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Rheumatoid Arthritis Treatment Potential of Stearic Acid Nanoparticles of Quercetin in Rats

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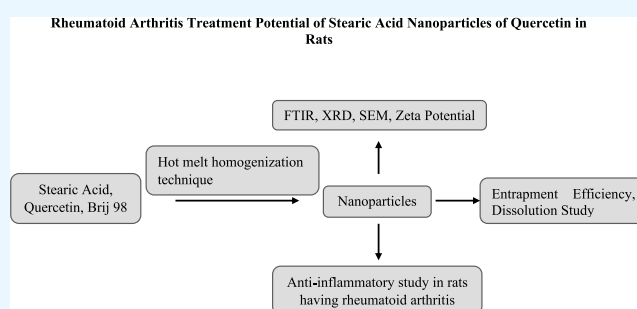
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ABSTRACT: This study aims to assess the anti-inflammatory potential of stearic acid nanoparticles of quercetin in an arthritic rat model. This article describes the fabrication of solid lipid nanoparticles (SLNs) using the hot melt encapsulation method, followed by the anti-inflammatory study of SLNs and other characterizations such as FTIR, XRD, and SEM. Thirty male healthy albino rats were taken and treated with FCA to induce rheumatoid arthritis. Quercetin loading of quercetin to stearic acid was confirmed by FTIR. The efficacy of quercetin-loaded SLNs to reduce inflammation was evaluated with the help of inflammatory biomarker levels. Quercetin-loaded stearic acid nanoparticles were successfully prepared by using a hot melt encapsulation method. Their average size and zeta potential were 100 nm and −25 mV, respectively. Rheumatoid arthritis was significantly ($p < 0.001$) reduced in the quercetin-loaded SLN group, as indicated by finding out the reduced levels of inflammatory mediators such as tumor necrosis factor (TNF-α) and rheumatoid factor. Quercetin-loaded stearic acid nanoparticles were found to be potentially effective in treating RA.

Rheumatoid Arthritis Treatment Potential of Stearic Acid Nanoparticles of Quercetin in Rats

Stearic Acid, Quercetin, Brij 98 → Hot melt homogenization technique → Nanoparticles → Entrapment Efficiency, Dissolution Study

Nanoparticles → FTIR, XRD, SEM, Zeta Potential

Nanoparticles → Anti-inflammatory study in rats having rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA), an inflammatory disease, is the most common type of arthritis, which results in the progressive destruction of bones, synovial joints, and cartilages.[1] It is an autoimmune disorder that affects 1−3% of the adult population. This low prevalence means that an average physician has little interaction with RA patients and their management.[2] It exists almost two to three times more frequently in women than in men. It can occur at any stage of life; however, the peak incidence occurs between 40 and 60 years of age.[3] The symptoms of RA vary from mild to severe. If these symptoms are mild or come and go, they should not be ignored because they become severe with the passage of time if left untreated. The chronic inflammation due to RA leads to deformity, disability, progressive joint destruction, and sometimes premature death. The criteria of the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) focus on early disease. With the early diagnosis of RA, it is possible to prevent disability and joint damage by suppressing the inflammatory activity.[4] The origin of RA is unknown; however, genetic and environmental factors such as smoke and dust exposure play an important role in the emergence of RA. Autoantibodies such as rheumatoid factors (RF) and antimodified protein antibodies (AMPA) are formed as the result of abnormalities in the humoral and cellular immune response. The formation of these antibodies leads to the immigration of T- and B-lymphocytes into the synovium. The innate immune system is also activated by the stimulation of monocytes and macrophages in the tissue sites.[5] Due to this, inflammatory mediators like tumor necrosis factor (TNF-α) and cytokines including interleukins (IL) start accumulating in the synovial joints. The production of proteolytic enzymes that degenerate the cartilages and bones increases due to the high levels of the production of C-reactive proteins by the hepatocytes as a result of the increased number of cytokines in the synovial cavity.[6] Cellular infiltration of kinins, prostaglandins, and histamines into the endothelium is increased by cytokines. These are the self-protective reactions of the body, which result in deformity, disability, severe tissue pain, and progressive joint damage and reduce the quality of life.[7]

Currently, there is no treatment for RA; however, early diagnosis and drugs can delay the progression of the disease.[8] The first-line therapy for the treatment of RA is drugs that suppress the inflammation such as nonsteroidal anti-inflam-

matory drugs (NSAIDs) and glucocorticoids. Dexamethasone, a glucocorticoid drug, is used to treat RA, and its antirheumatic potency is higher than any other steroid, about five to six times more than that of prednisolone.[9] These drugs act rapidly to treat swelling and pain. Disease-modifying antirheumatic drugs (DMARDs) such as hydroxychloroquine, methotrexate, and sulfasalazine are also used to treat RA. DMARDs not only improve symptoms but also show clinical progression. However, their onset of action is very low, so they are used with NSAIDs.[10] Gastrointestinal (GI) abnormalities like ulcers, bleeding, and perforations occur with the chronic use of NSAIDs.[11] The efficacy of these medications is very limited because the frequent use of NSAIDs and DMARDs results in joint destruction, inability to work, disability, and increased mortality.[12] Therefore, it is important to find natural, easily available, and biocompatible remedies that can slow down the progression of disease with minimum or no possible side effects.

Flavonoids are naturally available diphenylpropanoids that commonly exist in plant foods. Flavonoids have potent antioxidant and antiradical properties. They also possess anti-inflammatory potential in both the exudative and inflammatory phases by interfering with NF-κB and inhibiting the production of nitric oxide (NO), eicosanoids, and pro-inflammatory cytokines.

