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Phytosomal curcumin alleviates collagen-induced arthritis by downregulating Th17 and upregulating Treg cell responses in rats

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ABSTRACT

Objective: To explore the effects of a nano-formulation of curcumin (phytosomal curcumin) on the clinical and pathological symptoms of collagen-induced arthritis (CIA) in rats.

Methods: Forty male Wistar rats were immunized with an emulsion containing bovine type Ⅱ collagen and incomplete Freund's adjuvant and then administered phytosomal curcumin post-immunization. Clinical symptoms and histological analysis of the synovial tissues were performed. The effect of phytosomal curcumin on Th17 and Treg parameters was also evaluated.

Results: Phytosomal curcumin reduced the clinical severity and paw swelling in CIA-induced rats, which was accompanied by a reduction in the number of inflammatory cell infiltration in the synovial tissue. Additionally, treatment with phytosomal curcumin significantly inhibited CIA-associated mediators as well as increased the anti-inflammatory mediators in comparison to the control groups.

Conclusions: Phytosomal curcumin could improve CIA autoimmune responses and can be considered a potential candidate for the treatment of rheumatoid arthritis.

KEYWORDS: Phytosomal curcumin; Rheumatoid arthritis; Collagen; Th17; Treg; Anti-inflammation; *Curcuma longa*

Significance

Curcumin, a plant-based compound, is commonly used in traditional medicine because of its anti-inflammatory and immunomodulating properties. However, the main limitations of curcumin are low bioavailability and solubility. The present study showed the therapeutic effect of curcumin-loaded phytosome (phytosomal curcumin) on the clinical and pathological symptoms of collagen-induced arthritis. Therefore, it can be considered as a potential candidate for the treatment of rheumatoid arthritis.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease specified by inflammation and swelling of the joint synovium[1]. The annual incidence of recent cases of RA is about 5-50 cases per 100 000 which seriously affects the patient's quality of life and is a heavy burden for family and community[2]. It has been demonstrated that abnormal immune responses, especially inflammatory mediators, play a key role in disease pathogenesis[3].

Collagen-induced arthritis (CIA) is a well-established model for studying immunopathogenesis of RA in humans[4]. Synovitis studies have revealed the main mediators involved in the inflammatory cascade of arthritis are tumor necrosis factor-α (TNF-α), interleukin 17 (IL)-17, nitrite oxide (NO), and matrix metalloproteinases (MMPs)[5]. These mediators have a role in the recruitment of inflammatory cells into the synovium, subsequently leading to disease progression. It has been documented that, in addition to the increased level of inflammatory factors, the decreased level of regulatory elements, including transforming growth factor-β (TGF-β) and IL-10, are involved in the pathogenesis of RA and its animal model (CIA)[6].

Today, despite intensive research, RA/CIA has yet remained uncured. Pharmacotherapy for RA commonly involves treatment regimens, including non-steroidal anti-inflammatory drugs (NSAIDs), oral or intra-articular glucocorticoids, and disease-modifying anti-rheumatic drugs[7]. Unfortunately, the treatment with corticosteroids and NSAIDs is ineffective because of the long duration of therapy, several adverse effects, and high cost[8]. Therefore, new medications, based on natural and safe active compounds from medicinal plants with anti-inflammatory or immunomodulatory properties, are essential for disease management[9].

Curcumin, a natural pigment derived from the rhizome of *Curcuma longa*, is the active component in turmeric spice and has a wide range of biological effects, including modulation of many transcription factors and regulation of intracellular signaling pathways[10,11]. Furthermore, it is well demonstrated that the immunomodulatory effects of curcumin are due to its impact on the activity of inducible nitric oxide synthase, lipoxygenase, and cyclooxygenase-2 enzymes[12].

Recent studies have also reported that curcumin has anti-inflammatory and antioxidant activities. Thus, it seems that curcumin has therapeutic potential in RA treatment[13]. Furthermore, hydrophobic properties of curcumin reduce its accumulation at the disease site[14]. In order to overcome the challenge, a number of formulations and delivery systems have been developed for increasing the bioavailability of curcumin[15]. Phytosomes (transferosome), as a lecithin formulation, are also referred to as a new delivery system for conjugating phospholipids such as phosphatidylcholine. Phytosomes are recently introduced as a novel drug delivery system for herbal components. Manufacturing lipid-compatible molecular complexes aims to improve the delivery and bioavailability of the phytochemical cargo[16]. Phytosomes are successfully applied to enhance the efficacy of various types of herbal extracts such as ginkgo, milk thistle, and green tea, as well as phytochemicals, including curcumin, silybin, ginkgolides, and escin β-sitosterol with promising results in pharmacokinetic survives[17]. Therefore, the present study aimed to evaluate the therapeutic effect of curcumin-loaded phytosome (phytosomal curcumin) on the clinical and pathological symptoms of the RA model with a focus on the inflammatory and anti-inflammatory immune responses.

