization assay and bovine serum albumin (BSA) denaturation inhibition assay. These models provide preliminary evidence regarding membrane stabilizing activity and inhibition of inflammatory protein denaturation, which are important mechanisms involved in inflammation. The optimized formulation was subsequently evaluated in Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis model to determine its therapeutic efficacy [10].

Therefore, the present investigation aimed to develop and characterize a novel topical polyherbal ethosomal gel containing *Salvia officinalis* and *Sarcostemma brevistigma* extracts and to assess its potential as an effective, safe, and patient-friendly alternative for the management of rheumatoid arthritis. The study integrates herbal pharmacotherapy with advanced vesicular drug delivery technology to enhance topical delivery, improve anti-inflammatory activity, and provide a promising therapeutic approach for chronic inflammatory disorders.

Materials and Methods

Preparation of Polyherbal Ethosomal Gel

Fresh leaves of *Salvia officinalis* and stems of *Sarcostemma brevistigma* were collected, authenticated, washed thoroughly with distilled water, shade-dried at room temperature (25–30°C), and powdered after complete drying. The powdered plant materials were subjected to successive Soxhlet extraction using petroleum ether (60–80°C) followed by 95% ethanol. The petroleum ether extraction was initially carried out to remove non-polar components such as fats, waxes, and chlorophyll, while the ethanolic extraction was performed to obtain polar and moderately polar bioactive phytoconstituents. The ethanolic extracts were concentrated under reduced pressure and

stored under suitable conditions until further use. The total phenolic content of the extracts was estimated by UV spectrophotometric determination of gallic acid equivalents. A calibration curve of gallic acid was prepared at λ_{max} 260 nm, and the phenolic content of the extracts was calculated from the regression equation. The ethanolic extracts of *S. officinalis* and *S. brevistigma* showed gallic acid contents of 2.341% and 2.087%, respectively [11].

The polyherbal ethosomal gel formulations were prepared using the cold method by incorporating the ethanolic extracts of *S. officinalis* and *S. brevistigma* into an ethosomal vesicular system. Soya phosphatidylcholine was used as the primary phospholipid component, ethanol was incorporated as a penetration enhancer, and cholesterol was added to improve membrane stability. Different formulations (PEG1–PEG5) were prepared by varying the concentration of soya phosphatidylcholine (200–600 mg) and ethanol (20–40% v/v), while keeping the concentrations of herbal extracts and other excipients constant [12].

For ethosome preparation, phosphatidylcholine and cholesterol were dissolved in ethanol with continuous stirring to form the lipid phase. The aqueous phase containing distilled water and herbal extracts was added slowly under continuous mixing to produce a homogeneous ethosomal dispersion. The dispersion was sonicated to reduce vesicle size and obtain a uniform nanosized vesicular system. The prepared ethosomal dispersion was incorporated into Carbopol 940 gel base (1% w/w), previously hydrated with distilled water. Propylene glycol was used as a co-solvent and penetration enhancer, while methyl paraben and propyl paraben were added as preservatives. Triethanolamine was used for neutralization and adjustment of the gel pH. The final formulation weight was adjusted to 100 g using distilled water [13].

The prepared ethosomal gels were evaluated for physical appearance, homogeneity, pH, viscosity, spreadability, extrudability, drug content, vesicle size, polydispersity index (PDI), zeta potential, entrapment efficiency, in vitro drug release, and skin irritation potential. All formulations showed acceptable physicochemical properties suitable for topical application.

Selection Basis of PEG3 for In Vivo Anti-Arthritic Evaluation

Among all prepared polyherbal ethosomal gel formulations (PEG1–PEG5), PEG3 was selected as the optimized formulation for in vivo anti-arthritic evaluation based on its superior physicochemical characteristics and biological performance. PEG3 exhibited excellent homogeneity, acceptable skin-compatible pH (6.42 ± 0.03), optimum viscosity ($28,760 \pm 240$ cps), and maximum spreadability (22.6 ± 0.5 g·cm/s), indicating improved patient acceptability and ease of topical application. It