Quercetin is one of the most naturally occurring flavonoids that is found in broccoli, apples, onions, green leafy vegetables, and tea. Quercetin possesses a potent anti-inflammatory effect.[13] Quercetin, at low concentration, blocks the lipooxygenase pathway while, at high concentration, it blocks both the cyclooxygenase and lipooxygenase pathways.[14] Quercetin is a poorly absorbed substance and has a very poor oral bioavailability. Only 25% of the injectable dose of quercetin is absorbed from the small intestine.[15] The clinical use of quercetin is limited due to its low solubility and bioavailability. This problem can be solved with the help of nanocarriers suitable enough to solubilize, deliver the drug to the specific target sites, and provide maximum stability in the body fluids.

In recent years, the use of nanoparticles such as nanoemulsion, liposomes, polymeric nanoparticles, and lipid-based nanocarriers has been increased due to their enhanced chance of adhesion to the biological membranes. In recent reports, it is suggested that liposomes can produce an inflammatory response by stimulating the immune system with the release of pro-inflammatory cytokines.[16] There is a need for an alternative approach. Recently, solid lipid nanoparticles have been studied extensively because of their special attributes such as nontoxicity, drug targeting, improved body tolerance, and ability to entrap both hydrophilic and lipophilic drugs. The drawbacks associated with the use of liposomes, emulsions, and polymeric nanoparticles are minimized by SLNs.[17] Different routes of administration such as oral, intravenous, ocular, nasal, and pulmonary routes are used for the delivery of nanocarriers. The parenteral route is the most common route used for the delivery of nanoparticles; however, it is associated with less patient compliance. For clinical applications of nanocarriers, the best route is the oral route as it provides maximum patient compliance and comfort.[18]

Taking all of the above factors into account, this study was designed to check the anti-inflammatory potential of stearic acid nanoparticles of quercetin in an arthritic rat model. The efficiency of quercetin-loaded SLNs to reduce inflammation was evaluated with the help of inflammatory biomarker levels.

■ MATERIALS AND METHODS

Materials. Freund's complete adjuvant (FCA) was purchased from InvivoGen, France. Dexamethasone was acquired from Sigma (USA) and was dissolved in 0.9% saline. Quercetin was purchased from Santa Cruz, Germany. Ethyl alcohol (ETOH) and dimethyl sulfoxide (DMSO) were purchased from Merck, Germany. Stearic acid, ethanol, and hydrochloric acid were bought from Across Organics (a Thermo Fisher Scientific brand). The kits of ALP, AST, ALT, creatinine, and bilirubin were purchased from Shanghai Eliza Co., Ltd., China. The kits of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and ELISA were purchased from QCA, Spain. Throughout this experiment, deionized water was used. Brij-98 was purchased from Sigma-Aldrich (St. Louis, Missouri).

Synthesis of Quercetin-Loaded Stearic Acid Nanoparticles. The solid lipid nanoparticles of quercetin were prepared by a hot melt homogenization technique using a lipid (stearic acid), ethanol (95%), and Brij-98 at different proportions.[19] First of all, 100 mg of stearic acid was melted using a hot plate above its melting point, i.e., 69.3 °C. Quercetin was mixed with stearic acid in a concentration of 2.72 mg/mL. Then, a 3% solution of Brij-98 was prepared in 20 mL of deionized water on a hot plate above 60 °C with continuous stirring for 20 min. About 500 μL of ethanol (95%) was added to the lipids to ease the phase transfer. After the transfer of lipids, the temperature knob was turned off but the high-pressure homogenization was continued until the solution attained room temperature. Two different formulations were prepared by changing the concentration of the quercetin and centrifuging at ~14,600 rpm for 15 min using a Thermo Scientific Centrifuge machine for complete removal of surfactant. The pellets were redispersed in water after removal of the supernatant. The washing step was repeated several times, and nanoparticles were lyophilized for removal of water. Finally, the nanoparticle was obtained in form of powder and was stored at room temperature in a glass bottle (Table 1).

Table 1. Formulation of Quercetin SLNPs through a Hot Homogenization Method Using Different Concentrations of Quercetin

formulation	quercetin (mg)	lipids (mg)	Brij-98 (mg)	ethanol (μL)	water (ml)
QNP1	272	100	600	500	20
QNP2	544	100	600	500	20

Physiochemical Characterization of Solid Lipid Nanoparticles. The polydispersity index (PDI), zeta size, and zeta potential of the solid lipid nanoparticles were measured by dynamic light scattering through a Nano ZS machine (Malvern Instruments, Worcestershire, UK). The zetasizer measuring range is approximately 0.6 nm to 3 m. All of the samples were sterilized by exposure to ultraviolet light. All the SLN samples were diluted in distilled water, and the measurements were performed at 25 °C temperature.[20]

Entrapment Efficiency. The entrapment efficiency of quercetin is the amount of quercetin that is present in the nanoparticles. The suspensions of the quercetin-loaded nanoparticles were centrifuged at 17,000 rpm at 25 °C for

15−20 min. The difference between the total amount of drug used and the drug present in the supernatant aqueous phase was calculated. The UV/vis spectrophotometric (Elico SL 159, India) at 373 nm was used to find the nonentrapped quercetin from the supernatant obtained after centrifugation of nanoparticles. The calibration curve was obtained by plotting absorbance values against concentration. The concentration of quercetin in the supernatant is the amount of nonentrapped drug in the nanoparticles.[21] The encapsulation efficiency of quercetin was determined by the following equation:

$$\text{amount of loaded drugs}$$
$$= \text{total amount of drugs added} - \text{amount of non}$$
$$- \text{entrapped drugs}$$

$$EE(\%) = (\text{amount of drugs loaded in nanocarriers}$$
$$/\text{total amount of drugs}) \times 100$$

***In Vitro* Drug Release Studies.** *In vitro* release of quercetin from the SLNs was measured by using a biological shaker (LBS-030S-Lab Tech, Korea). Accurately weighed 10 mg of the quercetin was added into an equivalent 10 mL of 0.1 M phosphate buffer at pH 7.4 in a 50 mL volumetric flask. The pH and concentration of PBS buffer were the same as those of blood. This solution was incubated at 37 °C for 1 h with continuous shaking in a biological shaker at 100 rpm. The well-measured 0.5 mL aliquots were withdrawn from the dissolution medium at preselected time intervals, i.e., 0, 1, 1.5, 3, 6, 12, and 24 h, and the samples taken were replaced by the fresh phosphate buffer solution to maintain the sink conditions. The samples were transferred to a 1.5 mL microtube and centrifuged at 15,000 rpm. The supernatant was analyzed at maximum absorbance of 373 nm[22] by using a UV/vis spectrophotometer (Elico SL 159, India) with suitable dilution. All the measurements were taken in triplicate form (n = 3).