2. Materials and methods

2.1. Chemicals and reagents

Bovine type Ⅱ collagen was bought from Xi'an Harmonious Natural Bio-Technology Co., Ltd (China). Incomplete Freund's adjuvant (IFA) was from Sigma-Aldrich. Phytosomal curcumin (Meriva®; Indena S.p.A, Milan, Italy) contained a complex of curcumin and soy phosphatidylcholine in a 1:2 weight ratio, and 2 parts of microcrystalline to improve flow ability.

RNA extraction kit was purchased from Cinnagen, Iran (RNX-Plus kit, Cinnagen, Iran). cDNA synthesis kit was prepared by First Strand cDNA Synthesis Kit (Parstous, Iran). Real time SYBR-Green kit (Real Q Plus 2× Master Mix Green, Ampliqon, Denmark) was employed for real-time qPCR analysis. Rat IL-17A and IL-10 platinum ELISA were purchased from Bender Med systems, Austria.

2.2. Animals

In our pilot study, we compared the effect of free and nano form of curcumin (phytosomal curcumin) in the rat model of CIA. Our results showed that phytosomal curcumin had better effect than free curcumin on clinical score, paw volume and inflammation percentage (Supplementary Figure 1). Therefore, phytosomal curcumin was used for further experiment. In the present study, 40 male Wistar rats weighing (170±20) g were used. The animals were kept in customary laboratory conditions (12-hour light/dark cycles, 26-28 ℃). Animal cages were kept clean and rats received daily water and food. Rats were divided into five groups (n=8) as follows: The first group (control) received a normal diet and water without induction of CIA. The second group (CIA) was CIA induced and the rats received a normal diet and water. In the third group (positive control/IDM), the CIA rats received 200 mg/kg of indomethacin by gavage from day 7. For the treatment groups, two protocols were utilized. In the first treatment group (treatment protocol 1), 200 mg/kg phytosomal curcumin was orally administered from day 0 post-immunization until the end of the study. In the second treatment group (treatment protocol 2), phytosomal curcumin was orally administered, starting on day 7 post-immunization until the end of

the study. The dose of phytosomal curcumin was selected based on our pilot study (Supplementary material). These groups were named phyto-cur-0 and phyto-cur-7, respectively. At the end of the experimental period (21 d), the rats were sacrificed as per animal ethics.

2.3. Induction of CIA in rats

In order to induce CIA in rats, an emulsion containing bovine type Ⅱ collagen (CⅡ) and incomplete Freund's adjuvant (IFA) was used. Initially, 2 mg/mL collagen solution was dissolved in acetic acid 0.05 M and gently stirred overnight at 4 ℃. Then, it was emulsified in IFA in equal volume using a homogenizer (1 000 rpm, 60 min) (Labnet International Inc. USA). Next, this emulsion was injected in two areas: the first site was the plantar surface of the left hind paw as a starting dose and the second was an intradermal injection into the root of the tail as a booster dose on the same and the next day[9,18,19]. All collagen-treated rats developed paw edema, and displayed the first signs of disease onset on day 4, reaching a maximum inflammation on day 7. Treatment protocols 1 and 2 were started from days 0 and 7 until the end of the study (day 21), respectively (Figure 1).

2.4. Evaluation of CIA

Disease severity was evaluated by three indicators, including clinical score, paw volume, and anti-inflammatory percentage. The severity of arthritis was evaluated based on a scoring system as follows: 0=normal; 1=mild swelling and erythema; 2=moderate swelling and erythema; 3=erythema of the limb, and severe swelling; and 4=erythema, severe swelling, and disability to use the limb[20,21]. The clinical score was recorded once a week by three trained researchers unaware of the grouping of the mice.

Paw volume was measured by a weighing technique. Briefly, the paw was immersed up to a specified line into a container of water, which was placed on the scale with two decimal places. The weighing was done three times for each paw and the average was taken as paw volume due to the density of water (equal to 0.997 g/mL at 25 ℃)[19,22].

Also, the anti-inflammatory (improvement) percentage was calculated by the following equation:

Anti-inflammatory (improvement) percentage=[1–Vt/Vb]×100

Where Vt was paw volume in the treatment groups on the following day and Vb was paw volume of the CIA group on day 7 as an indicator of maximum inflammation[23].

2.5. Histopathological analysis

In order to analyze histopathological changes, a tissue sample was taken from the ankle joint of rats in the groups and fixed in 10% formalin. After that, the samples were kept in nitric acid (10%) until the tissue was decalcified. Then, the samples were embedded in paraffin and tissue sections at 5 μm were prepared for histological analysis. The prepared slides were stained with hematoxylin and eosin (H&E) and the photographs were taken using a microscope (Nikon, Japan).