demonstrated the highest drug content ($98.6 \pm 0.8\%$), confirming efficient incorporation of herbal phytoconstituents into the ethosomal system [14]. The vesicular characteristics of PEG3 were superior compared with other formulations, with the smallest particle size (198.3 ± 6.4 nm), lowest PDI value (0.216 ± 0.01), and highest negative zeta potential (-27.8 ± 0.9 mV). These characteristics indicated uniform vesicle distribution, enhanced colloidal stability, and improved potential for skin penetration. PEG3 also showed maximum entrapment efficiency ($86.7 \pm 1.0\%$), indicating efficient encapsulation of phytoconstituents within the phospholipid bilayer [15]. The optimized formulation demonstrated the highest in vitro drug release ($92.8 \pm 1.2\%$ at 8 h), suggesting improved diffusion and sustained delivery of bioactive compounds. In addition, PEG3 exhibited the strongest anti-inflammatory activity in HRBC membrane stabilization assay ($82.36 \pm 1.05\%$) and BSA protein denaturation inhibition assay ($86.38 \pm 1.05\%$), which was comparable to diclofenac sodium. The absence of skin irritation confirmed its dermal safety [16]. Based on these combined findings, PEG3 was selected as the optimized formulation for further evaluation in a Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis model.

Experimental Animals and Study Design

The in vivo anti-arthritic activity of the optimized polyherbal ethosomal gel (PEG3) was evaluated using Complete Freund's Adjuvant (CFA)-induced arthritis in Wistar albino rats. Healthy adult rats weighing 180–220 g were obtained from an approved animal facility and housed under standard laboratory conditions with controlled temperature ($22\text{--}25^\circ\text{C}$), relative humidity (45–55%), and a 12 h light/dark cycle. Animals were provided standard pellet diet and water ad libitum throughout the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) [17].

Table 1: Animals were randomly divided into four groups consisting of six animals in each group

Group	Treatment
Group I	Normal control (vehicle gel without CFA induction)
Group II	Arthritic control (CFA-induced arthritis + gel base)
Group III	Standard treatment (Diclofenac sodium 10 mg/kg topical)

Group IV	PEG3 polyherbal ethosomal gel (~500 mg/day topical application)
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Rheumatoid arthritis was induced by subplantar injection of 0.1 mL Complete Freund's Adjuvant (CFA) into the right hind paw of rats. The development of arthritis was monitored by measuring paw volume, arthritic score, and body weight changes throughout the experimental period. Treatment was initiated after arthritis development and continued for the predetermined study duration.

In Vivo Anti-Arthritic Activity

The anti-arthritic activity of PEG3 was evaluated by assessing inflammatory progression, physical changes, biochemical markers, organ indices, radiographic alterations, and histopathological changes [18].

Evaluation of Paw Edema and Arthritic Score

CFA administration produced progressive inflammation characterized by increased paw volume, swelling, redness, and joint stiffness in arthritic control animals. Paw volume was measured using a plethysmometer on predetermined days. Arthritic severity was scored using a standard clinical scoring system based on erythema and edema formation in the paws. The arthritic control group showed a significant increase in paw volume from day 7 onwards, confirming successful induction of arthritis [19].

Effect on Body Weight and Organ Weight

CFA-induced arthritis resulted in reduced body weight gain due to chronic inflammation and systemic disease progression. PEG3 treatment significantly improved body weight compared with arthritic control animals, indicating reduction of inflammatory stress and restoration of physiological function. The arthritic control group showed increased liver, kidney, and spleen weights due to inflammatory responses. PEG3 treatment significantly normalized these organ weights, suggesting protective effects against inflammation-associated tissue alterations [20].

Estimation of Inflammatory Biomarkers

Serum inflammatory markers including TNF- α , IL-1 β , IL-6, C-reactive protein (CRP), ESR, and rheumatoid factor (RF) were estimated to determine the effect of PEG3 on inflammatory pathways. CFA administration significantly increased TNF- α , IL-1 β , IL-6, CRP, ESR, and RF levels compared with normal animals. Treatment with PEG3 significantly reduced these inflammatory markers [21].

Radiographic and Histopathological Evaluation

Radiographic examination of ankle joints was performed to evaluate structural changes associated with arthritis. Arthritic control animals showed severe joint destruction, soft tissue swelling, narrowing of joint spaces, and bone erosion. PEG3 treatment significantly improved joint architecture

with reduced edema, preserved joint space, and minimal bone erosion. Histopathological examination of joint tissues demonstrated severe inflammatory infiltration, synovial hyperplasia, cartilage destruction, and bone abnormalities in CFA-induced arthritic rats [22].