Fourier Transmission Infrared (FTIR) Spectroscopy Analysis. The FTIR spectra of quercetin and quercetin-loaded stearic acid nanoparticles were recorded on ATR-FTIR spectrophotometry (PerkinElmer, Waltham, Massachusetts, USA). All the samples were in powdered form and scanned at 1 cm^{-1} resolution, within the range of 400−4000/cm.

Morphology of the Nanocarriers. Scanning electron microscopy (SEM) was performed to study the morphology of quercetin-loaded Stearic acid nanoparticles using a SEM (JEOL Tokyo, Japan).

X-ray Diffractometry (XRD). To observe the crystallinity of samples, XRD was studied using an X-ray diffractometer (Siemens D5000, Germany) with Ni-filtered Cu K radiation at a voltage of 40 kV and 25 mA. All the measurements were made in the triplicate form for each sample.[23]

In Vivo Studies. Thirty healthy male albino rats, 8 weeks old, weighing 170−200 g, were used in the *in vivo* studies. All the animals were kept in the animal house facility of the Department of Pharmacology and Physiology, University of Animal Sciences, Lahore, under a controlled temperature of 20−25 °C, relative humidity of 40−60%, and light/dark cycle of 12 h and having free access to diet and water. All of the experiments on these animals were conducted according to the ethical policies and guidelines approved by the Departmental Ethical Review Committee for the Use of Laboratory Animals, vide letter no. 3497. All of the animals were fed with autoclaved commercial rat feed and UV-radiation-treated water

throughout the experiment. A single injection of FCA (0.1 mL) was injected in the subplanter region of the left hind paw of all the rats to induce RA. All the animals were treated with FCA except the normal control group. FCA contains 0.1 mg of inactivated (dry-heat-dead) *Mycobacterium tuberculosis* in sterile paraffin oil and mannide monooleate. A Vernier caliper (Mitutoyo, Japan) was used to measure the ankle swelling (mm) in the paw due to the FCA injection every 7 days.[24] Experimental animals were divided into five groups with six rats in each group as follows:

1. Normal control (N) group: These animals received 0.9% NaCl solution as an oral vehicle and routine diet and water.
2. Arthritic control (AC) group: The rats treated with the FCA model received oral vehicle 0.9% NaCl solution and normal feed and water for 28 days.
3. Arthritic treated (H) group: RA rats received the quercetin suspension at a dosage of 15 mg/kg[13,14] and normal NaCl solution and a routine diet.
4. Arthritic treated (ST) group: All the rats in this group after induction of rheumatoid arthritis received the quercetin-loaded nanoparticles in suspension form at a dose of 15 mg/kg.
5. Dexamethasone-treated (H) group: The rheumatoid arthritis rats received the normal diet of water and dexamethasone suspension (at a dose of 0.3 mg per kilogram of rat weight).

On completion of the experiment, the rats were sedated with isoflurane and euthanized using cervical dislocation.

Therapeutic Assessment. Therapeutic efficacy of the prepared SLNs was assessed by testing ankle swelling,[13] biochemical and inflammatory biomarker levels,[5] and histopathological analysis of the ankle joint.[14]

Statistics. The data for the blood parameters were compared using one-way ANOVA using $p < 0.05$. The data for ankle joint swelling were analyzed by repeated ANOVA measures. A pairwise comparison was made using Tukey's post hoc test to check the difference between the mean values of different groups. These analyses were performed using Minitab (v. 18.0).

■ RESULTS

Zeta Size and Potential. The prepared nanoparticles of stearic acid had a particle size of 100 nm (Figure 1). Additionally, the SLNs were tested by NanoZeta to check the stability of the nanoparticles. The prepared SLNs have a zeta potential of −25 mV, which means that the nanoparticles are stable (Figure 2). The higher the values on the positive or negative side indicates that the nanoparticles are more stable.

SEM. The particles were spherical but not smooth, which means the drug was present on the surface of the nanoparticles (Figure 3).

