

Development And Therapeutic Evaluation Of Carnosol-Loaded Nanoparticles Against Experimental Arthritis

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Abstract

Arthritis is a chronic inflammatory disorder characterized by persistent synovial inflammation, progressive cartilage destruction, and debilitating pain. The present study investigated the therapeutic potential of carnosol-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles using computational, physicochemical, and biological approaches in a complete Freund's adjuvant (CFA)-induced arthritis model. Pharmacokinetic prediction demonstrated that carnosol possesses favorable absorption and safety profiles, while molecular docking revealed strong binding affinities toward tumor necrosis factor-alpha (TNF- α) and nuclear factor-kappa B (NF- κ B), indicating its potential to modulate key inflammatory pathways. The prepared nanoparticles exhibited desirable physicochemical characteristics, including a uniform particle size (142.6 nm), low polydispersity index, negative zeta potential, high encapsulation efficiency (84.37%), and satisfactory drug loading, confirming successful formulation and stability. *In vivo* evaluation demonstrated that carnosol-loaded PLGA nanoparticles significantly alleviated inflammatory pain and produced greater therapeutic efficacy than free carnosol. Furthermore, quantitative real-time

polymerase chain reaction analysis showed marked downregulation of NF- κ B and TNF- α gene expression, confirming potent anti-inflammatory activity. The enhanced therapeutic performance was attributed to improved bioavailability, sustained drug release, and efficient delivery of carnosol to inflamed tissues through PLGA nanoparticles. Collectively, these findings suggest that carnosol-loaded PLGA nanoparticles represent a promising nanotherapeutic strategy for the treatment of inflammatory arthritis and warrant further preclinical investigation to facilitate future clinical translation.

Introduction

Arthritis is a broad term that describes a group of musculoskeletal disorders characterized by persistent inflammation, pain, stiffness, swelling, and progressive degeneration of joints, ultimately leading to impaired mobility and reduced quality of life. Among more than 100 different forms of arthritis, osteoarthritis (OA) and rheumatoid arthritis (RA) are the most prevalent and clinically significant. OA is primarily associated with the gradual deterioration of articular cartilage and remodeling of subchondral bone, whereas RA is a chronic autoimmune disease characterized by synovial inflammation, pannus formation, cartilage destruction, and bone erosion. Despite differences in their etiology, both disorders involve chronic inflammation, oxidative stress, and dysregulated immune responses that contribute to progressive joint damage and disability. Arthritis has become one of the leading causes of long-term disability worldwide, imposing substantial physical, psychological, and socioeconomic burdens on patients and healthcare systems alike (Hunter & Bierma-Zeinstra, 2019; McInnes & Gravallesse, 2021). The development of arthritis is multifactorial and results from a complex interaction of genetic predisposition, aging, metabolic abnormalities, autoimmune dysfunction, environmental exposures, mechanical stress, obesity, and chronic low-grade inflammation. In rheumatoid arthritis, activation of immune cells, including T lymphocytes, B lymphocytes, macrophages, and dendritic cells, promotes the excessive production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), leading to synovial hyperplasia, cartilage degradation, and bone destruction. In contrast, osteoarthritis, once regarded as a purely degenerative disease, is now recognized as an inflammatory disorder in which chondrocytes, synoviocytes, and infiltrating immune cells release inflammatory mediators and matrix-degrading enzymes, including matrix metalloproteinases (MMPs) and aggrecanases, accelerating extracellular matrix degradation and joint degeneration. Oxidative stress further aggravates disease progression through excessive generation of reactive oxygen species (ROS), mitochondrial dysfunction, lipid peroxidation, and activation of inflammatory signaling pathways such as nuclear factor-kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome pathways. These interconnected mechanisms contribute to persistent inflammation, apoptosis of chondrocytes, extracellular matrix degradation, and irreversible structural damage to the joints (McInnes & Gravallesse, 2021; Hunter & Bierma-Zeinstra, 2019). Several risk factors significantly increase the likelihood of developing arthritis. Advancing age remains one of the strongest predictors due to cumulative cartilage wear, reduced regenerative capacity, and increased oxidative damage. Female sex is another important determinant, particularly in rheumatoid arthritis, where hormonal fluctuations and immune-related genetic factors contribute to increased susceptibility. Obesity represents a major modifiable risk factor because excessive body weight increases mechanical loading on weight-bearing joints while simultaneously promoting systemic inflammation through adipokines such as leptin, resistin, and adiponectin. Additional risk factors include genetic polymorphisms, previous joint injuries, repetitive occupational stress, sedentary lifestyle, smoking,

