ORIGINAL ARTICLE

Enhanced Anti-Arthritic Activity of a Garcinol-Loaded Liposomal Hydrogel in Complete Freund's Adjuvant-Induced Arthritic Rats

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder characterized by synovial inflammation, cartilage degradation, and bone erosion, leading to progressive joint dysfunction. Garcinol, a natural polyisoprenylated benzophenone isolated from *Garcinia indica*, possesses potent anti-inflammatory and antioxidant properties; however, its therapeutic application is limited by poor aqueous solubility and low bioavailability. The present study aimed to develop a garcinol-loaded liposomal hydrogel and evaluate its anti-arthritic efficacy in Complete Freund's Adjuvant (CFA)-induced arthritic rats. **Objective:** This study aimed to develop a nanocarrier-based topical formulation of garcinol by incorporating optimized liposomes into a hydrogel matrix and to investigate its physicochemical characteristics, *in vitro* release behavior, and anti-arthritic efficacy in a CFA-induced RA model. **Materials and Methods:** Garcinol-loaded liposomes were prepared using the thin-film hydration method employing Phospholipon® 90H and cholesterol. The optimized liposomal formulation was incorporated into a Carbopol® 934 hydrogel matrix to obtain a topical liposomal hydrogel. The formulation was characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, physicochemical properties, and *in vitro* drug release. Anti-arthritic activity was evaluated in CFA-induced arthritic rats by assessing paw thickness, paw edema volume, arthritis score, body weight, and histopathological alterations. Methotrexate gel (1% w/w) was used as the standard treatment. **Results:** The optimized garcinol-loaded liposomes exhibited a particle size of 162.4 ± 3.2 nm, PDI of 0.246 ± 0.02, zeta potential of −27.6 ± 1.5 mV, and entrapment efficiency of 81.55 ± 2.1%. The liposomal hydrogel showed suitable physicochemical characteristics with a pH of 6.82 ± 0.11 and drug content of 98.24 ± 1.36%. *In vitro* release studies demonstrated sustained drug release over 24 h. *In vivo* studies revealed that treatment with garcinol-loaded liposomal hydrogel significantly reduced paw thickness, paw edema volume, and arthritis score while improving body weight compared with the CFA control group ($P < 0.001$). Histopathological examination confirmed marked protection against inflammatory cell infiltration, synovial hyperplasia, cartilage destruction, and bone erosion. The anti-arthritic efficacy of the liposomal hydrogel was superior to garcinol-loaded liposomes and comparable to methotrexate gel. **Discussion:** The enhanced anti-arthritic activity of the garcinol-loaded liposomal hydrogel may be attributed to the synergistic benefits of liposomal encapsulation and hydrogel-based topical delivery. Liposomes improved drug solubility and retention, whereas the hydrogel matrix provided sustained release and prolonged localization at the inflamed site. The observed reduction in inflammatory manifestations and joint damage supports the therapeutic potential of garcinol as a topical anti-arthritic agent. **Conclusion:** The developed garcinol-loaded liposomal hydrogel demonstrated significant anti-arthritic activity in CFA-induced arthritic rats by reducing inflammation, improving clinical symptoms, and protecting joint architecture. The formulation showed superior efficacy compared with garcinol-loaded liposomes and comparable therapeutic performance to methotrexate gel, indicating its potential as a promising topical treatment strategy for RA.

Key words: Anti-arthritic activity, complete Freund's adjuvant, garcinol, histopathology, liposomal hydrogel, nanocarriers, rheumatoid arthritis, topical drug delivery

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Received: 22-05-2026
Revised: 24-06-2026
Accepted: 30-06-2026

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic immune-mediated disorder that primarily affects synovial joints and is marked by persistent inflammation of the synovial tissue. Continuous inflammatory activity promotes structural damage to articular cartilage and underlying bone, leading to progressive joint deterioration. Clinically, the disease manifests as pain, swelling, stiffness, and deformity of the affected joints, often resulting in impaired mobility and a substantial decline in overall quality of life.[1] Current treatment strategies rely on non-steroidal anti-inflammatory drugs, corticosteroids, and disease-modifying antirheumatic drugs to control inflammation and delay disease progression; however, chronic administration of these agents is frequently limited by adverse reactions and concerns regarding long-term safety and compliance.[2]

Garcinol, a bioactive phytochemical isolated from *Garcinia indica*, has been extensively investigated for its therapeutic potential due to its pronounced antioxidant and inflammation-regulating activities. Previous studies have shown that garcinol suppresses inflammatory mediators and signaling pathways involved in chronic inflammatory disorders, suggesting its potential therapeutic role in RA.[3]

Liposomes are widely investigated nanocarriers that can improve the solubility, stability, and bioavailability of poorly water-soluble drugs. Furthermore, incorporation of liposomes into hydrogel systems can enhance skin retention, provide sustained drug release, and improve localized therapeutic efficacy.[4] Such nanocarrier-based topical systems offer the advantage of delivering drugs directly to inflamed tissues while minimizing systemic exposure.