FTIR Spectroscopy. FTIR spectroscopy was used to identify the chemical structure by absorption peaks of various functional groups. We observed peaks of quercetin and quercetin-loaded NPs at 3223 cm^{-1} O−H stretching; 1613 cm^{-1} refers to the amide 1 band (N−H bending), and 1012 cm^{-1} C−O−C stretching (Figure 4), indicating the presence of the methoxyl group. It reveals that quercetin is well incorporated into the particles. Similarly, FTIR spectroscopy tells us of the successful formulation of quercetin-loaded stearic acid nanoparticles.
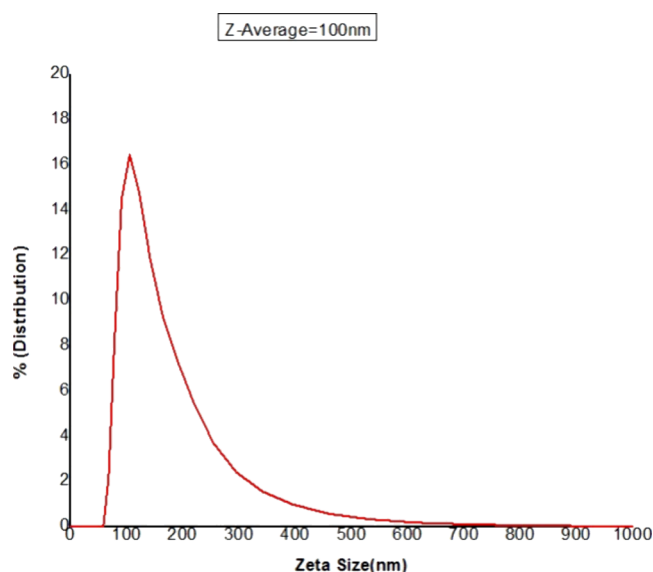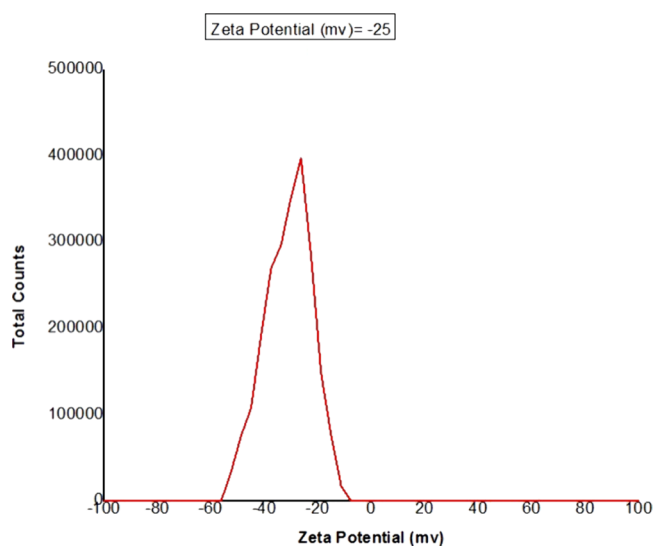
Figure 1. Zeta size of quercetin-loaded SLNs

Figure 2. Zeta potential of quercetin-loaded SLNs.
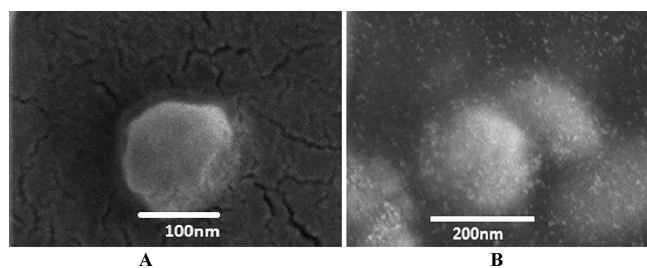

Figure 3. SEM of nanoparticles without quercetin (A) and quercetin-loaded nanoparticles (B).

XRD. XRD shows the patterns of pure quercetin and quercetin-loaded stearic acid nanoparticles. The sharp peaks were present in the quercetin at angles of 2θ 12.48, 15.86, 23.88, and 24.88°, hence representing that quercetin is present in crystalline form. The diffraction patterns of quercetin were only patterned by the large peaks, so there was no longer a distinction between the characteristics of the quercetin.
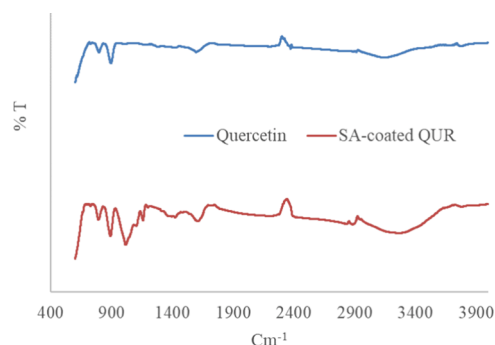

Figure 4. FTIR spectra of quercetin (QUR) and quercetin-loaded stearic acid nanoparticles (SA-coated QUR).

***In Vivo* Findings.** For the determination of ankle swelling, 30 healthy 8-week-old male albino rats were used in the experimental research. Animals were randomly divided into five groups (n = 5; Table 1) and kept in the animal facility of the Department of Pharmacology, UVAS, Lahore, Pakistan. All of the rats were fed with standard feed and UV-radiation-treated water throughout the experiment.

Ankle swelling was measured in all the experimental rats on day 1 before FCA administration and then on days 6, 9, 14, 18, and 21 with the help of a Vernier caliper (Kern, Germany). Ankle swelling was significantly ($p < 0.001$) decreased in the treated groups, ST and H, when compared with arthritic control groups on day 21, whereas there was a nonsignificant ($p > 0.001$) difference in the treated groups (Figures 5 and 6).
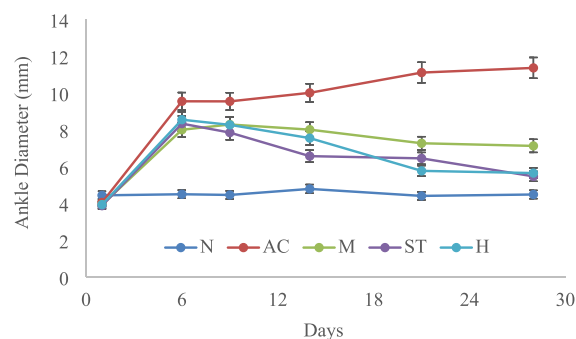

Figure 5. Data comparing ankle swelling concerning time intervals in the normal control (N), arthritic control (AC), quercetin suspension-treated (M), quercetin-loaded stearic acid NP-treated (ST), and dexamethasone-treated (H) groups (n = 5 rats per group). Ankle diameter is shown in millimeters (n = 3; mean ± SE). Bars with similar alphabet letters represent statistically nonsignificant ($p \geq 0.05$) differences from each other.