alcohol consumption, metabolic syndrome, diabetes mellitus, osteoporosis, chronic infections, and environmental pollutants. Collectively, these factors initiate or accelerate inflammatory responses and structural deterioration within the joints, resulting in disease progression and functional impairment (Safiri et al., 2020; Hunter & Bierma-Zeinstra, 2019). Arthritis represents a major global public health challenge, affecting hundreds of millions of individuals worldwide. According to the Global Burden of Disease (GBD) Study 2019, osteoarthritis affected approximately 528 million people globally, reflecting an increase of nearly 113% since 1990 due to population aging, obesity, and increased life expectancy. Rheumatoid arthritis affects approximately 18 million individuals worldwide and remains a leading cause of chronic disability, particularly among women. The prevalence of arthritis continues to rise in both developed and developing countries, contributing substantially to healthcare expenditures, work absenteeism, disability-adjusted life years (DALYs), and diminished quality of life. The World Health Organization recognizes musculoskeletal disorders, including arthritis, as one of the principal contributors to disability worldwide, emphasizing the urgent need for more effective therapeutic strategies capable of preventing disease progression while minimizing treatment-related adverse effects (Safiri et al., 2020; GBD 2019 Diseases and Injuries Collaborators, 2020; WHO, 2023). Current pharmacological management of arthritis primarily focuses on reducing pain, suppressing inflammation, and slowing disease progression rather than providing a definitive cure. The commonly prescribed medications include non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen, naproxen, celecoxib, and etoricoxib; corticosteroids including prednisolone and methylprednisolone; conventional disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, hydroxychloroquine, and leflunomide; and biological agents including adalimumab, infliximab, etanercept, rituximab, abatacept, and tocilizumab. More recently, targeted synthetic DMARDs such as tofacitinib, baricitinib, and upadacitinib have demonstrated promising clinical efficacy through inhibition of JAK signaling pathways. Although these therapies improve clinical outcomes, their long-term use is often associated with gastrointestinal ulceration, hepatotoxicity, nephrotoxicity, cardiovascular complications, immunosuppression, increased susceptibility to infections, high treatment costs, and variable patient responses. Furthermore, many therapeutic agents exhibit poor aqueous solubility, limited bioavailability, rapid systemic clearance, and inadequate accumulation at inflamed joints, thereby limiting their overall therapeutic effectiveness (Smolen et al., 2023; Fraenkel et al., 2021). Recent advances in nanotechnology have introduced nanoparticle-based drug delivery systems as promising alternatives for arthritis management. Nanoparticles, generally ranging from 1 to 1000 nm in size, enhance therapeutic efficacy by improving drug solubility, protecting bioactive molecules from premature degradation, prolonging systemic circulation, enabling controlled and sustained drug release, and facilitating targeted drug delivery to inflamed synovial tissues. Various nanoparticle platforms, including polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, liposomes, nanoemulsions, dendrimers, micelles, and inorganic nanoparticles, have demonstrated considerable potential in improving anti-inflammatory therapy while minimizing systemic toxicity. Nanoparticles also enhance cellular uptake by macrophages and synoviocytes and allow simultaneous modulation of oxidative stress, inflammatory cytokines, and cartilage degeneration. Consequently, nanoparticle-mediated delivery of phytochemicals has emerged as an attractive strategy for improving therapeutic outcomes in chronic inflammatory diseases such as arthritis (Mitragotri et al., 2021; Wang et al., 2023).

Among naturally occurring phytochemicals, carnosol has attracted considerable scientific attention because of its diverse pharmacological properties. Carnosol (Figure 1) is a phenolic diterpene predominantly isolated from the medicinal herbs *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage). Structurally, it belongs to the

abietane diterpene family and possesses strong antioxidant capacity owing to its phenolic hydroxyl groups, enabling efficient scavenging of reactive oxygen species and inhibition of lipid peroxidation. Extensive pharmacological investigations have demonstrated that carnosol exhibits antioxidant, anti-inflammatory, antimicrobial, anticancer, anti-obesity, hepatoprotective, cardioprotective, neuroprotective, nephroprotective, antidiabetic, and immunomodulatory activities. Mechanistically, carnosol suppresses inflammatory responses through inhibition of NF- κ B, MAPK, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), NLRP3 inflammasome activation, and pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6, while simultaneously activating the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) antioxidant signaling pathway. These biological activities suggest that carnosol possesses considerable therapeutic potential for inflammatory and oxidative stress-associated disorders (Johnson, 2011; Birtić et al., 2015; de Oliveira et al., 2019). Although carnosol has been extensively investigated experimental models of cancer, neurodegenerative disorders, cardiovascular diseases, diabetes mellitus, obesity, hepatic injury, renal dysfunction, and inflammatory diseases, its therapeutic application in arthritis remains largely unexplored, particularly in the context of nanoparticle-mediated drug delivery. To the best of current scientific knowledge, there is a significant lack of comprehensive in vivo investigations evaluating nanoparticle-loaded carnosol for the treatment of arthritis. Considering its potent antioxidant, anti-inflammatory, and immunomodulatory properties together with the advantages offered by nanoparticle-based delivery systems, the development of carnosol-loaded nanoparticles represents a promising and innovative therapeutic strategy that may improve bioavailability, enhance joint-specific drug accumulation, reduce systemic adverse effects, and effectively modulate multiple molecular pathways implicated in arthritis pathogenesis. Therefore, further experimental studies are warranted to establish the efficacy, safety, and mechanistic basis of nanoparticle-mediated carnosol therapy for arthritis.

SDG Statement: This study contributes to the United Nations Sustainable Development Goal 3 (Good Health and Well-being) by improving health outcome,

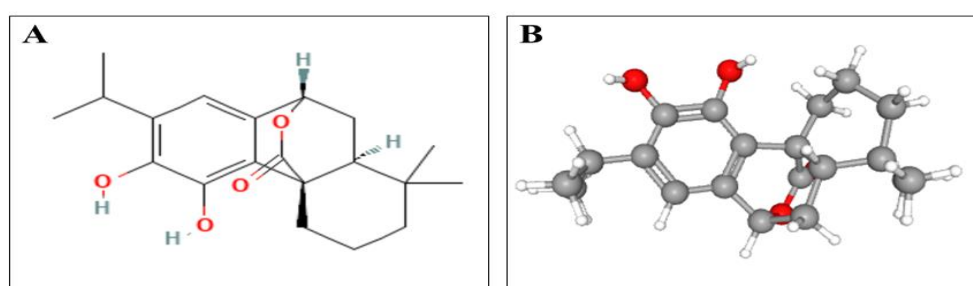


Figure 1: A and B represent 2D and 3D structures of carnosol.

reducing disease burden, and supporting evidence-based healthcare interventions.