2.6. Enzyme–linked immunosorbent assay (ELISA) assay

To determine the effect of phytosomal curcumin on the cytokine level, the rats were sacrificed at the end of the study. The serum from peripheral blood was collected and stored at −80 ℃ before the assay. The level of IL-17A and IL-10 in the serum was measured using ELISA kits (rat IL-17A and IL-10 platinum ELISA®, Bender Med systems, Austria) according to the manufacturer's instructions.

2.7. Quantitative real–time PCR (qPCR) for mRNA expression of inflammatory and anti–inflammatory factors

The effects of phytosomal curcumin on the mRNA expression of some inflammatory and anti-inflammatory factors were determined by real time-PCR. Total RNA was extracted from the blood samples of rats using RNX-Plus kit (Cinnagen, Iran) and their complementary DNA (cDNA) was prepared by First Strand cDNA Synthesis Kit (Parstous, Iran). In this study, the mRNA expressions of *IL–17A*, *IL–10*, *TNF–α*, *TGF–β*, *MMP–8*, and nitric oxide (*NO*) were measured by RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) in an ABI Step One Plus® system (Applied Biosystems™, USA). Gene expression was analyzed using semi-quantitative method $2^{-\Delta\Delta Ct}$ and *β–actin* as an internal control was used to normalize the data. The tests were repeated in triplicate and the sequences of primers are depicted in Table 1.

2.8. Statistical analysis

The results were expressed as mean±standard deviation and

Table 1. The sequences of primers in the study.

Genes	Forward primer 5'-3'	Reverse primer 5'-3'
MMP–8	TCCAGGTTACCCCACTAGCA	AGTGACTCTGCGACTGACAAG
NO	AGGTGCTATTCCCAGCCCAA	CGGGTCGATGGAGTCACATA
IL–10	TTGAACCACCCGGCATCTAC	CCAAGGAGTTGCTCCCGTTA
TGF–β	CACCTGCAAGACCATCGACA	ACTGGCGAGCCTTAGTTTGG
IL–17A	GTTCAGTGTGTCCAAACGCC	AGGGTGAAGTGGAACGGTTG
TNF–α	CCCAGACCACAATTCCC	CTCAAGCCCTGGTATGAGC
β–actin	TAAGGCCAACCGTGAAAAGA	GGTACGACCAGAGGCATACA

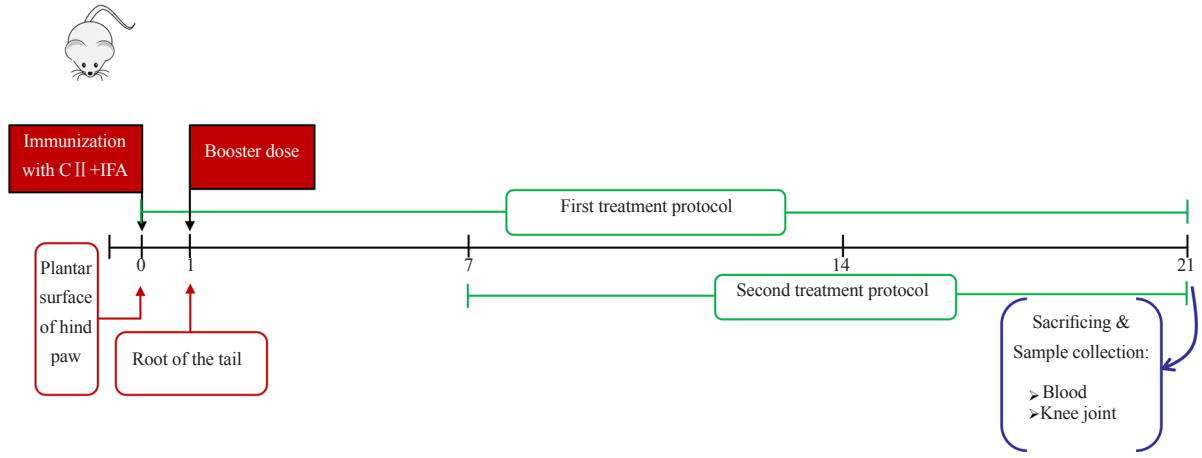

Figure 1. Schematic time-course of induction of collagen-induced arthritis and treatment with phytosomal curcumin in rats. C Ⅱ: bovine type Ⅱ collagen; IFA: incomplete Freund's adjuvant.

analyzed by one-way ANOVA followed by Tukey *post hoc* test using SPSS statistical package 15. Differences were reported to be statistically significant at $P<0.05$.

2.9. Ethical statement

All experiments were done in accordance with the NIH Guide for the Care and Use of Laboratory Animals, with approval from Animal Ethic Committee of Rafsanjan University of Medical Sciences (Ethical code: IR.RUMS.REC.1398.036).

3. Results

3.1. Phytosomal curcumin decreases paw swelling, clinical score, and inflammation

As shown in Figure 2A, severe RA symptoms developed in all rats of the CIA group with a maximum score of 4.00±0.29 on day 14 compared to the control group (0.50±0.23, $P<0.001$).