For further biochemical and inflammatory biomarker analysis, rats were euthanized at the end of the study period on the 21st day. Blood was collected in gel clot activator tubes for serum collection. Serum was separated by centrifugation at 10,000 rpm for 10 min and stored in a deep freezer for further analysis.

The RA factor was significantly ($p < 0.001$) increased in the AC group when compared with the normal control group (N), while the RA factor was significantly ($p < 0.001$) decreased in the quercetin-loaded stearic acid NP-treated group (ST) and dexamethasone-treated (H) group, and it was nonsignificantly ($p > 0.05$) reduced in the quercetin suspension-treated (M) group (Figure 7).
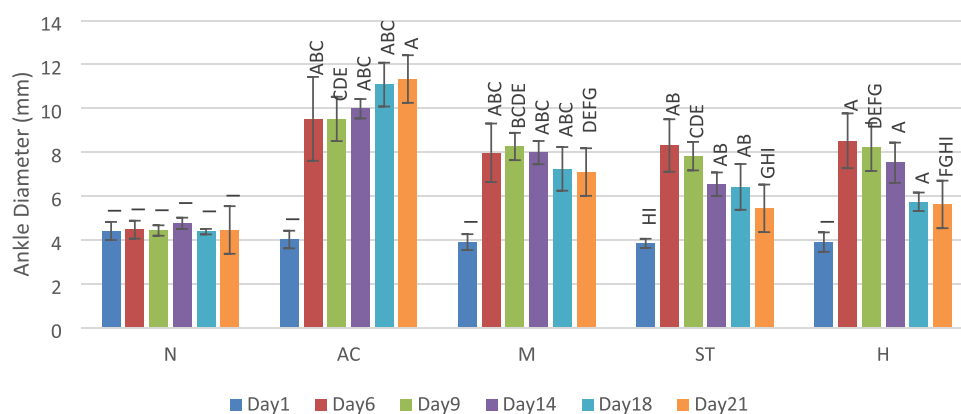
Figure 6. Data comparing ankle swelling for different treatments in the normal control (N), arthritic control (AC), quercetin suspension-treated (M), quercetin-loaded stearic acid NP-treated (ST), and dexamethasone-treated (H) groups ($n = 5$ rats per group). Ankle diameter is shown in mm ($n = 3$; mean ± SE). Bars with similar alphabet letters represent statistically nonsignificant ($p \geq 0.05$) differences from each other.
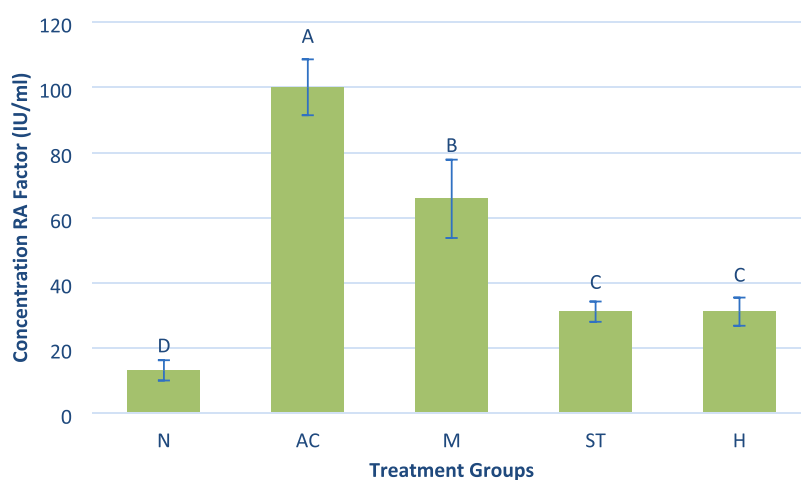

Figure 7. Rheumatoid arthritis (RA) factor in different groups. RA factor (mean ± SE) in normal (N), arthritic control (AC), quercetin suspension-treated (M), quercetin-loaded stearic acid NP-treated (ST), and dexamethasone-treated (H) groups ($n = 5$ rats per group). Bars with similar alphabet letters indicate statistically nonsignificant ($p \geq 0.05$) differences from each other.

To test the therapeutic potential, inflammatory markers (IL-6, TNF-α, Cox-2) were significantly ($p < 0.001$) increased in the AC group throughout the study when compared with the normal control group. These parameters were decreased in the quercetin-loaded stearic acid NP-treated (ST) and dexamethasone-treated (H) groups (Figure 8).

For safety analysis, biochemical parameters (AST, ALT, ALP, and bilirubin) were significantly ($p < 0.001$) increased in the RA group due to the FCA injection while these parameters tended to normal values after treatment (Figure 9).

For histopathological studies, all animals were dissected to collect the ankle joint tissue. These tissues were placed in a 10% formalin solution for histopathological studies. The samples were tested at the Department of Pathology, UVAS, Lahore. Histopathological findings are presented in Figure 10 and Table 2. In histopathological analysis, it was seen that the ankle joint was intact in the normal control group, whereas there was the presence of granuloma, inflammatory cells, and edema in the arthritic control group. In the treated group with quercetin solution, there was the presence of granuloma and mild inflammatory cells and edema, whereas in quercetin-loaded SLNs, it was seen that there is neither granuloma nor edema and very mild inflammatory cells; however, in the dexamethasone-treated group, there is no granuloma seen,

while there was the presence of mild inflammatory cells and edema.