Results and Discussion

Preparation of Polyherbal Ethosomal Gel

The polyherbal ethosomal gel containing *Salvia officinalis* leaf extract and *Sarcostemma brevistigma* stem extract was successfully developed using a cold dispersion method by incorporating variable concentrations of soya phosphatidylcholine and ethanol. Ethosomal vesicles were prepared to enhance the topical delivery of bioactive phytoconstituents through improved skin penetration and prolonged retention at the site of application. Carbopol 940 was selected as the gelling polymer due to its excellent viscosity-building ability, bioadhesive characteristics, and compatibility with topical formulations. Five formulations (PEG1–PEG5) were prepared by varying phosphatidylcholine concentration (200–600 mg) and ethanol concentration (20–40% v/v), while maintaining other formulation components constant. The prepared gels exhibited smooth, pale green appearance with no visible phase separation, indicating uniform incorporation of ethosomal vesicles into the gel matrix. All formulations showed acceptable pH values ranging from 6.12 ± 0.05 to 6.68 ± 0.05 , which was suitable for topical application and compatible with physiological skin pH. The vesicular characterization demonstrated that formulation variables significantly influenced ethosome properties. Particle size of prepared formulations ranged from 198.3 ± 6.4 nm to 286.4 ± 8.5 nm, with PEG3 exhibiting the smallest vesicle size (198.3 ± 6.4 nm). The reduced vesicle size of PEG3 can be attributed to the optimized concentration of ethanol and phosphatidylcholine, which enhanced membrane flexibility and prevented vesicle aggregation. The PDI values ranged from 0.216 ± 0.01 to 0.321 ± 0.01 , indicating relatively uniform vesicle distribution. PEG3 exhibited the lowest PDI value (0.216), suggesting formation of a homogeneous nanosized vesicular system. The zeta potential values of all formulations were negative, ranging from -18.5 ± 0.7 to -27.8 ± 0.9 mV. PEG3 showed the highest negative surface charge (-27.8 ± 0.9 mV), indicating improved electrostatic stability and reduced tendency for vesicle aggregation during storage. The entrapment efficiency ranged from $71.4 \pm 1.3\%$ to $86.7 \pm 1.0\%$, where PEG3 showed maximum encapsulation of phytoconstituents ($86.7 \pm 1.0\%$). Enhanced entrapment efficiency was attributed to the optimum phospholipid concentration, which provided sufficient bilayer

structure for incorporation of phenolic and flavonoid compounds present in both plant extracts. The in vitro release study revealed that PEG3 exhibited maximum cumulative release ($92.8 \pm 1.2\%$) after 8 h compared with other formulations. The improved release profile was associated with smaller vesicle size, higher entrapment efficiency, and optimized gel viscosity, facilitating controlled diffusion of phytoconstituents through the gel matrix. The optimized formulation demonstrated excellent spreadability (22.6 ± 0.5 g·cm/s), drug content ($98.6 \pm 0.8\%$), and acceptable viscosity ($28,760 \pm 240$ cps), indicating its suitability for topical administration.

Table 2: Evaluation Parameters of Polyherbal Ethosomal Gel Formulations

Parameter	PEG 1	PEG 2	PEG 3	PEG 4	PEG 5
Particle size (nm)	286.4 ± 8.5	241.7 ± 7.2	198.3 ± 6.4	214.5 ± 6.9	236.8 ± 7.5
PDI	0.321 ± 0.01	0.284 ± 0.02	0.216 ± 0.01	0.238 ± 0.02	0.267 ± 0.02
Zeta potential (mV)	-18.5 ± 0.7	-22.3 ± 0.8	-27.8 ± 0.9	-25.1 ± 0.8	-23.1 ± 0.7
Entrapment efficiency (%)	71.4 ± 1.3	78.9 ± 1.2	86.7 ± 1.0	84.2 ± 1.1	81.6 ± 1.2
Drug release at 8 h (%)	72.5 ± 1.5	81.3 ± 1.4	92.8 ± 1.2	89.6 ± 1.3	85.4 ± 1.4

Selection Basis of PEG3 for In Vivo Anti-Arthritic Activity

Among all developed formulations, PEG3 was selected as the optimized formulation for further pharmacological investigation due to its superior physicochemical characteristics and biological performance. PEG3 demonstrated the highest drug content ($98.6 \pm 0.8\%$), maximum entrapment efficiency ($86.7 \pm 1.0\%$), smallest particle size (198.3 ± 6.4 nm), lowest PDI (0.216 ± 0.01), highest negative zeta potential (-27.8 ± 0.9 mV), and maximum drug release ($92.8 \pm 1.2\%$). Furthermore, PEG3 showed superior anti-inflammatory activity in preliminary in vitro studies. In HRBC membrane stabilization assay,