The Complete Freund's Adjuvant (CFA)-induced arthritis model closely mimics the pathological and immunological features of human RA and is extensively used for evaluating anti-arthritic agents.[5] Therefore, the present study aimed to develop a garcinol-loaded liposomal hydrogel and evaluate its anti-arthritic efficacy in CFA-induced arthritic rats through assessment of paw thickness, paw edema volume, arthritis score, body weight, and histopathological changes.

MATERIALS AND METHODS

Preparation of garcinol-loaded liposomes

A thin-film hydration strategy was employed to formulate garcinol-loaded liposomes, enabling the generation of nanoscale phospholipid vesicles suitable for drug delivery applications.[6,7] Briefly, Garcinol (25 mg), Phospholipon® 90H (297.4 mg), and cholesterol (15.95 mg) were accurately weighed and dissolved in a chloroform: methanol mixture (2:1, v/v). A thin lipid film was generated by evaporating the organic solvent under reduced pressure using a rotary evaporator set at 40°C and 60 rpm. The deposited lipid layer was hydrated with 10 mL of phosphate-buffered saline (pH 7.4) maintained at 60°C to produce liposomal vesicles. To obtain a homogeneous nanosized formulation, the vesicular suspension was subsequently treated with probe sonication for 153 s.[8]

Characterization of optimized liposomes

Particle size, polydispersity index (PDI), and zeta potential

Characterization of the optimized liposomes included the assessment of particle size distribution, PDI, and surface charge (zeta potential). These parameters were evaluated to assess vesicle size distribution, homogeneity, and colloidal stability.[9]

Entrapment efficiency

Entrapment efficiency was evaluated by separating free drug from liposome-associated drug through centrifugation. Ultraviolet (UV)–visible spectrophotometry was employed to estimate the concentration of garcinol remaining in the supernatant, following which the percentage entrapment efficiency was determined using the appropriate calculation formula.

$$\text{Entrapment Efficiency (\%)} = \text{Total Drug} - \text{Free Drug} / \text{Total Drug} \times 100$$

High entrapment efficiency indicates efficient incorporation of the lipophilic drug within the phospholipid bilayer.[10]

Preparation of hydrogel

A Carbopol® 934-based hydrogel was prepared by dispersing the polymer (1.5% w/w) in distilled water followed by a hydration period of 24 h to facilitate gel formation.[11] Propylene glycol and glycerin were incorporated to enhance hydration and consistency, while methyl paraben and propyl paraben were used to prevent microbial contamination during storage. The pH was adjusted to 6.5–7.0 using triethanolamine to obtain a homogeneous hydrogel.

Preparation of garcinol-loaded liposomal hydrogel

The optimized garcinol-loaded liposomal dispersion was gradually incorporated into the Carbopol hydrogel base under continuous stirring to ensure uniform distribution of liposomes throughout the gel matrix. The prepared formulation was allowed to equilibrate for 24 h before evaluation.[12]

Evaluation of liposomal hydrogel

Physicochemical characterization of the liposomal hydrogel was carried out by evaluating parameters including visual

homogeneity, pH, viscosity, spreadability, extrudability, and drug content, which are critical indicators of its performance as a topical formulation.[11,12]

In vitro drug release study

Drug release studies were conducted using a Franz diffusion cell system to compare the release profiles of garcinol-loaded liposomes and liposomal hydrogel. Phosphate-buffered saline (pH 7.4) maintained at physiological temperature served as the receptor phase. Aliquots were withdrawn at regular intervals and analyzed using UV spectrophotometry for quantification of released drug.[13]

Experimental animals

Adult male Wistar rats weighing 180–220 g were employed in the study. The animals were kept under regulated laboratory conditions, including controlled temperature and light cycles, and were provided unrestricted access to standard feed and potable water.

Ethical approval

The proposed animal study protocol was reviewed and sanctioned by the Institutional Animal Ethics Committee (IAEC), Richa Brijesh Science LLP, Bhopal, India. Ethical approval was granted under reference number 2356/PO/Rc/S/2025/CCSEA before commencement of the experiments. All procedures involving animals were conducted in accordance with CPCSEA guidelines for the ethical care and use of laboratory animals.