■ DISCUSSION

RA is a progressive disease for which there is currently no treatment strategy. Drugs are available only to slow down its progression. Due to the anti-inflammatory effect of quercetin, stearic acid nanoparticles of quercetin are prepared to check their effect against inflammation. The SLNs were prepared by the hot melt encapsulation technique by just physical mixing of fatty acids, which is safe from other TDDS.[25] The parameters used in this technique were very similar to those required for conventional semisolid dosage forms, and it may be feasible for large-scale production. SLNs have many advantages from liposomes, niosomes, emulsions, micelles, and polymeric nanoparticles having high drug loading stability, biocompatibility, and ability to protect drugs from degradation.[26]

Quercetin is a hydrophobic drug that belongs to the flavonoid family and has very low bioavailability; therefore, we have chosen SLNs to nanoencapsulate quercetin. The zeta size and zeta potential of the prepared SLNs are very important for many reasons. First, these particles should be small enough to cross biological barriers like fenestrated blood vessels with a pore size of 50−150 nm. Second, nanoparticles should be
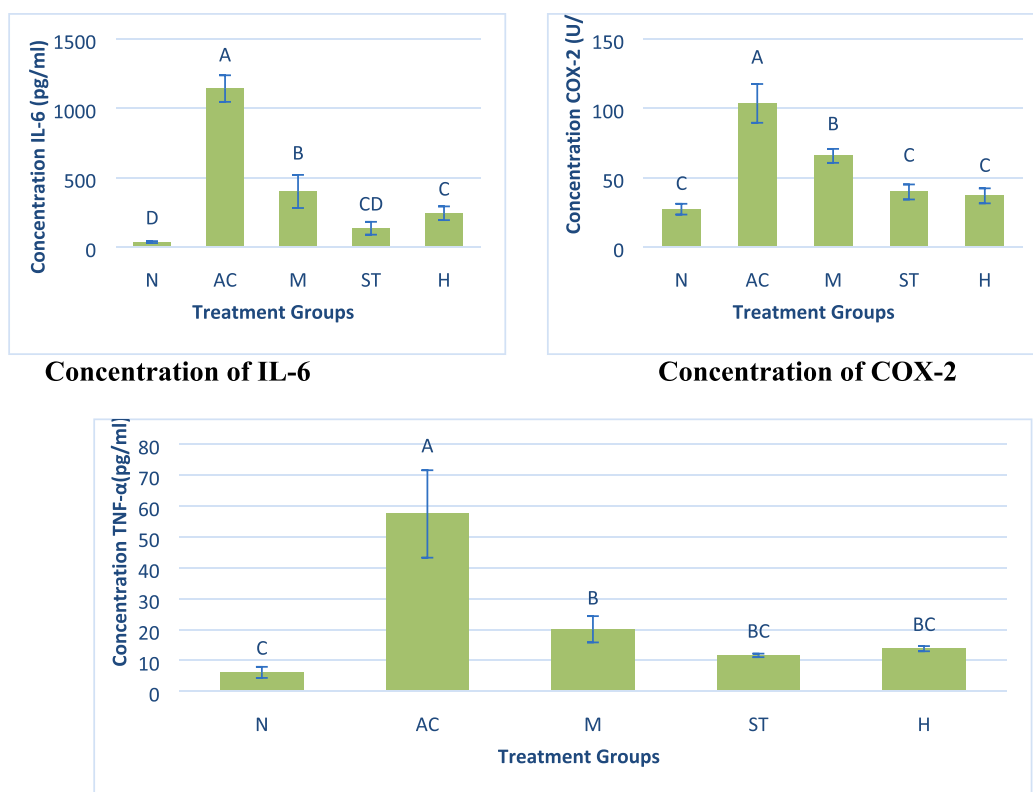
Figure 8. Concentration of TNF-α. Inflammatory markers (IL-6, TNF-α, Cox-2) (mean $\pm$ SE) in normal (N), arthritic control (AC), quercetin suspension-treated (M), quercetin-loaded stearic acid NP-treated (ST), and dexamethasone-treated (M) groups (n = 5 rats per group). Bars with similar alphabet letters show statistically nonsignificant ($p \geq 0.05$) differences from each other.

homogenized enough for better performance in vitro and in vivo. The zeta potential of the nanoparticles is very important to demonstrate the homogeneous dispersity in aqueous dispersion. The zeta potential of all formulations was in the acceptable range of −20 to −60 mV, which is a prerequisite for the long-term stability of an SLN dispersion. We have prepared nanoparticles with an average size of less than 200 nm and zeta potential −25 mV, which is good according to a previous study.[27] The SLNs reported here have a spherical shape, as confirmed by a SEM. According to these findings, the prepared nanoparticles are stable and can permeate the biological membranes.

FTIR studies demonstrated that the O−H stretching of quercetin is present at 3223 cm^{-1}, which indicates that quercetin was present intact in SLNs. N−H bending stretching at 1613 cm^{-1} and C−O−C stretching at 1012 cm^{-1} indicate the presence of quercetin in the prepared solid lipid nanoparticles. The low intensity of the quercetin peak was due to the amount of quercetin in the SLNs. The same types of peaks were reported for quercetin-loaded nanoparticles previously,[28] which is in agreement with our study. These results indicate that quercetin is in an intact form after loading to the nanoparticles.

There are many animal models for RA induction, but the FCA-induced arthritis model has proven its efficiency. Common RA symptoms can be observed including joint swelling edema, joint deformation, and cartilage degeneration. In this study, RA induction was carried out by FCA, which is composed of heat-killed mycobacteria, emulsifying agents, and mineral oil. Similar induction of RA by FCA has been reported previously.[29] There are many routes including the IV, oral,

topical, and intra-articular for the administration of SLNs, but the oral route is considered the goal standard due to patient compliance and cost-effectiveness. The oral route was chosen in this study due to ease of dose administration and compliance of the patient, while the intra-articular route has several limitations that include injection techniques and rapid clearance of drug via joint cavity.[30] Induction of arthritis with FCA injection under the left hind paw brings about swelling and edema of ankle joints and paws in rats. Significant progression of inflammation in the arthritic control group throughout an inflammatory reaction mediates as a primary lesion within 3 to 5 days after FCA injection followed by a secondary lesion after 11−12 days. Swelling of ankle joints is an indicator of arthritic activity. The ankle swelling was significantly decreased in the SLN-treated group.[31] Based on the results, it can be claimed that quercetin-loaded SLNs have promising efficacy against RA.