PEG3 produced $82.36 \pm 1.05\%$ membrane stabilization, whereas in BSA denaturation inhibition assay it demonstrated $86.38 \pm 1.05\%$ inhibition of protein denaturation. These results were comparable with diclofenac sodium, confirming its potent anti-inflammatory potential. The enhanced activity of PEG3 may be attributed to improved delivery of phytoconstituents from *Salvia officinalis* and *Sarcostemma brevistigma* through the ethosomal carrier system. Phenolic compounds, flavonoids, terpenoids, and antioxidant constituents present in these plants are known to suppress inflammatory mediators and oxidative stress pathways involved in rheumatoid arthritis progression.

Experimental Design and Animal Grouping

The optimized PEG3 formulation was evaluated for anti-arthritis activity using Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis model in Wistar albino rats. Animals were randomly divided into four groups containing six animals each. Arthritis was induced by subplantar injection of CFA into the right hind paw. Treatment was initiated after confirmation of arthritis development.

Table 3: Experimental Design for In Vivo Anti-Arthritic Study

Group	Treatment
Group I	Normal control (gel base only)
Group II	Arthritic control (CFA + gel base)
Group III	Standard treatment (Diclofenac sodium 10 mg/kg topical)
Group IV	PEG3 polyherbal ethosomal gel (~500 mg/day topical)

The animals were monitored for paw edema, body weight changes, arthritic score, inflammatory biomarkers, rheumatoid factor, CRP levels, radiographic changes, and histopathological alterations.

In vivo antiarthritic activity

The antiarthritic potential of the optimized polyherbal ethosomal gel formulation (PEG3) containing *Salvia officinalis* leaves and *Sarcostemma brevistigma* stems extracts was evaluated using a Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis model in Wistar rats. The CFA-induced arthritis model is a well-established experimental model that mimics several pathological characteristics of human rheumatoid arthritis, including persistent inflammation, joint swelling, increased inflammatory cytokines, immune activation, and progressive structural damage of articular tissues. Based on the superior physicochemical

characteristics and in vitro anti-inflammatory activity, PEG3 was selected for in vivo evaluation. The therapeutic efficacy of PEG3 was compared with the standard anti-inflammatory drug diclofenac sodium.

Effect of PEG3 on paw edema in CFA-induced arthritic rats

CFA administration produced a progressive increase in paw volume throughout the experimental period, confirming successful induction of arthritis. The arthritic control group showed a significant increase in paw volume from 0.83 ± 0.02 mL on day 0 to 2.18 ± 0.07 mL on day 21, indicating severe inflammatory edema. In contrast, normal control animals maintained a constant paw volume throughout the study period, demonstrating the absence of inflammatory changes. Topical administration of diclofenac sodium significantly reduced CFA-induced paw swelling, with paw volume decreasing to 0.96 ± 0.04 mL on day 21. Similarly, treatment with PEG3 significantly suppressed paw edema, reducing paw volume to 1.05 ± 0.04 mL on day 21 compared with the arthritic control group. Although the standard drug exhibited slightly higher inhibition of edema, PEG3 demonstrated considerable anti-inflammatory activity and effectively prevented the progression of CFA-induced inflammation. The reduction in paw swelling produced by PEG3 may be attributed to the synergistic action of bioactive phytoconstituents present in *S. officinalis* and *S. brevistigma*. Phenolic compounds, flavonoids, terpenoids, and steroidal constituents present in these plants are reported to inhibit inflammatory mediators such as prostaglandins, nitric oxide, TNF- α , and interleukins. Furthermore, the ethosomal delivery system enhances skin penetration and facilitates prolonged localization of phytoconstituents at the inflammatory site, thereby improving therapeutic effectiveness.

Table 4. Effect of Polyherbal Ethosomal Gel (PEG3) on Paw Volume in CFA-Induced Arthritic Rats

Group	Treatment	Day 0 (mL)	Day 7 (mL)	Day 14 (mL)	Day 21 (mL)
I	Normal control	0.82 ± 0.03	0.84 ± 0.03	0.83 ± 0.02	0.84 ± 0.03
II	Arthritic control (CFA)	0.83 ± 0.02	1.46 ± 0.05^a	1.89 ± 0.06^a	2.18 ± 0.07^a
III	Diclofenac sodium	0.82 ± 0.03	1.40 ± 0.04^a	1.19 ± 0.05^b	0.96 ± 0.04^b
IV	PEG3	0.83 ± 0.02	1.42 ± 0.04^a	1.31 ± 0.05^b	1.05 ± 0.04^b

Values expressed as Mean $\pm$ SEM (n=6).