Induction of arthritis using CFA

A RA-like condition was produced by delivering 0.1 mL of CFA into the left hind paw through the subplantar route. Clinical manifestations associated with arthritis were monitored throughout the study duration to evaluate disease development and treatment response.[5]

Experimental design and treatment protocol

Animals were randomly divided into five groups (n = 6) shown in Table 1.

Table 1: Experimental design

Group	Treatment
Group I	Normal saline (Normal control)
Group II	CFA only (Arthritic control)
Group III	Garcinol-loaded liposomes (Topical)
Group IV	Garcinol-loaded liposomal hydrogel (Topical)
Group V	Methotrexate gel 1% W/W (Topical standard)

Treatments were administered topically once daily for 24 days.

Measurement of paw thickness

Paw thickness was measured using a digital vernier caliper at predetermined intervals and used as an indicator of inflammatory swelling.[14]

Measurement of paw edema volume

Hind paw edema was monitored by measuring paw volume with a digital plethysmometer at baseline and subsequently on days 7, 14, 21, and 24 after treatment initiation.[14]

Assessment of arthritis score

Arthritis severity was assessed using a standard scoring system ranging from 0 (normal paw) to 4 (severe inflammation and deformity).

Measurement of body weight

Body weight was recorded periodically throughout the study using a digital balance.

Histopathological analysis

At the end of the experimental study, ankle joint specimens were isolated and preserved in 10% buffered formalin solution. Histological sections were prepared using standard tissue processing techniques, stained with hematoxylin and eosin, and examined microscopically for assessment of inflammatory and structural changes.[14]

Statistical analysis

For all measured parameters, results were expressed as mean ± standard error of the mean (SEM) based on observations from six rats per group. Comparative statistical assessment of the experimental groups was carried out using a single-factor analysis of variance (ANOVA). Subsequent pairwise comparisons were performed using Tukey's test to determine the source of significant variation. A P-value below 0.05 was taken as evidence of statistical significance.

RESULTS

Physicochemical characterization of optimized garcinol-loaded liposomes

Characterization studies revealed that the optimized garcinol-loaded liposomes displayed favorable physicochemical

properties. As shown in Table 2, the vesicles had an average diameter of 162.4 ± 3.2 nm [Figure 1] with a PDI of 0.246 ± 0.02, indicating a narrow size range. Furthermore, the zeta potential was recorded as −27.6 ± 1.5 mV [Figure 2], suggesting sufficient electrostatic stabilization of the formulation. Furthermore, the formulation exhibited a high entrapment efficiency of 81.55 ± 2.1%, confirming efficient incorporation of garcinol within the phospholipid bilayer.

Physicochemical evaluation of garcinol-loaded liposomal hydrogel

The prepared garcinol-loaded liposomal hydrogel was evaluated for various physicochemical parameters, and the results are presented in Table 3. The formulation appeared smooth, homogeneous, and free from phase separation. The pH was found to be 6.82 ± 0.11, indicating compatibility with skin pH. The hydrogel exhibited appropriate viscosity, satisfactory spreadability, and good extrudability. Drug content analysis demonstrated 98.24 ± 1.36% drug incorporation, indicating uniform distribution of garcinol within the hydrogel matrix.

In vitro drug release study

A comparative evaluation of garcinol release from the liposomal and liposomal hydrogel formulations was performed, with the release data summarized in Table 4. Both formulations exhibited sustained drug release over 24 h. The liposomal formulation showed a comparatively faster release, achieving 95.62 ± 3.34% cumulative drug release at 24 h. In contrast, the liposomal hydrogel exhibited a more controlled release profile with 91.26 ± 3.18% cumulative drug release at the end of 24 h. The slower release from the hydrogel may be attributed to the additional diffusion barrier provided by the Carbopol matrix.

Table 2: Physicochemical characteristics of optimized garcinol-loaded liposomes

Parameter	Result (Mean±SD, *n*=3)
Particle size (nm)	162.4±3.2
Polydispersity index (PDI)	0.246±0.02
Zeta potential (mV)	−27.6±1.5
Entrapment efficiency (%)	81.55±2.1

Table 3: Physicochemical evaluation of garcinol-loaded liposomal hydrogel

Parameter	Result
Appearance	Smooth, homogeneous gel
Color	Pale yellow
Homogeneity	Uniform, no phase separation
pH	6.82±0.11
Viscosity (cps)	18,420±315
Spreadability (g·cm/s)	21.84±0.92
Extrudability (g)	486±12
Drug content (%)	98.24±1.36

Effect of treatments on paw thickness

CFA-induced arthritic rats exhibited a progressive increase in paw thickness throughout the study, confirming the development of inflammatory arthritis. Treatment with garcinol-loaded liposomes and garcinol-loaded liposomal hydrogel significantly alleviated paw swelling compared with the CFA control group, demonstrating their anti-arthritic potential [Figure 3]. The liposomal hydrogel exhibited greater anti-inflammatory activity and showed results comparable to methotrexate gel [Table 5].

