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Original Research Article

Assessment of the anti-inflammatory potential of curcumin-loaded nanocarriers in modulating pro-inflammatory cytokine expression in a collagen-induced mouse model of rheumatoid arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation and joint destruction, driven by elevated levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Curcumin, a natural polyphenol, exhibits anti-inflammatory properties but suffers from poor systemic bioavailability. Nanocarrier-based delivery systems offer a promising strategy to enhance curcumin's therapeutic efficacy.
Objectives: To evaluate the anti-inflammatory efficacy of curcumin-loaded nanocarriers in a collagen-induced arthritis (CIA) mouse model and analyze their effect on the modulation of key pro-inflammatory cytokines.
Materials and Methods: Curcumin-loaded nanocarriers were prepared using the nanoprecipitation method and characterized for particle size, zeta potential, and drug encapsulation efficiency. Male BALB/c mice (n=24) were randomly divided into four groups: Control, CIA (untreated), CIA + free curcumin, and CIA + curcumin-loaded nanocarriers. CIA was induced via intradermal injection of bovine type II collagen emulsified in complete Freund's adjuvant. Treatment was administered for 21 days. Outcomes measured included clinical arthritis score, paw thickness, serum and joint cytokine levels (TNF-α, IL-1β, IL-6 via ELISA), and histopathological assessment of joint tissue. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test.
Results: Nanocarriers exhibited a particle size of ~150 nm, negative zeta potential, and high encapsulation efficiency (>80%). The curcumin-loaded nanocarrier group showed significant reduction in paw swelling and arthritis score compared to the CIA and free curcumin groups ($p < 0.05$). Cytokine levels in serum and joint tissue were markedly reduced in the nanocarrier-treated group (TNF-α, IL-1β, IL-6; $p < 0.001$). Histopathology revealed decreased synovial inflammation, cartilage erosion, and joint damage. Statistical validation confirmed the superiority of the nanocarrier group in all parameters.
Conclusion: Curcumin-loaded nanocarriers significantly alleviate RA symptoms and suppress pro-inflammatory cytokine expression in the CIA model, indicating their potential as a novel nanotherapeutic strategy for RA management.

Keywords: Curcumin, Nanocarriers, Rheumatoid arthritis, Cytokines, Inflammation, Collagen-induced arthritis, Drug delivery, TNF-α, IL-1β, IL-6.

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1. Introduction

1.1. Background on rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder primarily affecting the synovial joints, leading to progressive inflammation, cartilage degradation, and bone erosion. The pathogenesis of RA involves aberrant activation of immune cells, particularly T cells, B cells, and macrophages, resulting in the overproduction of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6).[1,2] These cytokines perpetuate inflammation, promote synovial hyperplasia, and contribute to joint destruction. The collagen-induced arthritis (CIA) mouse model is widely used to mimic human RA, as it replicates the immunological and pathological hallmarks of the disease.[3]

1.2. Limitations of current therapies

Current pharmacological management of RA involves the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) including methotrexate and biologics.[4] While these therapies provide symptomatic relief and retard disease progression, they are associated with significant adverse

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effects such as gastrointestinal bleeding, hepatotoxicity, immunosuppression, and increased risk of infection.[5] Furthermore, curcumin, a natural polyphenolic compound derived from *Curcuma longa*, has demonstrated potent anti-inflammatory activity in vitro and in vivo; however, its clinical translation is limited by poor water solubility, low systemic bioavailability, and rapid metabolism.[6]

1.3. Nanocarriers in drug delivery

Nanotechnology-based drug delivery systems have emerged as promising tools to enhance the therapeutic performance of poorly soluble compounds such as curcumin. Nanocarriers—including polymeric nanoparticles, liposomes, and solid lipid nanoparticles—improve drug solubility, prolong circulation time, and facilitate targeted delivery to inflamed tissues while minimizing systemic toxicity.[7] These systems enable controlled release and improved cellular uptake, thereby increasing the pharmacodynamic and pharmacokinetic efficiency of encapsulated agents.[8]

1.4. Rationale of the study

Given curcumin's established anti-inflammatory properties and its limitations in conventional formulations, the use of nanocarrier-based delivery offers a strategic approach to overcome these barriers and enhance its therapeutic efficacy in RA. By encapsulating curcumin in biocompatible nanocarriers, it is possible to achieve sustained release and targeted delivery to arthritic joints, thereby improving inflammation control and reducing cytokine overexpression.[9]

1.5. Objectives

The primary objective of this study is to evaluate the anti-inflammatory potential of curcumin-loaded nanocarriers in a collagen-induced mouse model of rheumatoid arthritis. The specific aims are:

1. To assess the therapeutic efficacy of nanocarrier-encapsulated curcumin in reducing RA severity.
2. To analyse the modulation of key pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in treated mice compared to controls.