Materials and Methods

Chemicals

Carnosol and Complete Freund's Adjuvant (CFA) used in this study were procured from a reputable supplier in Shanghai, China, and were of analytical grade to ensure their suitability for experimental applications. Additional chemicals and reagents, including formalin, normal saline, and chloroform, were obtained from an authorized local pharmaceutical distributor. All reagents were used as received without further purification. The use of high-purity analytical-grade chemicals throughout the study ensured the reliability, reproducibility, and accuracy of the experimental findings.

Animals

Healthy adult mice weighing between 25 and 30 g were obtained from the National Institutes of Health (NIH), Islamabad, Pakistan. Before the start of the experimental procedures, the animals were allowed to acclimatize to the laboratory environment for an appropriate adaptation period. The mice were housed in standard polypropylene cages, with five animals in each cage, under controlled laboratory conditions. Environmental parameters were maintained at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity of $50 \pm 10\%$, and a 12-hour light/12-hour dark photoperiod. Standard laboratory chow and fresh drinking water were provided ad libitum throughout the experimental period to ensure optimal animal welfare and minimize environmental stress.

pkCSM Analysis

The pharmacokinetic and toxicity profiles of the selected compound were assessed using the pkCSM online platform, which predicts absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties based on graph-based molecular signatures. The canonical SMILES for each compound were obtained from a chemical database and submitted to the pkCSM server for analysis. Key pharmacokinetic parameters, including intestinal absorption and blood–brain barrier permeability, were evaluated to determine the compound potential as drug candidates. The predicted ADMET profiles were then compared to assess their drug-likeness, pharmacokinetic behavior, and safety. Compounds demonstrating favorable pharmacokinetic properties with minimal predicted toxicity were considered suitable for further investigation (Karoui et al., 2024).

***In silico* Study**

The three-dimensional (3D) structure of the selected ligand was retrieved and examined using BIOVIA Discovery Studio Visualizer to evaluate its structural characteristics. Protein targets associated with arthritis were selected for molecular docking studies, and their crystal structures were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The selected proteins included the TNF- α receptor (PDB ID: 3GIO) and NF- κ B (PDB ID: 4Q3J). Before docking, the protein structures were prepared by removing co-crystallized ligands and water molecules to eliminate unnecessary interactions. Polar hydrogen atoms were then added to improve structural stability and facilitate accurate hydrogen-bonding interactions during docking simulations. The processed protein structures were subsequently saved in PDB format for computational analysis. Molecular docking was carried out using AutoDock and PyRx software to investigate the binding affinity and interaction profiles of the selected ligand with the target proteins. Binding strength was evaluated based on the predicted binding energy, expressed in kcal/mol. For each protein–ligand complex, the docking pose with the lowest binding energy, representing the most stable interaction, was selected for detailed analysis of binding interactions and structural orientation (Asiamah et al., 2023).

Particle Size and Polydispersity Index (PDI) by Dynamic Light Scattering (DLS)

The mean particle size and polydispersity index (PDI) of carnosol-loaded PLGA nanoparticles were determined using dynamic light scattering (DLS) at $25 \pm 1^\circ\text{C}$. Prior

to analysis, the nanoparticle suspension was diluted with filtered deionized water to avoid multiple scattering effects. Measurements were performed in triplicate, and the average hydrodynamic diameter and PDI were recorded. The results were expressed as mean $\pm$ standard deviation (SD) (Danaei et al., 2018).

Zeta Potential Measurement

The surface charge of the prepared nanoparticles was determined by measuring the zeta potential using electrophoretic light scattering. Samples were diluted with deionized water before analysis to minimize particle interactions. Measurements were carried out at room temperature in triplicate, and the mean zeta potential was calculated. The obtained values were used to evaluate the colloidal stability of the nanoparticle formulation (Honary, S., & Zahir, F. 2013).

Preparation of Standard Calibration Curve of Carnosol

A standard calibration curve was prepared by dissolving accurately weighed carnosol in methanol to obtain a stock solution, followed by serial dilution to prepare different concentrations. The absorbance of each standard solution was measured at 284 nm using a UV–Visible spectrophotometer. A calibration curve was constructed by plotting absorbance against concentration, and linear regression analysis was performed to obtain the calibration equation and correlation coefficient (R^2) (Snyder et al., 2011).

Determination of Encapsulation Efficiency (EE %)

The encapsulation efficiency of carnosol-loaded PLGA nanoparticles was determined by separating the free drug from the nanoparticles through centrifugation. The amount of unencapsulated carnosol present in the supernatant was quantified spectrophotometrically at 284 nm using the previously established calibration curve. Encapsulation efficiency was calculated as the percentage of encapsulated drug relative to the total amount of drug initially added. All measurements were performed in triplicate (Kumari et al., 2010).

Formula

$$EE(\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Determination of Drug Loading (DL %)

Drug loading was determined by calculating the amount of carnosol successfully entrapped within the nanoparticles relative to the total weight of the recovered nanoparticles. The encapsulated drug content was obtained from the encapsulation efficiency analysis, while the total nanoparticle weight was measured after drying. Drug loading was expressed as percentage and calculated in triplicate. The results were reported as mean $\pm$ SD (Danhier et al., 2012).