Effect of treatments on paw edema volume

The CFA control group showed a significant increase in paw edema volume throughout the study period.

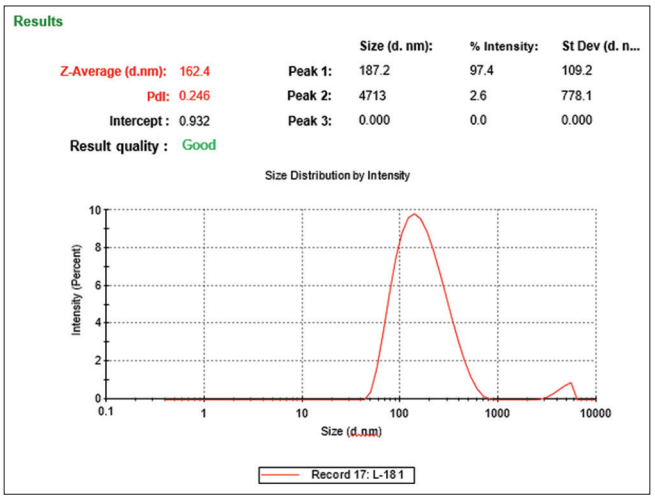

Figure 1: Particle size distribution of optimized garcinol-loaded liposomes

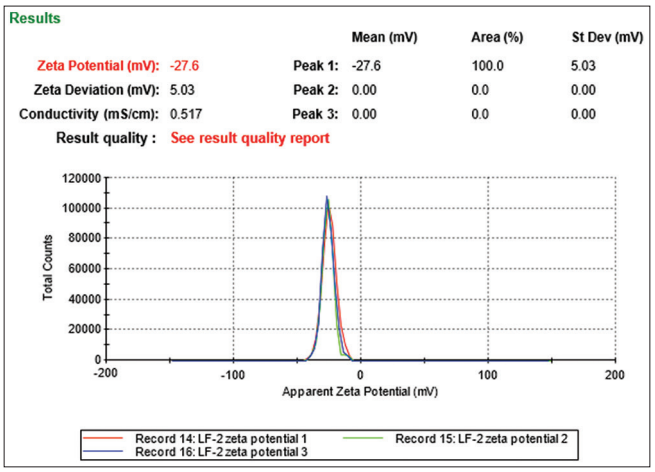

Figure 2: Zeta potential distribution of optimized garcinol-loaded liposomes

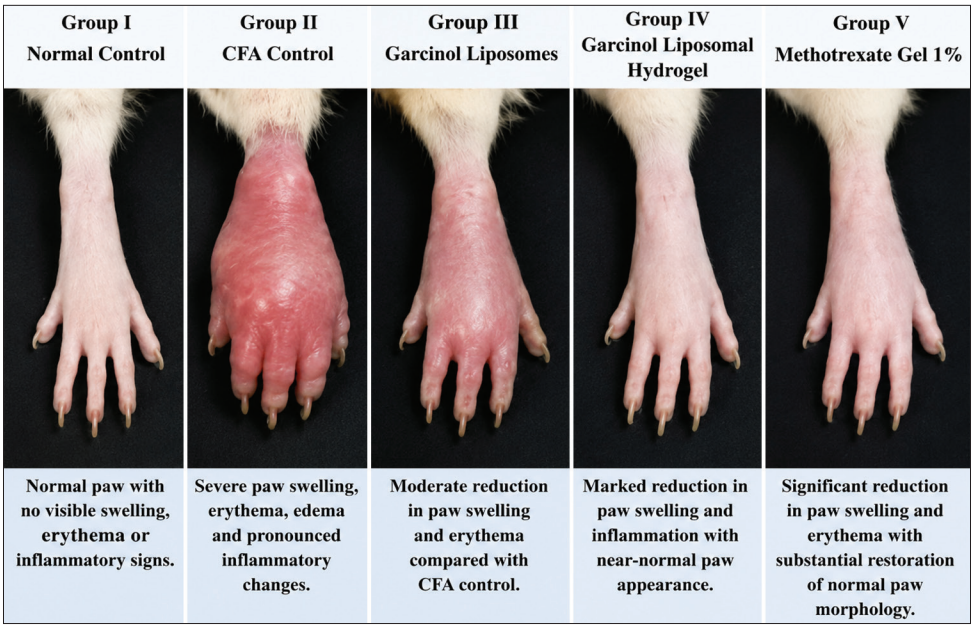

Figure 3: Effect of treatments on paw thickness

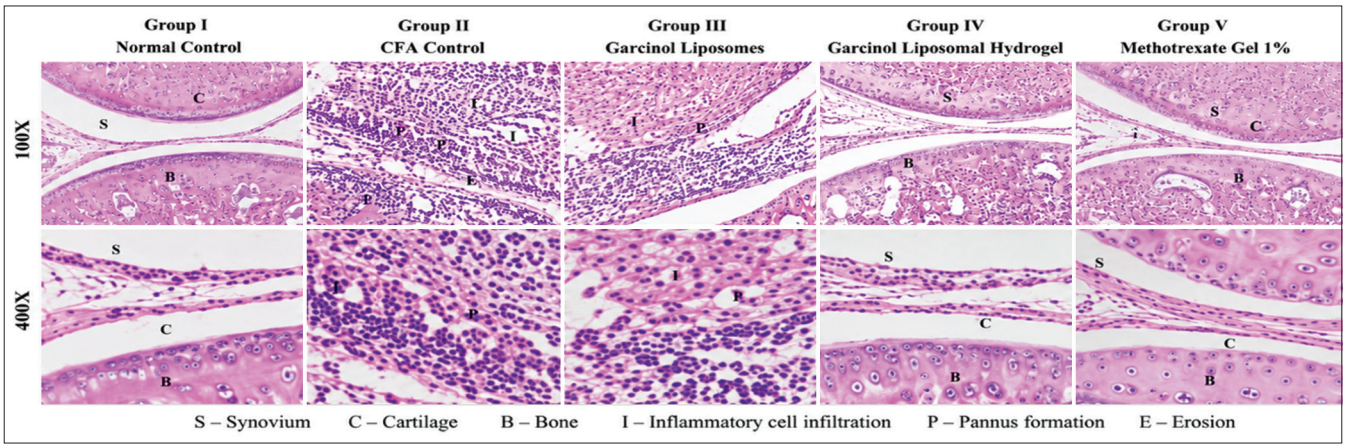

Figure 4: Histopathological assessment of anti-arthritic activity of garcinol-loaded liposomal hydrogel in complete Freund's adjuvant-induced arthritic rats using hematoxylin and eosin-stained ankle joint sections

Treatment with garcinol-loaded liposomes and garcinol-loaded liposomal hydrogel significantly suppressed edema formation. The liposomal hydrogel group demonstrated greater inhibition of paw swelling than the liposome-treated group and approached the efficacy of methotrexate gel [Table 6].

Effect of treatments on arthritis score

A progressive increase in arthritis score was observed in the CFA control group, indicating severe arthritic manifestations. Treatment with garcinol-loaded formulations significantly reduced arthritis severity. The garcinol-loaded liposomal hydrogel exhibited superior efficacy compared with garcinol-loaded liposomes and produced a marked reduction in arthritis score [Table 7].

Effect of treatments on body weight