In the treatment groups, phtosomal curcumin improved the clinical score following sensitization. As shown in Figures 2A and B, phtosomal curcumin significantly reduced the severity of arthritis compared to the CIA group ($P<0.05$ for the phyto-cur-0 group and $P<0.01$ for the phyto-cur-7 group).

In addition, a high degree of paw swelling was observed in the CIA group compared with the control group and this symptom continued until the end of the study (Figures 2C and D). Treatment with phtosomal curcumin significantly mitigated paw swelling [CIA (2.85±0.17) *vs.* phypo-cur-0 (1.75±0.13), $P<0.01$, and *vs.* phyto-cur-7 (1.50±0.15), $P<0.01$].

Moreover, phtosomal curcumin resulted in a significant reduction in the percentage of inflammation ($P<0.01$) and an increase in the percentage of improvement over the CIA group ($P<0.001$) (Figures 2E-H).

3.2. Phytosomal curcumin reduces inflammation and bone erosion in CIA rats

Clinical evaluation of rats during CIA showed that curcumin in phytosomal form modulated the course/severity of CIA. To examine underlying reasons for the improvement in clinical signs, histological analyses were used to evaluate the hyperplasia of the synovial tissue in the paw. The CIA rats had apparent hyperplasia of the synovial tissue with extensive infiltration of inflammatory immune cells compared to the control group that had a clear structure of the joint synovial tissue without any hyperplasia (Figure 3). Consistent with the clinical results, CIA rats receiving phytosomal curcumin from day 7 showed a decreased pro-inflammatory cell infiltration and hyperplasia of synovial tissue compared to the CIA group (Figure 3). These results show that phytosomal curcumin could reduce inflammation, death of chondrocytes, cartilage surface erosion, and bone erosion in CIA rats, in part due to reduced cellular infiltration into the synovial tissue.

3.3. Phytosomal curcumin reduces pro-inflammatory and increases anti-inflammatory cytokines in CIA rats

CIA rats showed significantly increased IL-17A levels while decreasing IL-10 levels in comparison with control rats (Figures 4A and B, $P<0.05$). Treatment with phytosomal curcumin from day 7 markedly inhibited IL-17A production and increased IL-10 level in comparison with the CIA group ($P<0.01$ for IL-17A and $P<0.05$ for IL-10). However, treatment with phytosomal curcumin from day 0 only inhibited IL-17A, but had no significant effect on IL-10 compared with the CIA group ($P<0.05$ for IL-17A).

3.4. Effects of phytosomal curcumin on the mRNA expression of pro-inflammatory and anti-inflammatory factors

To evaluate the therapeutic potential of phytosomal curcumin on some inflammatory/anti-inflammatory factors, real-time PCR was