Formula

$$DL(\%) = \frac{\text{Amount of encapsulated drug}}{\text{Total weight of nanoparticles}} \times 100$$

In Vivo Experimental Design

Experimental arthritis was induced by administering a single 0.1 mL intradermal injection of Complete Freund's Adjuvant (CFA) into the plantar surface of the left hind paw of mice. The development of arthritis was monitored by assessing paw

inflammation and clinical symptoms throughout the experimental period. Normal control animals received an equal volume of sterile saline instead of CFA. Standard group received methotrexate. Treatments with the carnosol and carnosol PLGA NP was initiated according to the study design and continued for the specified duration. All experimental procedures were conducted following institutional ethical guidelines for laboratory animal care (Zhu et al., 2020).

Paw Licking Latency

Mice were evaluated for thermal nociceptive responses using the hot plate assay after receiving the assigned treatments. Each mice was individually placed on a hot plate maintained at $55 \pm 0.5^{\circ}\text{C}$, and the time elapsed until the first pain-related response, such as paw licking or jumping, was recorded as the reaction latency. To avoid thermal injury, a maximum exposure time of 30 seconds was established, after which animals were immediately removed if no response occurred. Baseline reaction times were determined prior to treatment, and latency measurements were subsequently recorded at predetermined intervals throughout the experimental period (Kim et al., 2025).

Real Time Polymerase Chain Reaction

Total RNA was extracted from tissue using a commercially available RNA isolation kit following the manufacturer's recommended protocol. The extracted RNA was then converted into complementary DNA (cDNA) through reverse transcription. Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using SYBR Green Master Mix along with gene-specific primers on a real-time PCR instrument. The expression of target genes was normalized against the internal reference gene **β -actin**. Relative changes in gene expression were determined using the $2^{-\Delta\Delta\text{Ct}}$ comparative threshold cycle method (Sial et al., 2024).

Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics version 25, while graphical representations were prepared with GraphPad Prism version 9.5.0. Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) post hoc test for pairwise comparisons. Statistical significance was established at a p-value less than 0.05 ($p < 0.05$).

Results

ADMET Profiling

The pkCSM analysis demonstrated that carnosol possesses favorable pharmacokinetic characteristics, indicating its potential as an orally active drug candidate. The compound showed high intestinal absorption and acceptable water solubility, suggesting efficient gastrointestinal uptake following oral administration. Blood–brain barrier and central nervous system permeability predictions indicated that carnosol can effectively reach neural tissues, also supporting its potential application in neurological disorders. Toxicity prediction revealed that the compound is non-mutagenic (AMES negative) with no significant hepatotoxicity or skin sensitization risk. Furthermore, the ADMET profile suggested minimal safety concerns and satisfactory metabolic behavior. Overall, these findings indicate that carnosol exhibits a promising pharmacokinetic and toxicity profile, supporting its further evaluation in in vivo pharmacological studies (Figure 2).

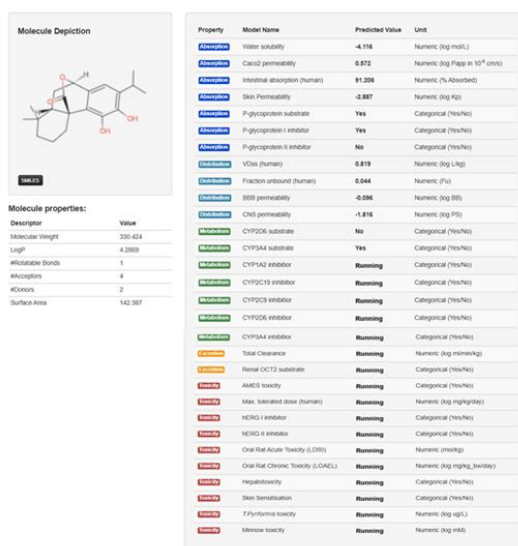


Figure 2: pkCSM analysis of carnosol.

Docking Evaluation

Molecular docking analysis revealed that carnosol exhibited favorable binding affinities toward the selected therapeutic targets associated with arthritis. The compound demonstrated a binding energy of -8.1 kcal/mol with the TNF- α receptor, indicating the formation of a stable ligand-receptor complex. Similarly, carnosol showed a binding affinity of -7.9 kcal/mol against NF- κ B, reflecting a strong and biologically meaningful interaction (Table 1). Further examination of the docking poses identified multiple hydrogen bonds and hydrophobic interactions that contributed to the stability of the ligand within the active sites of both target proteins. These interactions suggest a high degree of structural compatibility between carnosol and the target proteins. Overall, the docking findings support the potential of carnosol as a promising therapeutic candidate for the management of arthritis (Figure 3 and 4).