CFA-induced arthritic rats showed a significant reduction in body weight compared with normal animals. Treatment with garcinol-loaded liposomes and garcinol-loaded liposomal hydrogel significantly improved body weight gain. The liposomal hydrogel group demonstrated better restoration of body weight than the liposome-treated group and showed results comparable to methotrexate gel [Table 8].

Histopathological analysis

Histological assessment of ankle joint tissues indicated normal morphology and structural integrity in the healthy control animals. However, the CFA-treated arthritic group displayed severe inflammatory responses characterized by

Table 4: *In vitro* drug release profile of garcinol-loaded liposomes and garcinol-loaded liposomal hydrogel

Time (h)	Garcinol-loaded liposomes (% cumulative drug release)	Garcinol-loaded liposomal hydrogel (% cumulative drug release)
0	0.00±0.00	0.00±0.00
1	12.84±1.21	8.25±0.92
2	22.68±1.48	15.72±1.15
4	38.52±2.14	28.64±1.86
6	52.75±2.48	40.82±2.15
8	64.38±2.76	51.45±2.38
10	72.64±2.92	60.82±2.54
12	79.28±3.08	68.54±2.68
16	86.42±3.15	78.36±2.85
20	91.84±3.22	85.48±3.04
24	95.62±3.34	91.26±3.18

dense inflammatory cell accumulation, synovial thickening, pannus formation, progressive cartilage damage, and erosion of the underlying bone tissue. Treatment with garcinol-loaded liposomes significantly reduced these pathological changes, whereas the garcinol-loaded liposomal hydrogel showed marked preservation of joint architecture and reduced inflammatory damage [Figure 4]. The therapeutic effect of the liposomal hydrogel was comparable to methotrexate gel [Table 9].