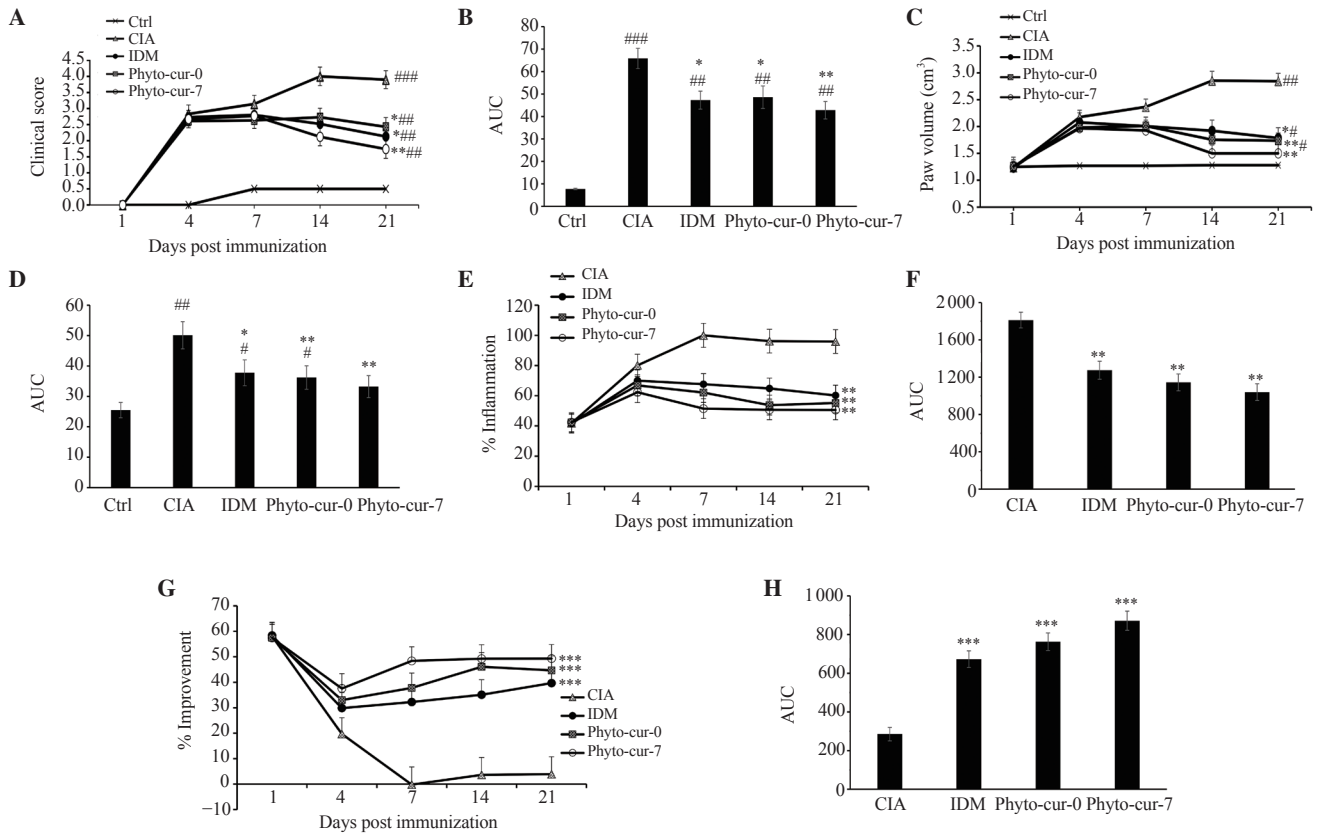

Figure 2. Effect of phytosomal curcumin on clinical symptoms associated with collagen-induced arthritis (CIA). Rats were immunized with C Ⅱ and IFA and then administered with phytosomal curcumin or vehicle starting from day 0 and 7 post-immunization. Rats were monitored for signs of CIA, and the results are presented as (A) clinical scores, (C) paw volume, (E) percent of inflammation and (G) percent of improvement. (B), (D), (F), and (H) show total area under curve (AUC) for clinical scores, paw volume, percent of inflammation, and percent of improvement, respectively. Data are presented as mean±SD and analyzed by one-way ANOVA followed by Tukey *post hoc* test. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ versus the CIA group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ versus the control group. Ctrl: healthy rats without CIA induction; CIA: CIA was induced and treated with vehicle; IDM: CIA was induced and treated with indomethacin; Phyto-cur-0: CIA was induced and treated with phytosomal curcumin from day 0 and Phyto-cur-7: CIA was induced and treated with phytosomal curcumin from day 7.

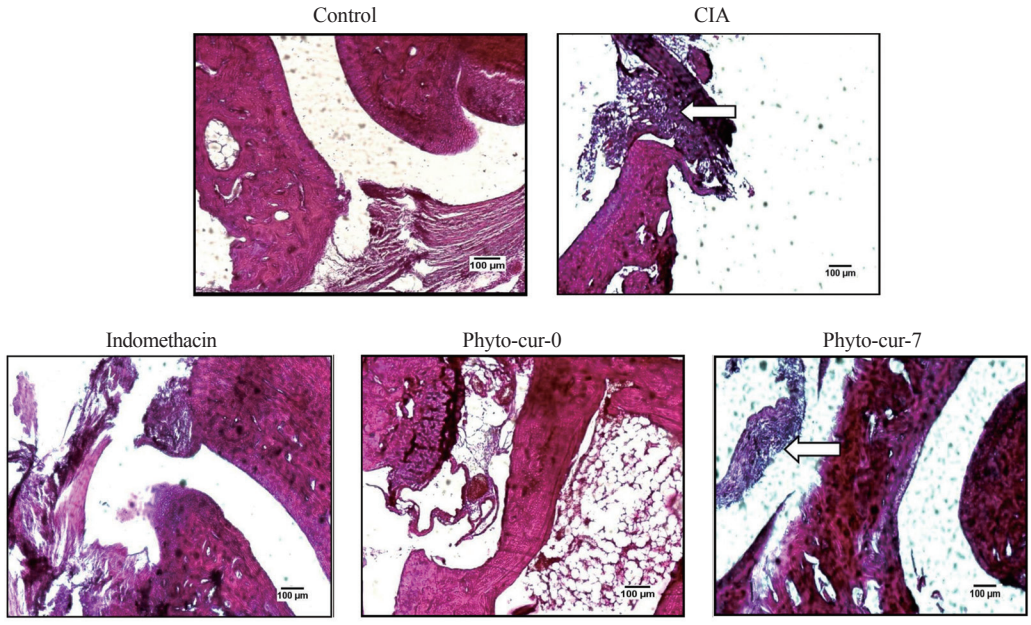

Figure 3. Phytosomal curcumin reduces inflammation and bone erosion in CIA rats. Tissue samples from ankle joint of each rat (collected on day 21 post-immunization) were fixed, and embedded in paraffin. Sections (5 μm) were prepared and the tissues were then stained with hematoxylin and eosin (Magnification: ×20; scale bar: 100 μm). The CIA group shows severe inflammation and infiltration of immune cells into the synovial tissue (arrow) compared to the control group. The groups treated with indomethacin and phytosomal curcumin from day 0 show moderate inflammation. The group treated with phytosomal curcumin from day 7 shows a decreased pro-inflammatory cell infiltration and hyperplasia of synovial tissue (arrow).

performed. The mRNA expressions of *IL–17A*, *TNF–α*, and *MMP–8* were increased (P<0.05 for *IL–17A*; P<0.01 for *TNF–α* and P<0.001 for *MMP–8*; Figures 5A-C) in the CIA group, while those of *IL–10, TGF–β*, and *NO* were significantly decreased compared with the normal control group (P<0.001; Figures 5D-F).