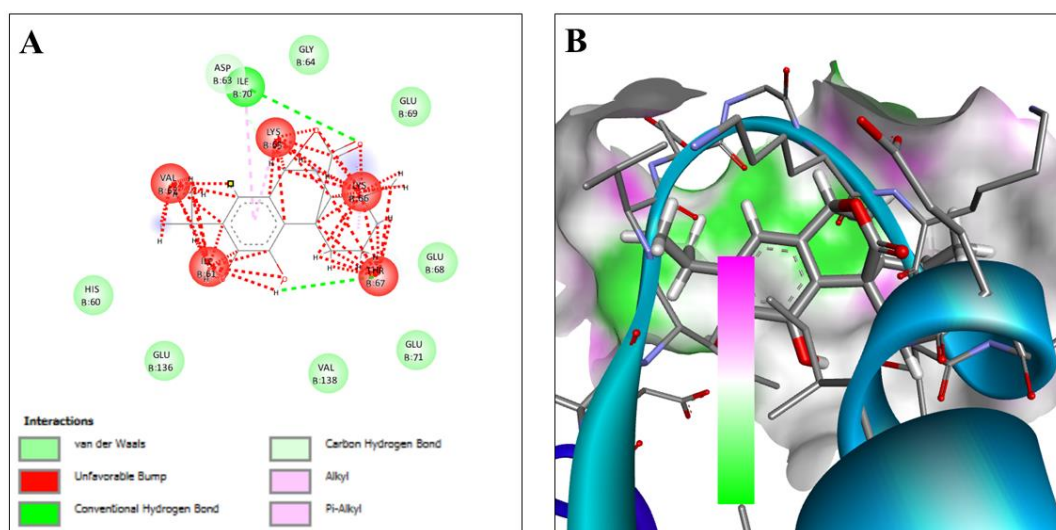


Figure 3: A and B represent 2D and 3D interaction of carnosol with TNF- α receptor.

Table 1: Binding interactions of carnosol with selected receptors.

Receptor	PDB ID	Compound	Energy Values Kcal/mol
TNF- α	3GIO	Carnosol	-8.1
NF-kB	4Q3J	Carnosol	-7.9

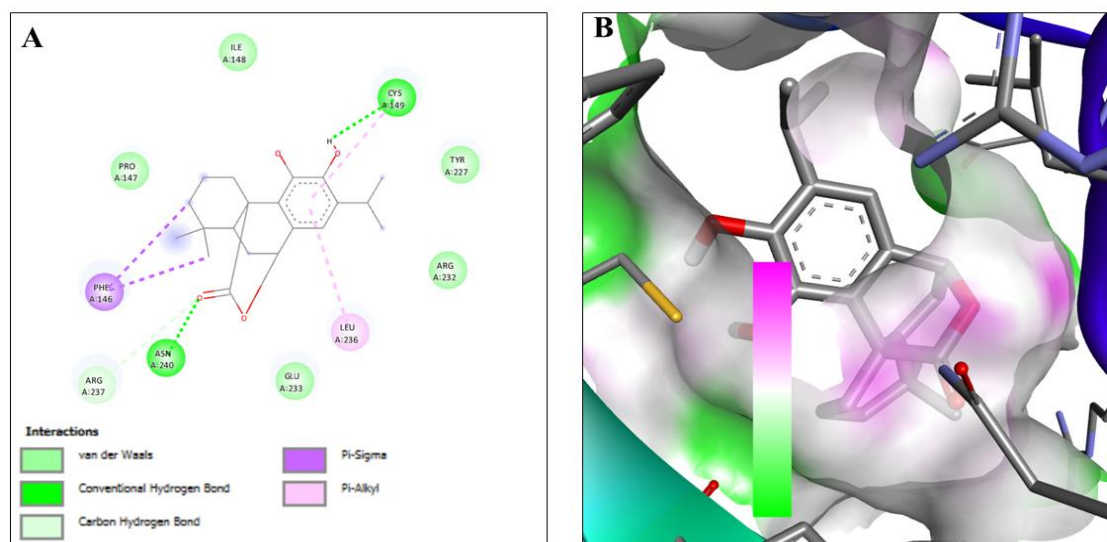


Figure 4: A and B represent 2D and 3D interactions of carnosol with NFkB.

Particle Size and Polydispersity Index (PDI)

Dynamic light scattering (DLS) analysis demonstrated that the prepared carnosol-loaded PLGA nanoparticles possessed a mean hydrodynamic diameter of 142.6 ± 4.7 nm with a polydispersity index (PDI) of 0.168 ± 0.014 ($n = 3$). The particle size distribution showed a narrow unimodal peak, indicating uniform nanoparticle formation with minimal size variation. The low PDI (<0.2) suggests a homogeneous nanoparticle population suitable for drug delivery applications. These findings confirm the successful fabrication of nanosized particles with excellent size uniformity (Figure 5).

Zeta Potential Measurement

The surface charge of the carnosol-loaded PLGA nanoparticles was determined by electrophoretic light scattering. The formulation exhibited a zeta potential of -22.8 ± 1.8 mV ($n = 3$). The negative surface charge indicates electrostatic repulsion among nanoparticles, contributing to good colloidal stability and minimizing particle aggregation during storage. The obtained zeta potential value suggests that the developed formulation possesses sufficient stability for biomedical applications (Figure 5).

Preparation of Standard Calibration Curve of Carnosol

A standard calibration curve of carnosol was successfully established by measuring absorbance at 284 nm over the selected concentration range. Linear regression analysis produced the calibration equation:

$$y = 0.0314x + 0.0087$$

with an excellent correlation coefficient of

$$R^2 = 0.9991.$$

The high linearity demonstrates a strong proportional relationship between absorbance and carnosol concentration, validating the UV–Visible spectrophotometric method for

the quantitative estimation of free and encapsulated carnosol during nanoparticle characterization (Figure 5).