Statistical interpretation

Results are presented as mean ± SEM (n = 6). Statistical evaluation was performed using one-way ANOVA with Tukey's multiple comparison test. Symbol ### denotes $P < 0.001$ versus the normal control group. Symbols ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively, versus the CFA-induced arthritic group.

The significant reduction in histopathological score observed in the garcinol-loaded liposomal hydrogel-treated group confirms its potent anti-arthritic activity and demonstrates its ability to protect against CFA-induced joint inflammation, cartilage degradation, and bone destruction.

Table 5: Effect of treatments on paw thickness

Day	Normal control	CFA control	Garcinol liposomes	Garcinol liposomal hydrogel	Methotrexate gel
0	5.25±0.21	5.28±0.24	5.27±0.22	5.26±0.23	5.24±0.21
1	5.26±0.22	5.85±0.25	5.82±0.24	5.78±0.22	5.75±0.21
2	5.28±0.22	6.35±0.28	6.22±0.26	6.08±0.24	6.02±0.23
3	5.29±0.23	6.82±0.31	6.65±0.29	6.42±0.26	6.32±0.24
4	5.30±0.22	7.12±0.33	6.92±0.30	6.58±0.27	6.45±0.25
5	5.31±0.23	7.42±0.34	7.08±0.31	6.65±0.28	6.50±0.26
6	5.31±0.22	7.65±0.35	7.15±0.32	6.68±0.28	6.48±0.26
7	5.32±0.22	7.85±0.35	7.12±0.31	6.68±0.28	6.42±0.26
8	5.33±0.23	8.05±0.36	7.25±0.32	6.72±0.28	6.40±0.25
9	5.34±0.23	8.22±0.38	7.38±0.33	6.75±0.29	6.38±0.25
10	5.35±0.23	8.38±0.39	7.52±0.34	6.82±0.29	6.42±0.25
11	5.36±0.24	8.55±0.40	7.65±0.35	6.88±0.30	6.48±0.26
12	5.37±0.24	8.72±0.41	7.78±0.35	6.92±0.30	6.55±0.27
13	5.37±0.24	8.82±0.42	7.82±0.36	6.95±0.30	6.62±0.27
14	5.38±0.24	8.92±0.42	7.86±0.36	6.95±0.30	6.70±0.28
15	5.39±0.23	9.02±0.43	7.78±0.35	6.88±0.29	6.58±0.27
16	5.40±0.23	9.12±0.44	7.68±0.35	6.80±0.29	6.48±0.27
17	5.40±0.23	9.18±0.44	7.58±0.34	6.72±0.28	6.40±0.26
18	5.41±0.23	9.24±0.45	7.48±0.34	6.65±0.28	6.32±0.26
19	5.41±0.23	9.28±0.45	7.42±0.34	6.58±0.28	6.25±0.26
20	5.42±0.23	9.30±0.46	7.36±0.33	6.52±0.29	6.18±0.26
21	5.42±0.23	9.35±0.46	7.30±0.33	6.45±0.28	6.10±0.25
22	5.43±0.22	9.42±0.47	7.25±0.33	6.35±0.28	6.02±0.25
23	5.44±0.22	9.50±0.48	7.22±0.32	6.28±0.27	5.96±0.24
24	5.45±0.22	9.58±0.48	7.18±0.32	6.21±0.27	5.92±0.24

Table 6: Effect of treatments on paw edema volume

Day	Normal control	CFA control	Garcinol liposomes	Garcinol liposomal hydrogel	Methotrexate gel 1%
0	0.45±0.03	0.45±0.03	0.45±0.03	0.45±0.03	0.45±0.03
7	0.46±0.04	2.22±0.18###	1.58±0.11**	1.28±0.10***	1.15±0.09***
14	0.48±0.05	3.10±0.21###	2.28±0.15**	1.72±0.13***	1.55±0.12***
21	0.49±0.04	3.55±0.22###	2.82±0.18**	1.95±0.15***	1.78±0.14***
24	0.50±0.04	3.85±0.20###	2.72±0.17**	1.88±0.16***	1.70±0.15***

Symbol ### denotes P < 0.001 versus the normal control group. Symbols ** and *** indicate P < 0.01 and P < 0.001, respectively, versus the CFA-induced arthritic group.