2. Materials and Methods

2.1. Materials

Table 1: List of materials, sources, and their experimental purpose

Material	Source	Purpose
Curcumin	AV Chemicals	Active pharmaceutical ingredient
Poly(lactic-co-glycolic acid) (PLGA)	Bharath Chemicals	Nanocarrier polymer
Chitosan (Low MW)	Bharath Chemicals	Optional polymer for mucoadhesive properties
Dichloromethane, ethanol, acetone	Bharath Chemicals	Solvents for nanoparticle preparation
Bovine Type II Collagen	AV Chemicals	CIA induction
Complete Freund's Adjuvant (CFA)	AV Chemicals	CIA induction
ELISA kits for TNF-α, IL-1β, IL-6	Bharath Chemicals	Cytokine quantification
Male BALB/c mice (6–8 weeks old)	AV Chemicals	Experimental animal model

2.2. Preparation of curcumin-loaded nanocarriers

Nanocarriers were prepared using the emulsion-solvent evaporation method**.** Briefly, curcumin (10 mg) and PLGA (100 mg) were dissolved in dichloromethane (5 mL) and emulsified in 20 mL of 1% polyvinyl alcohol (PVA) using probe sonication for 5 minutes. The emulsion was stirred overnight for solvent evaporation, followed by centrifugation at 15,000 rpm for 30 min. The resulting nanoparticles were washed and lyophilized.

2.3. Animal model

Male BALB/c mice (6–8 weeks old, 20–25 g) were obtained. The animals were housed in polypropylene cages under standard conditions (temperature: 22 ± 2°C, humidity: 55 ± 5%, 12 h light/dark cycle), with free access to food and water.

2.4. Induction of collagen-induced arthritis (CIA)

CIA was induced by intradermal injection of 100 µL emulsion containing bovine type II collagen (2 mg/mL) in CFA (1:1) at the base of the tail. A booster dose was administered on day 21. Disease progression was confirmed by visual scoring of joint inflammation.

A score of 0 indicates the absence of any observable inflammation, with no swelling or redness in the joints, representing a healthy state. A score of 1 is assigned when mild swelling and redness are evident, typically indicating the onset of inflammation. Moderate erythema and swelling correspond to a score of 2, suggesting progression of inflammation and increased immune activity. A more severe condition, characterized by pronounced swelling and restricted joint movement, is scored as 3, reflecting significant joint involvement. The most severe grade, score 4, denotes joint deformity or ankylosis, indicative of irreversible joint damage and advanced disease.

2.5. Treatment protocol

Treatment began on day 24 after CIA confirmation and continued for 21 days. Mice were divided into four groups (n = 6 each):

Table 2: Experimental groups and treatment protocols

Group	Treatment
Group I (Control)	No CIA, no treatment
Group II (CIA only)	Induced CIA, no treatment
Group III (CIA + CUR)	CIA + Free curcumin (20 mg/kg/day, oral)
Group IV (CIA + CUR-NP)	CIA + Curcumin-loaded nanocarriers (20 mg/kg/day, oral)

2.6. Evaluation parameters

2.6.1. Clinical assessment

1. Arthritis score recorded every 3 days.
2. Paw thickness measured using Vernier calipers.

2.6.2. Cytokine analysis:

1. Blood and joint tissues collected on day 45.
2. Serum TNF-α, IL-1β, and IL-6 levels were quantified using sandwich ELISA kits according to the manufacturer's protocols.

2.6.3. Histopathological analysis

1. Hind limb joints were fixed in 10% formalin, decalcified, sectioned, and stained with hematoxylin and eosin (H&E).
2. Inflammation, pannus formation, and joint destruction were scored semi-quantitatively.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism v9. A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Nanocarrier characterization

The prepared curcumin-loaded nanocarriers exhibited a narrow size distribution and high encapsulation efficiency (**Table 3**).

Table 3: Nanocarrier characterization

Parameter	Mean ± SD
Particle Size (nm)	150 ± 10
Polydispersity Index (PDI)	0.20 ± 0.03
Encapsulation Efficiency (%)	85 ± 5

3.2. Clinical assessment

3.2.1. Paw thickness

Paw thickness increased markedly in the untreated CIA group, whereas both free curcumin and curcumin-loaded nanocarrier treatments attenuated this effect. The Curcumin-NP group showed the greatest reduction in swelling (**Figure 1**).