However, treatment with phytosomal curcumin from day 7 reversed the CIA-induced changes in the expression of inflammatory (*IL–17A*, *TNF–α*, and *MMP–8*) and anti-inflammatory (*IL–10* and *TGF–β*) mediators (Figures 5A-E). In contrast, in rats receiving phytosomal curcumin from day 0, only the expression of *MMP–8* was markedly reduced (P<0.05) with no significant effect on the mRNA expression of other mediators. Moreover, *NO* was significantly reduced in the group receiving phytosomal curcumin from day 7 compared with the CIA group (P<0.05) (Figure 5F).

4. Discussion

In this research, the efficacy of a new formulation of curcumin in treating CIA was investigated. The reason for choosing phytosome

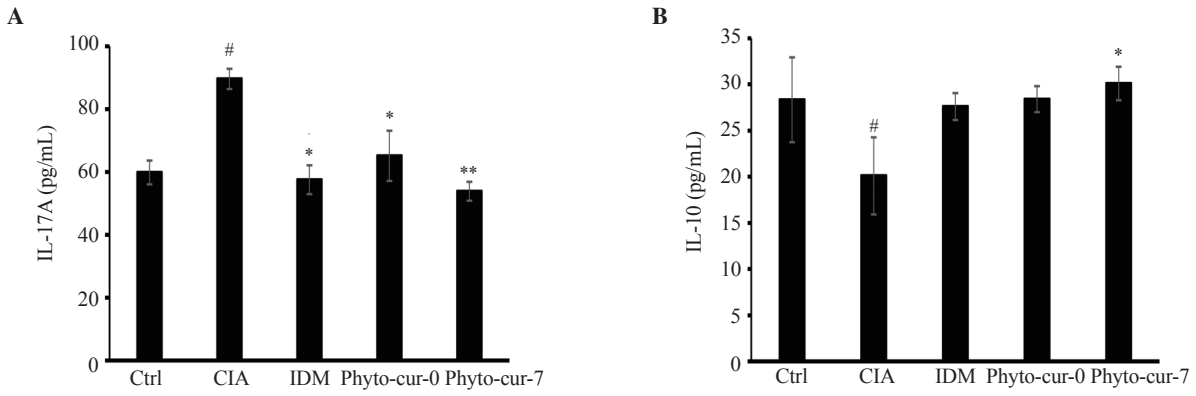

Figure 4. Effects of phytosomal curcumin on the levels of (A) IL-17 and (B) IL-10. Values are presented as mean±SD and analyzed by one-way ANOVA followed by Tukey *post hoc* test. $^{*}P$<0.05, $^{**}P$<0.01 versus the CIA group and $^{\#}P$<0.05 versus the control group.