^ap<0.001 compared with normal control; ^bp<0.001 compared with arthritic control.

Effect of PEG3 on body weight changes in arthritic rats

CFA-induced arthritis resulted in significant suppression of body weight gain due to chronic inflammation, reduced food intake, metabolic disturbance, and systemic stress associated with rheumatoid arthritis. The normal control group showed a progressive increase in body weight from 181.52 ± 2.61 g to 224.84 ± 3.12 g, representing normal physiological growth. However, arthritic control animals exhibited significantly lower weight gain, reaching only 191.38 ± 2.87 g at the end of the study. Treatment with diclofenac sodium significantly improved body weight gain (218.65 ± 3.05 g). Similarly, PEG3-treated animals showed significant recovery in body weight (214.42 ± 2.96 g) compared with untreated arthritic animals. The restoration of body weight in PEG3-treated animals indicates reduction of systemic inflammatory stress and improvement in overall physiological status. The beneficial effect may be associated with suppression of inflammatory cytokines and oxidative stress by phytoconstituents present in the herbal extracts.

Table 5. Effect of PEG3 on Body Weight in CFA-Induced Arthritic Rats

Group	Treatment	Initial Weight (g)	Final Weight (g)	Weight Change (g)
I	Normal control	181.52 ± 2.61	224.84 ± 3.12	$+43.32 \pm 2.18$
II	Arthritic control	182.07 ± 2.45	$191.38 \pm 2.87^{**}$	$+9.31 \pm 1.46^{**}$
III	Diclofenac sodium	181.89 ± 2.74	$218.65 \pm 3.05^{##}$	$+36.76 \pm 1.91^{##}$
IV	PEG3	181.74 ± 2.58	$214.42 \pm 2.96^{##}$	$+32.68 \pm 1.84^{##}$

^{***}p<0.001 compared with normal control; ^{###}p<0.001 compared with arthritic control.

Effect of PEG3 on arthritic score index

The severity of arthritis was evaluated by measuring clinical arthritic scores over 28 days. CFA injection produced progressive joint inflammation, resulting in increased arthritic scores from day 7 onwards. The arthritic control group showed a maximum score of 4.36 ± 0.09 on day 28, indicating severe joint inflammation and deformity. Treatment with diclofenac sodium significantly reduced arthritic severity, with the score decreasing to 0.52 ± 0.06 at day 28. PEG3 administration also significantly reduced arthritis progression, lowering the score to 0.88 ± 0.08 . The

reduction in arthritic score demonstrates the ability of PEG3 to suppress inflammatory progression and joint damage. The antiarthritic activity of PEG3 may be attributed to inhibition of inflammatory pathways and modulation of immune responses. Rosmarinic acid, carnosic acid, and flavonoids from *S. officinalis* together with phenolic and steroidal constituents of *S. brevistigma* may contribute through antioxidant activity and inhibition of inflammatory mediators.

Table 6. Effect of PEG3 on Arthritic Score Index in CFA-Induced Arthritis

Group	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal control	0	0	0	0	0
II	Arthritic control	0	1.82 ± 0.12	2.96 ± 0.14	3.74 ± 0.11	4.36 ± 0.09
III	Diclofenac sodium	0	1.79 ± 0.10	$1.68 \pm 0.09^{**}$	$1.02 \pm 0.08^{**}$	$0.52 \pm 0.06^{**}$
IV	PEG3	0	1.81 ± 0.11	$2.08 \pm 0.10^{*}$	$1.42 \pm 0.09^{**}$	$0.88 \pm 0.08^{**}$

Effect of PEG3 on inflammatory biomarkers

CFA induction significantly elevated inflammatory biomarkers including TNF- α , IL-1 β , IL-6, CRP, and ESR compared with normal animals. The arthritic control group showed marked elevation of TNF- α (95.74 ± 2.84 pg/mL), IL-1 β (84.31 ± 2.41 pg/mL), and IL-6 (102.46 ± 3.12 pg/mL), confirming activation of inflammatory pathways. Treatment with PEG3 significantly reduced inflammatory cytokines, lowering TNF- α to 43.56 ± 1.74 pg/mL, IL-1 β to 35.72 ± 1.42 pg/mL, and IL-6 to 42.64 ± 1.65 pg/mL. Diclofenac sodium produced slightly greater suppression; however, PEG3 demonstrated comparable anti-inflammatory activity. The reduction in inflammatory cytokines indicates that

PEG3 effectively regulates inflammatory cascades involved in rheumatoid arthritis progression. The observed activity may result from inhibition of NF- κ B-mediated inflammatory signaling and reduction of oxidative stress.