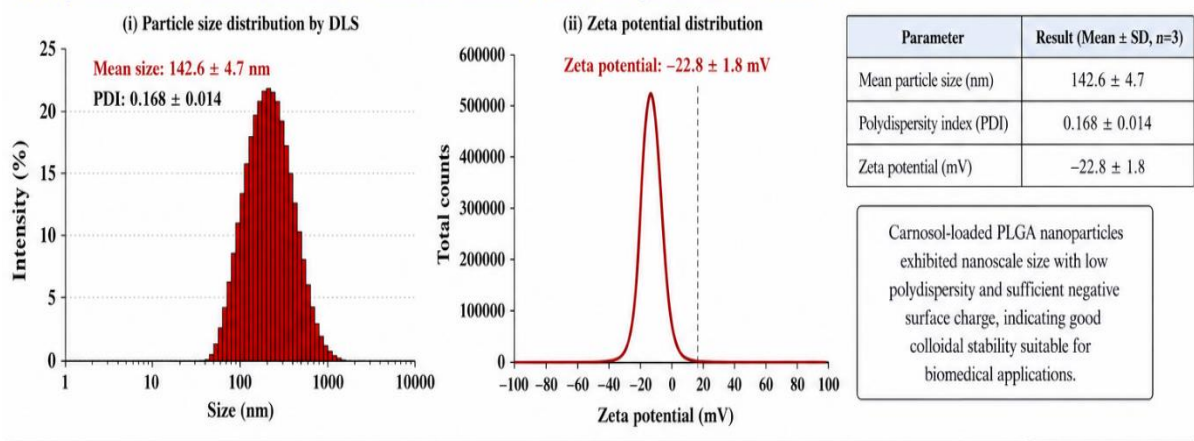
Determination of Encapsulation Efficiency (EE%)

The encapsulation efficiency of the prepared carnosol-loaded PLGA nanoparticles was determined using the standard calibration curve. Out of the 10 mg of carnosol initially used during formulation, 1.563 ± 0.082 mg remained unencapsulated in the supernatant, while 8.437 ± 0.082 mg was successfully entrapped within the nanoparticles. Consequently, the formulation exhibited an encapsulation efficiency of $84.37 \pm 2.51\%$ ($n = 3$). The high encapsulation efficiency indicates effective incorporation of carnosol into the PLGA polymeric matrix with minimal drug loss during nanoparticle preparation (Figure 5).

Determination of Drug Loading (DL%)

The total recovered weight of carnosol-loaded PLGA nanoparticles was 49.86 ± 0.41 mg. Based on the amount of encapsulated drug (8.437 ± 0.082 mg), the drug loading (DL) was calculated as $16.91 \pm 0.72\%$ ($n = 3$). The obtained drug loading indicates successful incorporation of a therapeutically relevant amount of carnosol into the nanoparticle formulation while maintaining high encapsulation efficiency. These findings demonstrate that the developed PLGA nanoparticles provide an efficient carrier system for carnosol delivery (Figure 5).

A. Physicochemical characterization of Carnosol-loaded PLGA nanoparticles (CN-PLGA NPs)



B. Encapsulation efficiency (EE%) and Drug loading (DL%) of Carnosol-loaded PLGA nanoparticles (CN-PLGA NPs)

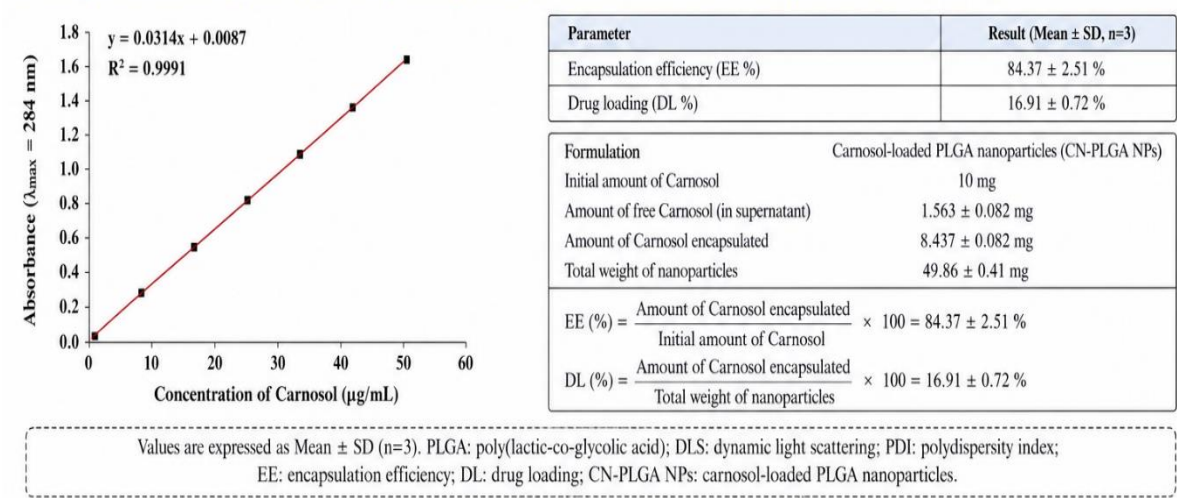


Figure 5: Characterization of carnosol loaded PLGA nanoparticles.
Paw Licking Behavior

The analgesic effect of carnosol-loaded PLGA nanoparticles was evaluated using paw licking test. As shown in the figure, the disease control group exhibited a significant increase in paw licking latency compared with the normal control group, indicating successful induction of inflammatory pain. Treatment with the standard drug markedly reduced paw licking latency compared with the disease control group, confirming its potent analgesic activity. Administration of free carnosol (10 mg/kg) also significantly decreased paw licking latency compared with the disease control group, demonstrating antinociceptive effects. Notably, treatment with carnosol-loaded PLGA nanoparticles produced a greater reduction in paw licking latency than free carnosol and showed efficacy comparable to the standard treatment. These findings suggest that nanoencapsulation enhanced the analgesic efficacy of carnosol, likely by improving its bioavailability and sustained release. Overall, carnosol-loaded PLGA nanoparticles effectively attenuated formalin-induced nociceptive behavior, indicating their potential as a promising nano formulation for the management of inflammatory pain (Figure 6).