Table 7: Effect of treatments on arthritis score

Day	Normal control	CFA control	Garcinol liposomes	Garcinol liposomal hydrogel	Methotrexate gel 1%
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7	0.00±0.00	1.35±0.12###	0.95±0.09*	0.72±0.07**	0.62±0.06***
14	0.00±0.00	2.32±0.18###	1.65±0.13**	1.18±0.10***	1.02±0.08***
21	0.00±0.00	3.05±0.22###	2.28±0.16**	1.55±0.12***	1.32±0.10***
24	0.00±0.00	3.30±0.24###	2.15±0.17**	1.48±0.12***	1.22±0.11***

Symbol ### denotes P < 0.001 versus the normal control group. Symbols ** and *** indicate P < 0.01 and P < 0.001, respectively, versus the CFA-induced arthritic group.

Table 8: Effect of treatments on body weight

Day	Normal control	CFA control	Garcinol-loaded liposomes	Garcinol-loaded liposomal hydrogel	Methotrexate gel 1%
0	198.5±4.2	199.2±4.5	198.8±4.3	199.0±4.1	198.7±4.4
7	204.8±4.6	191.6±4.1###	195.8±4.0*	198.9±4.3**	200.5±4.5**
14	212.3±5.1	182.4±3.8###	192.8±4.2**	202.6±4.8***	205.8±4.9***
21	216.8±5.3	177.2±3.6###	195.5±4.4**	205.4±4.9***	209.2±5.1***
24	220.5±5.5	173.8±3.5###	198.4±4.5**	208.7±5.1***	212.8±5.3***

Symbol ### denotes P < 0.001 versus the normal control group. Symbols ** and *** indicate P < 0.01 and P < 0.001, respectively, versus the CFA-induced arthritic group.

Table 9: Histopathological scores of ankle joint tissues

Group	Treatment	Histopathological score
Group I	Normal Control	0.33±0.21
Group II	CFA Control	3.85±0.15###
Group III	Garcinol Liposomes	2.42±0.18**
Group IV	Garcinol Liposomal Hydrogel	1.28±0.12***
Group V	Methotrexate Gel	0.95±0.10***

Symbol ### denotes P < 0.001 versus the normal control group. Symbols ** and *** indicate P < 0.01 and P < 0.001, respectively, versus the CFA-induced arthritic group.

DISCUSSION

The objective of the present investigation was to develop a liposomal hydrogel incorporating garcinol and to examine its effectiveness as a localized treatment strategy for RA. The optimized liposomal formulation exhibited desirable physicochemical characteristics, including nanosized vesicles (162.4 ± 3.2 nm), narrow size distribution (PDI 0.246 ± 0.02), adequate zeta potential (−27.6 ± 1.5 mV), and high entrapment efficiency (81.55 ± 2.1%), indicating successful formulation of a stable nanocarrier system. Incorporation of the optimized liposomes into a Carbopol® hydrogel matrix produced a homogeneous formulation with suitable pH, viscosity, spreadability, and drug content, making it appropriate for topical application.

The *in vitro* drug release study demonstrated sustained release of garcinol from both liposomes and liposomal hydrogel over 24 h. However, the liposomal hydrogel exhibited a comparatively slower and more controlled release profile than liposomes alone, which may be attributed to the additional diffusion barrier provided by the hydrogel matrix. Sustained release is advantageous for topical therapy because it prolongs drug retention at the site of inflammation and may reduce the frequency of administration.[6,11]

The CFA-mediated arthritis model is considered a clinically relevant experimental approach because it replicates major inflammatory and structural abnormalities characteristic of RA, including synovial hyperplasia, pannus formation, cartilage damage, and bone erosion.[5] In the present study, CFA administration produced a marked increase in paw thickness, paw edema volume, and arthritis score, accompanied by body weight loss and severe histopathological alterations, confirming successful induction of arthritis.