Table 7. Effect of PEG3 on Inflammatory Markers in CFA-Induced Arthritic Rats

Group	TNF- α (pg/m L)	IL-1 β (pg/m L)	IL-6 (pg/m L)	CRP (mg/L)	ESR (mm/h)
Normal control	24.83 $\pm$ 1.26	18.42 $\pm$ 0.91	21.56 $\pm$ 1.12	1.21 $\pm$ 0.08	4.32 $\pm$ 0.31
Arthritic control	95.74 $\pm$ 2.84 ^a	84.31 $\pm$ 2.41 ^a	102.4 $\pm$ 3.12 ^a	7.84 $\pm$ 0.29 ^a	18.72 $\pm$ 0.64 ^a
Diclofenac sodium	37.18 $\pm$ 1.53 ^b	29.46 $\pm$ 1.24 ^b	35.83 $\pm$ 1.47 ^b	2.34 $\pm$ 0.11 ^b	6.41 $\pm$ 0.38 ^b
PEG3	43.56 $\pm$ 1.74 ^b	35.72 $\pm$ 1.42 ^b	42.64 $\pm$ 1.65 ^b	2.91 $\pm$ 0.13 ^b	7.23 $\pm$ 0.42 ^b

Radiographic / Radiological Observations

Radiological observations remind an important diagnostic tool to study the progression of RA and also to confirm the therapeutic effect of anti-arthritic drugs. Periarticular soft tissue swelling / accompanied by marginal erosions is considered the earlier radiographic sign in RA. Prominent changes include narrowing of the joint spaces and bony erosions that result from the destructive activity of the pannus. Bone erosions have been found to be a specific and sensitive sign which indicates pathophysiologic process of the RA disease in the developed stage.

Table 8: Radiographic scoring of ankle joints in CFA-induced arthritic rats after topical treatment

Group	Treatment	Dose	Radiographic Score (0–4)	Radiographic Findings
Group I	Normal Control	Gel base	0.18 $\pm$ 0.06	Normal joint

		(Topical)		architecture, intact articular space, no bone erosion or soft tissue swelling.
Group II	Arthritic Control	CFA + Gel base	3.82 $\pm$ 0.15*	Severe soft tissue swelling, marked joint space narrowing, extensive bone erosion, periosteal reaction, and deformity.
Group III	Standard	CFA + Diclofenac sodium (10 mg/kg, topical)	1.12 $\pm$ 0.10###	Significant reduction in inflammation and edema with preservation of joint space and minimal bone erosion.

Group IV	PEG3	CFA + Polyherbal Ethosomal Gel (≈500 mg/day, topical)	1.46 ± 0.12###	Considerable reduction in soft tissue swelling, mild bone erosion, improved joint architecture, and partial restoration of joint space.



ol (A) Diseased Control (B) Standard (C) Polyherbal ethosomalgel treated (D)

Figure 1: Radiographic analysis showing of CFA induced arthritis in rats at 42 days of treatment after CFA injection

The in vivo findings demonstrated that the optimized polyherbal ethosomal gel (PEG3) exhibited significant antiarthritic activity against CFA-induced rheumatoid arthritis. PEG3 effectively reduced paw edema, improved body weight gain, decreased arthritic scores, normalized inflammatory biomarkers, and protected against systemic inflammatory alterations. The therapeutic efficacy of PEG3 was comparable to diclofenac sodium, indicating that the ethosomal carrier system successfully enhanced delivery and biological availability of herbal phytoconstituents. The observed antiarthritic activity can be attributed to the synergistic effects of phenolics, flavonoids,

terpenoids, and steroidal constituents from *Salvia officinalis* and *Sarcostemma brevistigma*. The ethosomal formulation further enhanced skin penetration, sustained release, and localization of active constituents at inflammatory sites. Therefore, PEG3 represents a promising topical herbal therapeutic approach for the management of rheumatoid arthritis with potential advantages of improved safety and reduced adverse effects associated with conventional anti-inflammatory drugs.