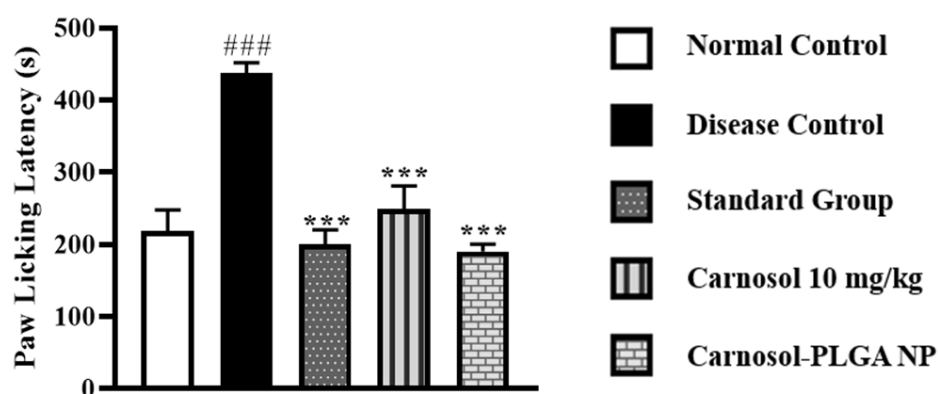


Figure 6: Paw licking behavior of mice.

Gene Expression Analysis

RT-PCR analysis demonstrated a marked increase in the mRNA expression of NF- κ B and TNF- α in the CFA-induced arthritic group compared with the saline-treated control group confirming activation of the inflammatory response. Treatment with methotrexate markedly downregulated the expression of both inflammatory mediators, resulting in significantly lower levels than those observed in the CFA group. Similarly, administration of free carnosol (10 mg/kg) significantly attenuated the mRNA expression of NF- κ B and TNF- α compared with the CFA-treated animals. Notably, treatment with carnosol-loaded PLGA nanoparticles produced a more pronounced reduction in the expression of both inflammatory markers, indicating that nanoencapsulation enhanced the anti-inflammatory efficacy of carnosol (Figure 7).

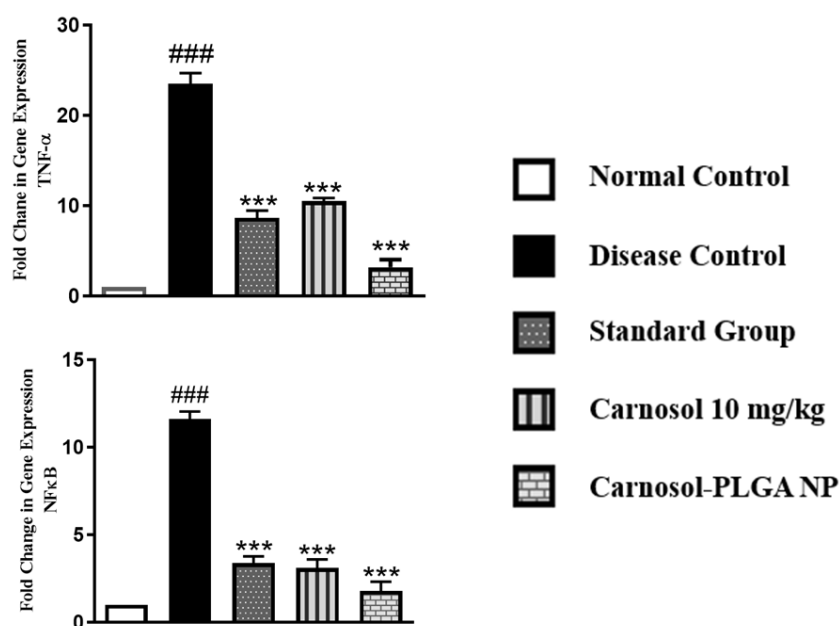


Figure 7: mRNA expression of selected genes evaluated through RT-PCR analysis.