The RA factor is a key inflammatory biomarker in RA and is more significantly decreased in the SLN-treated group. Also, the reduction in RA factor with SLNs was reported, which is in agreement with this study. TNF-α, interleukin-6, and COX-2 are key inflammatory biomarkers in RA. their role predominates in the progression and invasiveness of RA because these have an important role in the fine-tuning of other inflammatory biomarkers. TNF-α, interleukin-6, and COX-2 levels are higher in the RA group, and these levels are significantly decreased treated with quercetin solution and quercetin SLNs and dexamethasone but the superiority of reduction of RA was seen with quercetin-loaded stearic acid nanoparticles when comparing with the arthritic control group.[32] Quercetin can inhibit the

AST concentration   **ALT concentration**
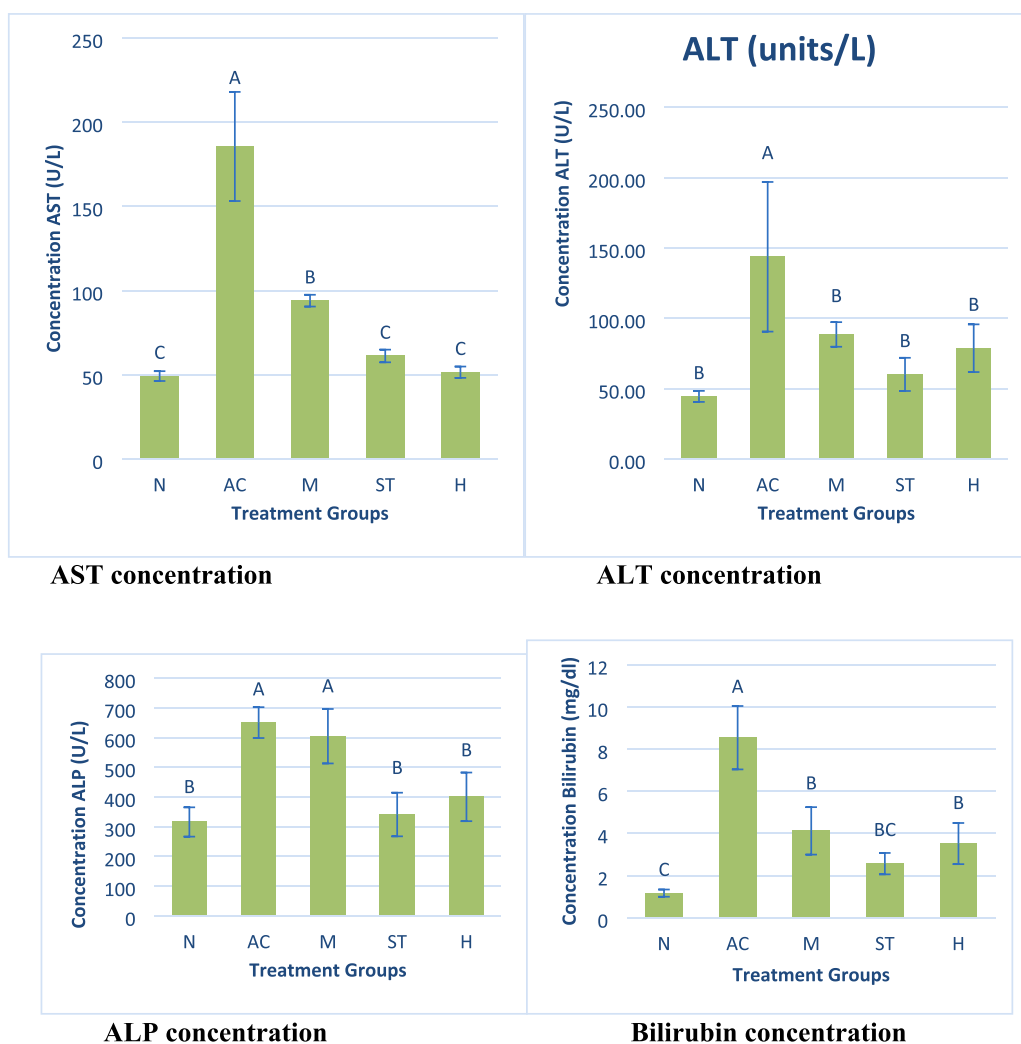
ALP concentration   **Bilirubin concentration**

Figure 9. Biochemical parameters AST, ALT, ALP, and bilirubin (mean ± SE) in normal (N), arthritic control (AC), quercetin suspension-treated (M), quercetin-loaded stearic acid NP-treated (ST), and dexamethasone-treated (H) groups (n = 5 rats per group). Bars with similar alphabet letters reflect statistically nonsignificant ($p \geq 0.05$) differences from each other.

production of T cells, pro-inflammatory cytokines, NF-κB, and osteoclast function that result in reduced joint inflammation.

The superiority of quercetin-loaded nanoparticles over quercetin suspension may be attributed to these reasons: First, nanoencapsulation of quercetin may increase its bioavailability that enhances its interstitial permeation up to 1.8 fold, proving that nanostructured lipid carriers retained more than 66% of its initial content in the lipid matrix followed by passage through GIT indicating that SLNs are a good candidate for oral drug delivery.[33] The second of the solid lipid nanoparticles may be attributed to the anti-inflammatory goal of the stearic acid that reduces the pro-inflammatory cytokine, and eventually inflammation is alleviated.