Paw thickness and paw edema volume are important indicators of inflammatory severity and disease progression. The CFA control group showed progressive paw swelling throughout the study period, reflecting inflammatory cell infiltration, vascular permeability, and synovial inflammation. Treatment with garcinol-loaded liposomes significantly reduced paw swelling; however, the garcinol-loaded liposomal hydrogel exhibited superior anti-inflammatory activity. The enhanced reduction in paw thickness and edema volume observed in the liposomal hydrogel group may be attributed to improved drug retention, sustained release, and enhanced skin permeation achieved through the combination of liposomal and hydrogel-based delivery systems.[10,12]

Similarly, arthritis scores were markedly elevated in the CFA control group, indicating severe disease progression. Treatment with the garcinol-loaded liposomal hydrogel significantly reduced arthritis severity compared with the arthritic control and liposome-treated groups. The reduction in arthritis score suggests effective suppression of inflammatory processes and protection against joint damage. These findings are consistent with previous reports demonstrating the anti-inflammatory potential of garcinol and nanocarrier-mediated drug delivery systems.[3,7]

Body weight loss is commonly observed in chronic arthritis due to systemic inflammation, reduced food intake, and metabolic disturbances. In the present study, arthritic animals exhibited significant body weight reduction, whereas treatment with the garcinol-loaded liposomal hydrogel restored body weight toward normal levels. This improvement may reflect attenuation of systemic inflammatory burden and improvement in overall physiological status.

Histopathological evaluation provided additional support for the therapeutic efficacy of the developed formulation. The CFA control group exhibited severe joint abnormalities, including extensive inflammatory cell infiltration, synovial tissue proliferation, pannus formation, cartilage degeneration, and bone erosion, which are characteristic features of inflammatory arthritis. In contrast, treatment with garcinol-loaded liposomal hydrogel markedly preserved joint architecture and significantly reduced histopathological damage. The histopathological score of the liposomal hydrogel group (1.28 ± 0.12) was substantially lower than that of the CFA control group (3.85 ± 0.15), indicating significant protection against arthritis-induced joint destruction.

The anti-arthritic effects observed in the present study may be linked to the pharmacological properties of garcinol. Previous reports have shown that this bioactive compound interferes with nuclear factor kappa B-mediated signaling and reduces the expression of several inflammatory cytokines and enzymes, such as tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6, cyclooxygenase-2, and inducible nitric oxide synthase, thereby limiting inflammation and oxidative stress.[3,7,8] Although inflammatory cytokines were not quantified in the present study, the significant improvement in clinical and histopathological parameters strongly supports the anti-inflammatory potential of Garcinol.

A notable finding of the present investigation was the superior efficacy of the garcinol-loaded liposomal hydrogel compared with garcinol-loaded liposomes alone. This enhancement may be attributed to the synergistic advantages of liposomal encapsulation and hydrogel-based topical delivery. Liposomes improve drug solubility and skin permeation, while the hydrogel matrix prolongs residence time and provides controlled drug release, resulting in improved local therapeutic efficacy.[10-12]

Overall, the anti-arthritic activity exhibited by the garcinol-loaded liposomal hydrogel was comparable to that of the standard methotrexate gel. These findings suggest that the developed formulation may represent a promising nanocarrier-based topical therapeutic approach for RA. However, further investigations involving inflammatory cytokine analysis, oxidative stress biomarkers, pharmacokinetic evaluation, and long-term safety studies are warranted to fully establish its therapeutic potential and clinical applicability.

CONCLUSION

The findings of the present work confirm the successful development of a garcinol-loaded liposomal hydrogel as an advanced topical drug delivery platform. The optimized formulation demonstrated desirable vesicular characteristics, efficient encapsulation of garcinol, and satisfactory physicochemical performance. The hydrogel matrix further enhanced formulation stability and provided controlled release characteristics suitable for localized therapeutic applications.

Administration of the garcinol-loaded liposomal hydrogel effectively alleviated the clinical manifestations of CFA-induced arthritis, as reflected by reductions in paw thickness, edema volume, and arthritis score. Improvements in body weight and preservation of joint morphology were also observed. Histopathological findings revealed diminished inflammatory responses and reduced structural damage within the joint tissues. The developed hydrogel demonstrated enhanced efficacy compared with liposomal garcinol and exhibited therapeutic effects comparable to the standard methotrexate formulation.

The enhanced therapeutic performance may be attributed to the combined advantages of liposomal encapsulation and hydrogel-based topical delivery, which improved drug retention, sustained release, and localized anti-inflammatory activity. Overall, the findings suggest that the developed garcinol-loaded liposomal hydrogel represents a promising nanocarrier-based topical therapeutic approach for RA and warrants further investigation through cytokine profiling, pharmacokinetic studies, and long-term safety evaluation to support its potential clinical application.

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Source of Support: Nil. **Conflicts of Interest:** None declared.