Conclusion

The in vivo antiarthritic activity of the optimized polyherbal ethosomal gel (PEG3) was evaluated in Complete Freund's Adjuvant (CFA)-induced rheumatoid arthritis in rats. Topical administration of PEG3 significantly reduced paw edema, arthritic score, and inflammatory progression compared with the arthritic control group. The formulation restored body weight, normalized spleen, liver, and kidney indices, and significantly decreased serum inflammatory markers including TNF- α , IL-1 β , IL-6, CRP, ESR, and rheumatoid factor. Radiographic evaluation demonstrated reduced soft tissue swelling, preservation of joint space, and decreased bone erosion. The observed activity may be attributed to enhanced skin penetration and sustained delivery of bioactive phytoconstituents from *Salvia officinalis* and *Sarcostemma brevistigma* through the ethosomal system. Overall, PEG3 exhibited promising antiarthritic potential comparable to diclofenac sodium, supporting its therapeutic application as a topical herbal intervention for rheumatoid arthritis.

References

- Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388(10055):2023-38. doi:10.1016/S0140-6736(16)30173-8.
- McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med*. 2011;365(23):2205-19. doi:10.1056/NEJMr1004965.
- McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol*. 2007;7(6):429-42. doi:10.1038/nri2094.
- Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA*. 2018;320(13):1360-72. doi:10.1001/jama.2018.13103.
- Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Control Release*. 2000;65(3):403-18. doi:10.1016/S0168-3659(99)00222-9.
- Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev Res*. 2000;50(3-4):406-15. doi:10.1002/1098-2299(200007/08)50:3/4<406::AID-DDR23>3.0.CO;2-M.
- Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. *Int J Pharm*. 2006;322(1-2):60-6. doi:10.1016/j.ijpharm.2006.05.027.
- Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. *Pharmazie*. 2007;62(2):133-7. doi:10.1691/ph.2007.2.6082.
- Kare PK, Malviya K. Pharmacognostical, phytochemical and in vitro anti-inflammatory potential of ethanolic extract of *Sarcostemma brevistigma* stems. *J Adv Zool*. 2022;43(1). doi:10.53555/jaz.v43i1.4623.
- Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 1999;299:152-78. doi:10.1016/S0076-6879(99)99017-1.
- Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol*. 1968;20(3):169-73. doi:10.1111/j.2042-7158.1968.tb09718.x.
- Rainsford KD. Adjuvant polyarthritis in rats: is this a satisfactory model for screening anti-arthritis drugs? *Agents Actions*. 1982;12(4):452-8. doi:10.1007/BF01965926.
- Lam FY, Ko IWM, Ng ESK, Tam LS, Leung PC, Li EKM. Analgesic and antiarthritic effects of Lingzhi and San Miao San supplementation in a rat model of arthritis induced by Freund's complete adjuvant. *J Ethnopharmacol*. 2008;120(1):44-50. doi:10.1016/j.jep.2008.07.028.
- Chillingworth NL, Donaldson LF. Characterisation of a Freund's complete adjuvant-induced model of chronic arthritis in mice. *J Neurosci Methods*. 2003;128(1-2):45-52. doi:10.1016/S0165-0270(03)00147-X.
- International Council for Harmonisation. Stability testing of new drug substances and products Q1A(R2). Geneva: ICH; 2003.
- Smolen JS, Landewé RBM, Bergstra SA, Kerschbaumer A, Sepriano A, Aletaha D, et al. EULAR recommendations for the management of rheumatoid arthritis with

- synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann Rheum Dis.* 2023;82(1):3-18. doi:10.1136/ard-2022-223356.
17. Finckh A, Gilbert B, Hodgkinson B, Bae SC, Thomas R, Deane KD, et al. Global epidemiology of rheumatoid arthritis. *Nat Rev Rheumatol.* 2022;18:591-602.
 18. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. *JAMA.* 2018;320(13):1360-1372.
 19. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med.* 2011;365(23):2205-2219.
 20. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet.* 2016;388(10055):2023-2038.
 21. Sparks JA. Rheumatoid arthritis. *Ann Intern Med.* 2019;170(1):ITC1-ITC16.
 22. Fraenkel L, Bathon JM, England BR, St Clair EW, Arayssi T, Carandang K, et al. 2021 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Care Res (Hoboken).* 2021;73(7):924-939.