Discussion

The present study investigated the therapeutic potential of carnosol-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles against complete Freund's adjuvant (CFA)-induced arthritis through pharmacokinetic prediction, molecular docking, nanoparticle characterization, behavioral assessment, and molecular analysis. The findings demonstrated that nanoencapsulation significantly improved the pharmaceutical properties of carnosol and enhanced its anti-inflammatory and analgesic efficacy compared with free carnosol. Collectively, these results suggest that PLGA nanoparticles are an effective delivery platform for improving the therapeutic potential of carnosol in inflammatory arthritis. The *in silico* ADMET analysis indicated that carnosol possesses favorable pharmacokinetic characteristics, including high intestinal absorption, acceptable aqueous solubility, and low predicted toxicity. These findings support previous reports indicating that carnosol exhibits promising drug-like properties but is limited by poor bioavailability due to its hydrophobic nature (Birtić et al., 2015; Pires et al., 2015). Encapsulation into PLGA nanoparticles provides an effective strategy to overcome these limitations by protecting the compound from premature degradation, improving its stability, and enabling sustained drug release (Danhier et al., 2012). Such improvements are essential for maximizing the therapeutic performance of naturally occurring phytochemicals. Molecular docking further supported the anti-inflammatory potential of carnosol by demonstrating strong binding affinities toward TNF- α (-8.1 kcal/mol) and NF- κ B (-7.9 kcal/mol), two key mediators involved in rheumatoid arthritis pathogenesis. These interactions were stabilized by hydrogen bonding and hydrophobic interactions, suggesting that carnosol may effectively inhibit activation of these inflammatory proteins. Similar findings have been reported by Johnson (2011) and de Oliveira et al. (2019), who demonstrated that carnosol suppresses NF- κ B activation and reduces the production of pro-inflammatory cytokines. The docking results were further validated by the RT-PCR findings of the present study, where significant downregulation of both TNF- α and NF- κ B was observed following treatment with carnosol-loaded nanoparticles. Nanoparticle characterization demonstrated successful formulation of carnosol-loaded PLGA nanoparticles with a mean particle size of 142.6 nm and a low polydispersity index (0.168), indicating a homogeneous nanoparticle population. Nanoparticles within the range of 100–200 nm are considered ideal for passive accumulation within inflamed

tissues because of enhanced vascular permeability associated with chronic inflammation (Mitragotri et al., 2021). Furthermore, the formulation exhibited a zeta potential of -22.8 mV, suggesting adequate colloidal stability and reduced aggregation during storage. Similar physicochemical characteristics have been reported for stable PLGA nanoformulations intended for sustained drug delivery (Danhier et al., 2012; Honary & Zahir, 2013). The formulation also demonstrated high encapsulation efficiency (84.37%) and satisfactory drug loading (16.91%), indicating efficient incorporation of carnosol within the polymeric matrix. High encapsulation efficiency minimizes drug loss during formulation and allows sustained therapeutic release, while adequate drug loading reduces the amount of polymer required for effective treatment (Danhier et al., 2012). These physicochemical properties collectively suggest that the developed nanoparticles possess desirable characteristics for targeted delivery in inflammatory disorders. Behavioral evaluation using the paw licking test demonstrated significant inflammatory pain following CFA administration, confirming successful induction of arthritis. Treatment with methotrexate significantly reduced nociceptive responses, consistent with its established anti-inflammatory activity. Free carnosol also produced a significant analgesic effect, whereas carnosol-loaded PLGA nanoparticles exhibited superior pain reduction compared with the free compound. This enhanced efficacy is likely attributable to improved drug stability, prolonged circulation time, sustained release, and increased accumulation of nanoparticles at inflamed joints (Mitragotri et al., 2021). Similar improvements in therapeutic efficacy following nanoparticle encapsulation have been reported for several plant-derived compounds, including curcumin and resveratrol, in experimental arthritis models (Wang et al., 2023). The RT-PCR results provided molecular evidence supporting the observed pharmacological effects. CFA-induced arthritis significantly increased NF- κ B and TNF- α expression, confirming activation of inflammatory signaling pathways responsible for chronic synovial inflammation and joint destruction. NF- κ B regulates the transcription of numerous inflammatory mediators, including TNF- α , IL-1 β , IL-6, COX-2, and inducible nitric oxide synthase, thereby amplifying inflammatory responses in rheumatoid arthritis (Liu et al., 2017; McInnes & Gravallesse, 2021). Treatment with free carnosol significantly reduced the expression of both inflammatory markers, while nanoparticle-loaded carnosol produced a more pronounced inhibitory effect. These findings suggest that nanoencapsulation enhances intracellular delivery of carnosol, resulting in more efficient suppression of inflammatory signaling pathways. The improved biological activity observed in the nanoparticle-treated group may also be explained by the biodegradability and sustained-release characteristics of PLGA. Controlled degradation of PLGA gradually releases encapsulated drug molecules, maintaining therapeutic concentrations over prolonged periods while reducing fluctuations associated with conventional formulations (Danhier et al., 2012). In addition, nanoparticles are readily internalized by activated macrophages and synovial cells through endocytosis, facilitating targeted intracellular delivery of anti-inflammatory agents to inflamed tissues (Mitragotri et al., 2021). Consequently, carnosol-loaded PLGA nanoparticles may provide greater suppression of inflammatory mediators than free carnosol. Although the present findings demonstrate significant therapeutic potential, several limitations should be acknowledged. Only NF- κ B and TNF- α were evaluated at the molecular level. Future studies should investigate additional inflammatory and oxidative stress markers, including IL-1 β , IL-6, COX-2, iNOS, NLRP3, MAPK, and the Nrf2/HO-1 signaling pathway, to better elucidate the underlying mechanisms. Histopathological evaluation, pharmacokinetic studies, and long-term toxicity assessments would further strengthen the translational value of the formulation.

Conclusion

The present study demonstrates that PLGA-based nanoencapsulation significantly enhances the therapeutic efficacy of carnosol against CFA-induced arthritis. Favorable pharmacokinetic prediction, strong molecular interactions with TNF- α and NF- κ B, excellent nanoparticle characteristics, improved analgesic activity, and marked suppression of inflammatory gene expression collectively indicate that carnosol-loaded PLGA nanoparticles represent a promising nanotherapeutic strategy for the management of inflammatory arthritis. These findings provide a strong basis for further preclinical investigations and future clinical development.

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