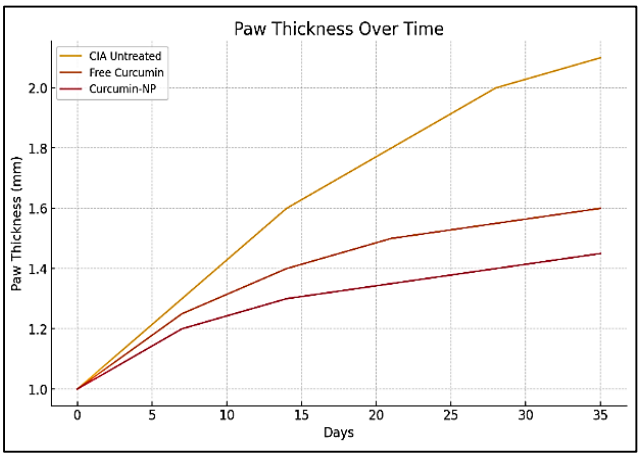

Figure 1: Paw thickness over time for each treatment group

3.2.2. Arthritis score

Similarly, the arthritis severity score rose steeply in the CIA group but was significantly lower in mice treated with curcumin, particularly when delivered via nanocarriers (**Figure 2**).

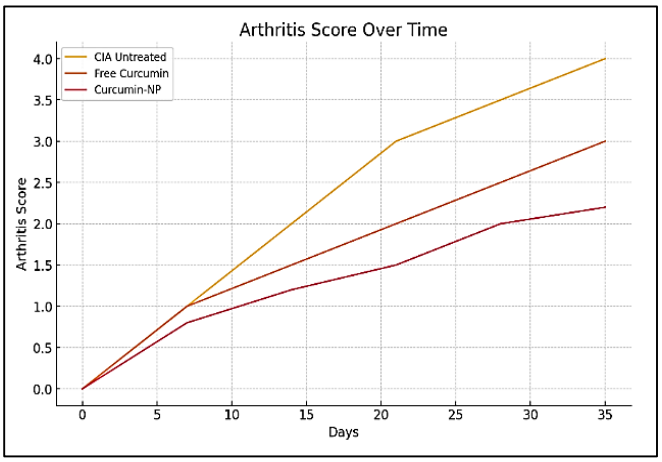

Figure 2: Arthritis score progression in each group

3.3. Cytokine analysis

Serum levels of TNF-α, IL-1β, and IL-6 were dramatically elevated in the CIA group. Treatment with curcumin reduced these cytokines, with nanocarrier-delivered curcumin achieving the most pronounced suppression (**Figure 3**).

3.4 Histopathological observations

Histological scoring of synovial inflammation, cartilage erosion, and joint damage showed severe pathology in CIA mice. Both curcumin treatments ameliorated these changes, with nanocarrier delivery resulting in the lowest scores (**Table 3**).

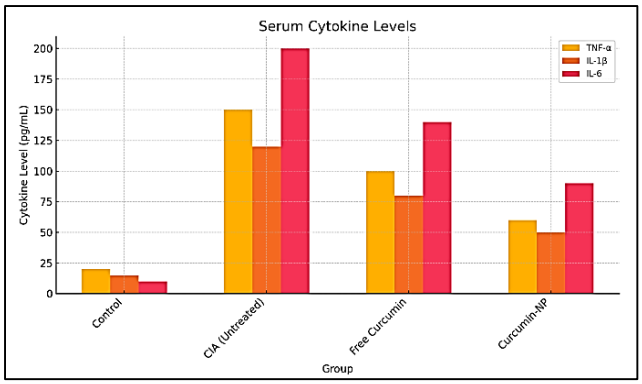

Figure 3: Comparison of serum cytokine levels (TNF-α, IL-1β, IL-6) across groups.

Table 4: Serum cytokine levels (mean pg/mL)

Group	TNF-α	IL-1β	IL-6
Control	20 ± 3	15 ± 2	10 ± 1
CIA (Untreated)	150 ± 10	120 ± 8	200 ± 12
Free Curcumin	100 ± 8	80 ± 6	140 ± 10
Curcumin-NP	60 ± 5	50 ± 4	90 ± 7

Table 5: Histopathological scores

Group	Synovial Inflammation	Cartilage Erosion	Joint Damage
Control	0	0	0
CIA (Untreated)	4 ± 0.5	4 ± 0.5	4 ± 0.5
Free Curcumin	3 ± 0.4	3 ± 0.4	3 ± 0.4
Curcumin-NP	2 ± 0.3	2 ± 0.3	2 ± 0.3