The FCA model is also used to determine the biochemical parameter differences. The elevated level of biochemical parameters is due to stress induced by inflammation. Serum ALT and AST are linked with enhanced bradykinin levels in inflammation and depict hepatotoxicity, whereas the ALP level in FC arthritis is linked with bone erosion and is an estimation of lysosomal integrity. In this study, elevated levels were seen in arthritic control rats; nevertheless, treatment of arthritic rats with SLNs considerably reduced these levels to the normal values due to the anti-inflammatory effect of quercetin.[34]

Bilirubin and creatinine levels also increased in the arthritic control groups due to the inflammatory stress of FCA; however, it was seen that these levels were significantly decreased in the treated groups and superiority was seen with the quercetin-loaded lipid nanoparticles. Thus, the results of the biochemical analysis confirmed the hepatoprotective effect of quercetin. Hence, these findings suggested its potent antiarthritic potential. The same findings have been reported, which justified our study.[34]

■ LIMITATIONS

As a limitation of this study, the safety and toxicity of the solid lipid nanoparticles prepared in this experiment have not been confirmed in vitro, which will be conducted in future experiments.

■ CONCLUSIONS

In conclusion, SLNs were prepared with an average size <200 nm and a zeta potential of −25 mV with a spherical shape prepared with the hot melt encapsulation technique. FTIR spectra also showed the presence of quercetin in the SLNs. Ankle swelling, RA factor, and key inflammatory biomarkers were significantly reduced with treatments, whereas a better
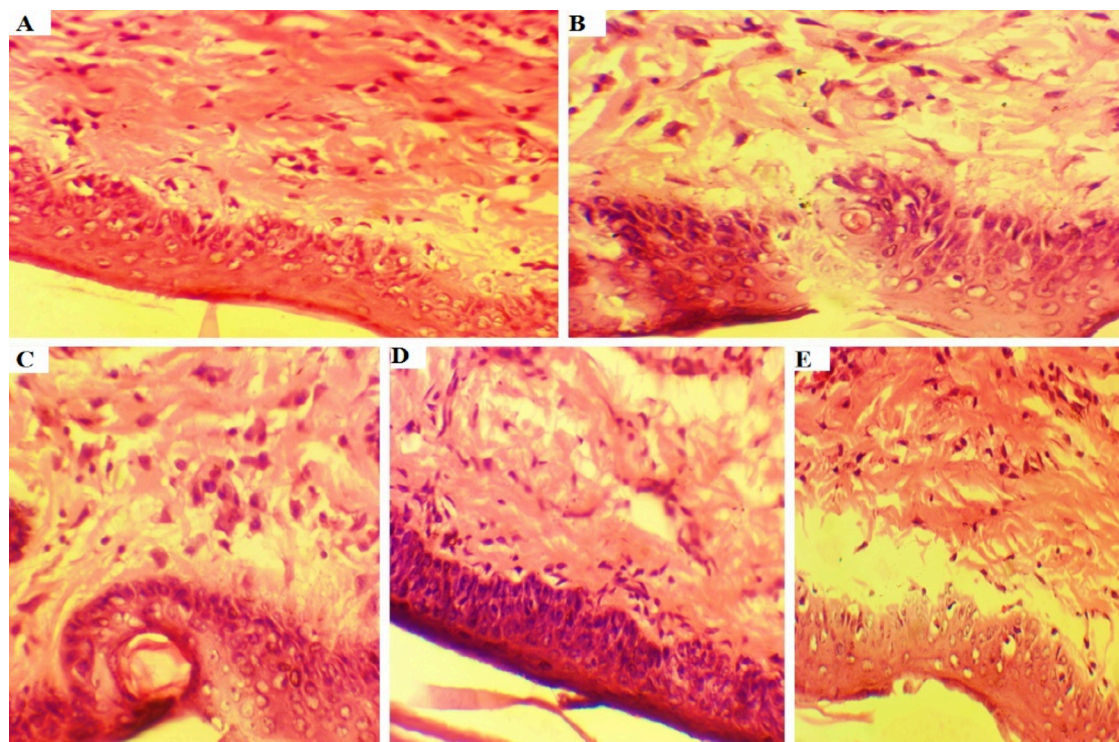
Figure 10. Comprehensive histopathological analysis of the ankle joint. (A) Normal control, (B) arthritic control, (C) rats treated with quercetin suspension, (D) rats treated with quercetin-loaded stearic acid NPs, and (E) rats treated with dexamethasone given orally in rats (n = 5/group) (stained with hematoxylin and eosin, magnification = 40×).

Table 2. Histopathological Analysis of Ankle Joint[a]

groups	edema	degree of erosion	infiltration of cells	connective tissue proliferation
normal control	0	0	0	0
arthritic control	++++	++++	++++	++++
arthritic rats treated with quercetin suspension	+++	++	+++	+++
rats treated with quercetin-loaded NPs	+	+	++	+
rats treated with dexamethasone	+	++	++	+

[a]Note: 0: no abnormality seen, +: damage changes up to 25%, ++: damage changes up to 50%, +++: damage changes up to 75%, ++++: damage changes more than 75%.

efficacy was seen with SLNs due to the synergistic effect of lipids with quercetin as an anti-inflammatory. Histopathological changes were also seen in the treated groups, compared to intact ankle joints in the healthy group. Therefore, herein we have proposed the administration of quercetin through solid lipid nanoparticles for enhancing its therapeutic potential.

■ ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, M.K.W., upon reasonable request.

■ AUTHOR INFORMATION

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Author Contributions

All the authors have significant scientific contributions.

Notes

The authors declare no competing financial interest.

All the experiments on these animals were conducted according to the ethical policies and guidelines approved by the Ethical Review Committee for the Use of Laboratory Animals, vide letter No. 3497.

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