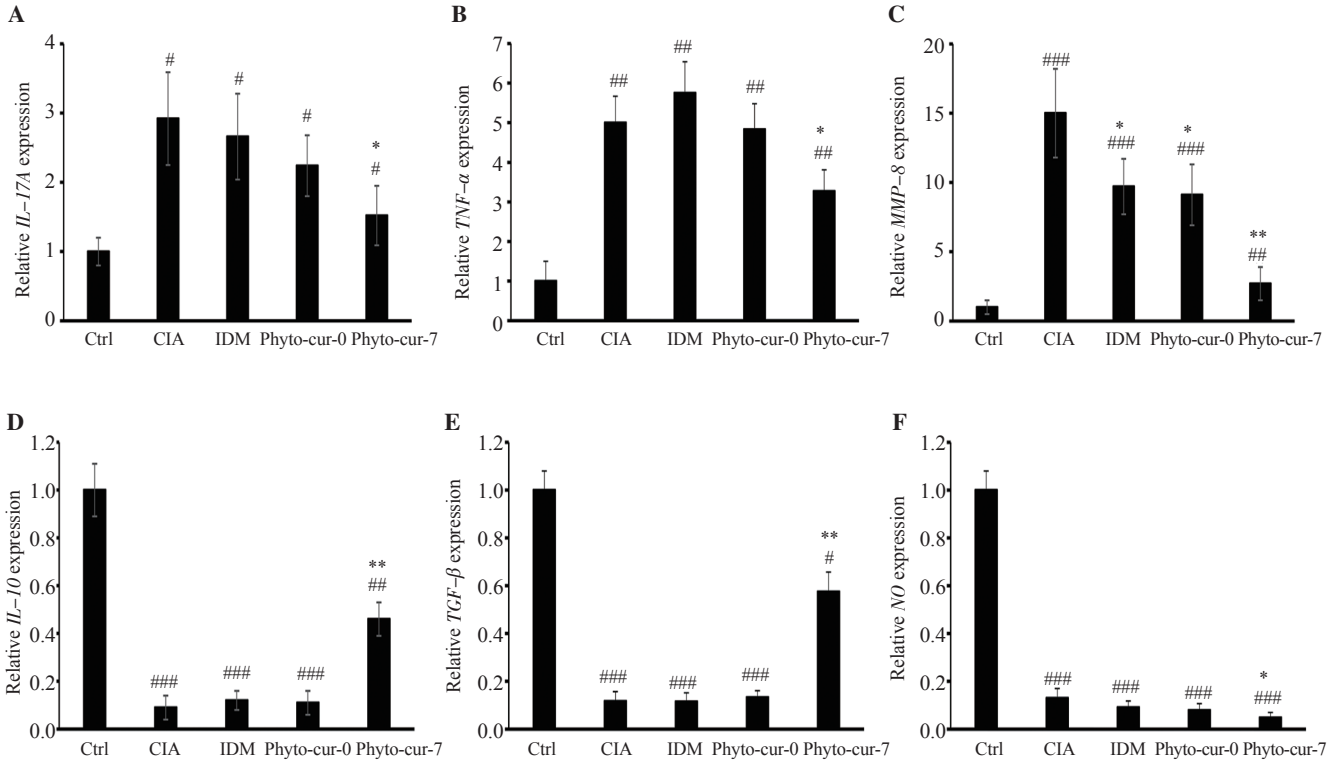

Figure 5. Effects of phytosomal curcumin on the mRNA expression of pro-inflammatory and anti-inflammatory factors. The mRNA expressions of (A) *IL–17A*, (B) *TNF–α*, (C) *MMP–8*, (D) *IL–10*, (E) *TGF–β* and (F) *NO* were determined by quantitative real time PCR and *β–actin* was used as a house keeping gene. Results are expressed as mean±SD and analyzed by one-way ANOVA followed by Tukey *post hoc* test. $^{*}P$<0.05 and $^{**}P$<0.01 versus the CIA group and $^{\#}P$<0.05, $^{\#\#}P$<0.01 and $^{\#\#\#}P$<0.001 versus the control group.

was due to its ability in drug delivery and bioavailability of the phytochemical cargo[24], as well as stability under most *in vivo* conditions.

Several researchers have used phytosome for increasing the efficacy of various types of herbal components. In order to improve the pharmaceutical and therapeutic effectiveness of curcumin, we used phytosome directly as a drug carrier. Therefore, loading curcumin by phytosome gives the additional capability and enables the drug as one of the best formulations for biomedical applications.

In our study, CIA was induced in rats, and a remarkable increase in the severity of arthritis and hind paw volume was observed in the CIA group compared to the control group. Our study found that phytosomal curcumin significantly decreased paw volume and disease severity compared to the CIA group. These results were also confirmed with a reduction in inflammation and an increase in anti-inflammatory percentage in rats treated with the drug. Although the exact mechanisms underlying how phytosomal curcumin reduces paw volume and disease severity are still unknown, it seems likely that the reduction of inflammatory cell infiltration into synovial tissues would lead to a decrease in disease severity, paw volume, and inflammation percentage in the treatment groups. Such an outcome was consistent with the pathological results that showed less severe joint damage and infiltration of immune cells in the treatment groups in comparison with what was noted in tissues from the CIA rats. In parallel with our results, several researchers have reported that curcumin can be considered an anti-inflammatory and immunomodulatory agent in inflammatory disorders such as inflammatory bowel disease[25], psoriasis[26], depression[27], atherosclerosis[28], Alzheimer's disease[29], and COVID-19[30].

In addition, a marked reduction in IL-17A and a prominent elevation in IL-10 expression in the treatment groups were observed. The researchers have established that the pathogenesis of CIA is multifactorial; however, inflammatory and anti-inflammatory cytokines play a pivotal role in the pathogenesis of CIA. It is well known that IL-17A is a signature cytokine for Th17 (as an inflammatory cell) and IL-10 is a crucial cytokine for Treg cell function. As a result, it seems that phytosomal curcumin can improve CIA disease through inhibition of Th17 function and promotion of Treg cell actions.

Previous investigations also demonstrated that curcumin plays as an anti-inflammatory agent and, hence, can regulate the differentiation of $CD4^+$ T cells to Th17 and promote the Treg cell subset[31]. Recently, a study on patients with COVID-19 indicated that curcumin supplementation leads to the downregulation of Th17 and the upregulation of Treg cell cytokines[32].