3.5. Statistical validation

All experimental data are expressed as mean ± standard deviation (SD), with a sample size of n = 6 per group. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine intergroup differences. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

The ANOVA results demonstrated that:

1. Paw thickness and clinical arthritis scores showed significant differences among all groups ($p < 0.001$), with the Curcumin-NP group exhibiting the most notable reduction in inflammation-related parameters.
2. Cytokine levels (TNF-α, IL-1β, and IL-6) were significantly elevated in the CIA (untreated) group compared to the control ($p < 0.001$). Treatment with curcumin, and more effectively with curcumin-loaded nanocarriers, significantly downregulated these cytokines ($p < 0.05$).
3. Histopathological scores (synovial inflammation, cartilage erosion, joint damage) were markedly improved in the Curcumin-NP group compared to the CIA and free curcumin groups ($p < 0.05$), indicating superior protective effects of the nanocarrier system.

These findings confirm that curcumin-loaded nanocarriers significantly enhance therapeutic efficacy in collagen-induced arthritis (CIA) through greater suppression of inflammatory markers and structural joint preservation compared to free curcumin administration.

4. Discussion

4.1. Interpretation of results

The present study demonstrated that curcumin-loaded nanocarriers significantly attenuated rheumatoid arthritis (RA) symptoms in a collagen-induced mouse model, as evidenced by reduced paw swelling, lower arthritis scores, decreased pro-inflammatory cytokine levels, and improved joint histology. Compared to free curcumin, nanocarrier-based delivery showed superior efficacy, likely due to enhanced systemic availability and sustained release, allowing for consistent therapeutic levels at the target site. These results are in line with earlier findings that curcumin, when encapsulated in nanocarriers, exhibits better pharmacological activity due to improved solubility and bioavailability.[6,10]

4.2. Mechanistic insights

The observed reduction in TNF-α, IL-1β, and IL-6 levels indicates that curcumin may exert its anti-inflammatory effects through inhibition of NF-κB signaling, which is a central regulator of pro-inflammatory cytokine production in RA pathogenesis.[11] Curcumin is also known to modulate JAK/STAT pathways, reducing the transcription of cytokines and other inflammatory mediators.[12] The nanoparticle formulation potentially facilitates intracellular delivery, enhancing its bioactivity within immune cells.

4.3. Comparison with previous studies

Previous studies using free curcumin in RA models reported modest benefits due to its poor absorption and rapid metabolism.[13] In contrast, nanocarrier formulations have shown more consistent results in preclinical models. For example, Yallapu et al. demonstrated increased anti-inflammatory efficacy using PLGA-encapsulated curcumin in inflammatory disorders,[7] supporting our current findings. Moreover, studies utilizing liposomal and micellar forms of curcumin have similarly reported improved therapeutic indices.[14,15]

4.4. Advantages of nanocarrier formulation

Nanocarriers offer multiple advantages over traditional drug delivery systems. In this study, the use of biodegradable polymers such as PLGA enabled targeted and sustained release, minimizing systemic toxicity and reducing the frequency of administration. This is particularly beneficial in chronic diseases like RA, where patient compliance is

crucial. Additionally, enhanced cellular uptake of nanoparticles may lead to greater suppression of intracellular inflammatory signaling pathways.[16]

4.5. Limitations

Despite promising findings, there are limitations. The study duration was relatively short, and long-term safety and efficacy remain unassessed. Additionally, while the mouse model provides useful mechanistic insight, translation to human physiology is not straightforward. Differences in immune system complexity and pharmacokinetics must be addressed in future clinical studies.

4.6. Future directions

Future work should focus on clinical validation of this approach in RA patients. Investigating curcumin-loaded nanocarriers in combination with existing DMARDs or biologics may reveal synergistic effects. Moreover, this nanocarrier platform can be extended to other phytochemicals with anti-inflammatory properties such as resveratrol, quercetin, or boswellic acids. Studies exploring oral or intra-articular routes may further optimize delivery strategies.

5. Conclusion

The present study demonstrates that curcumin-loaded nanocarriers exhibit significant therapeutic potential in the treatment of rheumatoid arthritis (RA), as evidenced by reduced paw swelling, lower arthritis scores, decreased levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), and improved histopathological outcomes in the collagen-induced arthritis (CIA) mouse model. Compared to free curcumin, the nanocarrier formulation provided enhanced bioavailability, targeted delivery, and sustained anti-inflammatory effects. These findings suggest that nanocarrier-based delivery of curcumin may serve as a promising and more effective alternative to conventional RA therapies, warranting further investigation in long-term preclinical and clinical studies to validate its translational potential.

6. Source of Funding

None.

7. Conflict of Interest

None.

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