It is worth mentioning that the possible mechanism involved in RA/CIA disease is the expression of MMP. MMP-8 (collagenase-2) is an enzyme involved in the cleavage of cartilage proteoglycan and type Ⅰ, Ⅱ, and Ⅲ collagens. In RA patients, MMP-8 contributes to joint damage and inflammation[33]. Lou *et al.* also showed the level of MMP-8 and MMP-9 was increased in the joint cavity and cell apoptosis and osteoarthritis were promoted in rats with diabetic osteoarthritis[34]. Our findings showed that phytosomal curcumin could inhibit MMP-8 production in the treatment groups compared to the CIA group. These results suggest an inhibitory effect of the drug on inflammatory factors in an animal model of arthritis when the drug was administered during the disease.

However, our results differed from those in a previous study by Nicoliche T *et al*. on rats with osteoarthritis, which showed the level of MMP-8 was increased in the curcumin-treated group compared with the control. This research also showed that curcumin increased the level of MMP-8 only in the surface layer of articular cartilage. Conversely, in the deep layer of articular cartilage, MMP-8 was decreased[35]. Another study showed that high-dose curcumin increased IL-6 and MMP-2 levels, while low-dose curcumin decreased IL-6 and MMP-2 levels. Therefore, it seems that the effect of curcumin in inflammatory processes is dose-dependent[36].

In addition, to identify the anti-inflammatory activity of phytosomal curcumin, our research evaluated the level of TNF-α (as a pro-inflammatory cytokine) in rats treated with the drug. It is well documented that TNF-α plays an essential role in the pathogenesis of many inflammatory and autoimmune diseases, including atherosclerosis, RA, and inflammatory bowel disease[37]. In the current study, treatment with phytosomal curcumin from day 7 significantly reduced TNF-α levels. Consistent with our results, a previous study indicated that supplementation with curcumin could downregulate human TNF-α production[38]. An *in vitro* study by Wanga *et al*. showed that curcumin significantly inhibits the upregulation of TNF-α and IL-6 in adipocytes *via* suppressing NF-κB and JNK signaling pathways[39]. In an animal model of breast cancer, Ji *et al*. have also reported that treatment with curcumin leads to a decrease in TNF-α level, which was associated with a reduction in the synthesis and release of several inflammatory mediators[40].

In addition, to identify the oxidant/anti-oxidant activity of phytosomal curcumin, this research evaluated NO expression in rats. It is well known that NO is a free radical, and seems to be an oxidizing agent. This mediator is produced by the effect of NO synthase through converting amino acid *L*-arginine to *L*-citrulline[41]. Several studies have indicated that treatment with curcumin can suppress the production of NO in the models of *in vivo* and *in vitro* research. Moran *et al*. showed that curcumin decreased NOS expression and NO levels, as well as inhibited the proliferation of human osteoblast-like cells[42]. The *in vivo* studies also reported that curcumin inhibited NO production in a murine Meth-A ascites cancer model[43]. Furthermore, an *in vitro* research reported that curcumin could potentially decrease NO levels in mouse macrophages (RAW 264.7 cells). This study also confirmed that curcumin suppresses inducible nitric oxide synthase, an enzyme that is essential for NO production[44]. The result of the present study was consistent with aforementioned results of other studies, suggesting that phytosomal curcumin has an antioxidant and immunomodulatory effect by reducing NO levels. Therefore, the other possible mechanism

involved in the improvement of joint lesions may be through the antioxidant activity of phytosomal curcumin.

Interestingly, our data revealed the immunomodulatory and anti-inflammatory effect of phytosomal curcumin, especially in the group treated from day 7 post-immunization, which was more effective than that of the group treated from day 0.

It can be concluded that treatment from day 0 is associated with the onset of host inflammatory responses and the production of numerous inflammatory mediators, which can reduce the effectiveness of the drug, while one week after induction of the disease, the inflammatory responses are downregulated and anti-inflammatory responses are upregulated. Therefore, administration of phytosomal curcumin on day 7 has a better therapeutic effect than on day 0. In addition, these results also indicate that the impact of phytosomal curcumin on CIA rats is therapeutic, not preventive.

Taking all of the results together, the present study showed that phytosomal curcumin not only decreases the expressions of pro-inflammatory mediators but also enhances those of anti-inflammatory factors, which ameliorates CIA. Therefore, it can be concluded that phytosomal curcumin suppresses CIA *via* inhibition of Th17 cells and activation of Treg cells. Although our research showed that phytosomal curcumin could alleviate CIA mainly by the effect on inflammatory and anti-inflammatory parameters in the blood, further research should be conducted to verify the effect of phytosomal curcumin on inflammatory makers in secondary lymph organs or directly in the affected paws.

Conflict of interest statement

The authors declare no competing interests.

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Authors' contributions

MR and RN wrote the manuscript; NZ and MRR performed experiments and collected data. YY and MA analyzed the data. ZT performed pathological experiments, and RN designed the study and revised the manuscript.

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