



## RESEARCH ARTICLE

## Immune Modulatory effect of Curcumin NPs on Induced Osteoarthritis in Rats

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ARTICLE INFO	ABSTRACT
Received: May 29, 2024 Accepted: Jul 1, 2024	This experimental study, we investigated the immune-modulatory effects of curcumin nanoparticles (NPs) on induced osteoarthritis in rats. Thirty male albino rats were divided into six groups, with osteoarthritis induced in all groups except the negative control. Treatment groups received chitosan, curcumin-loaded on chitosan (150 mg/kg and 300 mg/kg), or ibuprofen. The preparation and characterization of curcumin NPs were described, and inflammatory markers (TNF- $\alpha$ , IL-6, IL-10) were assessed using ELISA. Results showed significant anti-inflammatory effects in the curcumin-treated groups, particularly with the higher dose of 300 mg/kg, supporting the potential of curcumin-loaded on chitosan NPs as a therapeutic approach for osteoarthritis management.
<b>Keywords</b> Osteoarthritis Curcumin nanoparticles Chitosan, anti-inflammatory Rats.	
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### INTRODUCTION

Osteoarthritis (OA) it is known as degenerative joint disease, OA most generally occurs in the hands, hips, and knees, with OA, the cartilage within a joint begins to break down and the underlying bone starts to turn, these changes usually develop slowly and get worse after some time, osteoarthritis OA can cause pain, stiffness, and swelling [1].

Osteoarthritis is an important disease due to its broad impact on public health, [2]. According to global statistics, the prevalence of osteoarthritis has increased significantly in recent years [3], is the most frequent form of arthritis, it affects over 32.5 million US adults, this disease causes reduced mobility and degenerates the quality of life of affected individuals, this is accompanied by enormous opportunity losses resulting from medical treatments and surgical procedures, in addition to the psychological trouble that patients bear [4].

OA progresses pro-degenerative cytokines such as interleukins (ILs) and tumor suppressor factor- $\alpha$  TNF- $\alpha$  [5]. Treatments for OA are mainly divided into: pharmacological treatments limited to analgesics and/or nonsteroidal anti-inflammatory drugs (NSAIDs), and surgical treatments [6]. Anti-inflammatory medications may cause many side effects, such as stomach and intestinal upset, and in some cases, they can lead to liver and kidney problems[7].

Previous studies have shown that curcumin, extracted from turmeric, has anti-inflammatory and antioxidant properties [8]. One of the most studied medicinal herbs is turmeric, turmeric is the dried rhizome powder of the *Curcuma longa* plant, composed of many phytochemicals [9], [10]. Concerning the approximate composition, turmeric is composed of water (80–90%), followed by carbohydrates

(around 13%), proteins (2%), minerals (2%), and lipids (<1%) [11]. Among the minor components of turmeric, curcuminoids have a central role and may compose up to 10% of dry turmeric powder, this category mainly comprises curcumin, dimethoxy-curcumin, and bisdemethoxycurcumin, which can compose 62–90, 9–23, and 0.3–14 mg/g of commercial turmeric products (extracts and powders), respectively. Additionally, more than 50 curcuminoids (such as bisabocurcumin, curcumalongin, cyclocurcumin, and terpecurcumin) have been identified in turmeric [12], which produce the yellow color of turmeric.

Aim of study: Preparation and Characterization of Curcumin Nanoparticles and Study its Anti-inflammatory Action in Rats with Osteoarthritis

## **MATERIALS AND METHODS**

### **Experimental animals, housing and adaption:**

Thirty 12-weeks mature male Albino rats weighing [180-210g] were supplied from Veterinary Medicine Laboratories veterinary medicine college, university of Tikrit-Iraq. Rats were housed in animal house of faculty of science, kufa university-Iraq and kept in well ventilation under controlled temperature between 23 °c and 25 c.

Animals were fed with commercial food from the manufacturer green world company food and water provided ad libitum throughout the experimental periods. Rats were assigned randomly one week before the experimental period for adaptation, during the lab work in a lab animal house using lab coats, gloves and a face mask which is surgical disposable.

The rats were anesthetized with ketamine [0.05]- xylazine [0.1] mixture mg/kg [b.w.] for each rat by intramuscular injection with an insulin syringe, after 5-minute of anesthesia the permanent marker was used to surround the shaving area in the right knee joint of each rat. Shaving was done by Braun shaving machine, the work on rats was done in a sterile surgical area.

### **Study Design**

Thirty adult male rats were randomly assigned into six groups (5 rats in each group). All groups induced OA by injected intra-articular in the knee joint with 3 mg of MIA expected Group I utilized in this group (normal saline) are considered to be the negative control group. Other groups were treated for 21 days as following .

Group II: Rats utilized in this group were injected intra-articular in the knee joint with 3 mg of MIA and considered the positive control group.

Group III: Rats administered chitosan in a dose of 200 mg/kg orally.

Group IV: Rats treated with curcumin loaded on chitosan in a dose of 150 mg/kg orally.

Group V: Rats administered curcumin loaded on chitosan in a dose of 300 mg/kg orally.

Group VI: Rats administered ibuprofen in a dose of 40 mg/kg orally via gavage tube 7 days after osteoarthritis for 21 days.

### **Preparation Chitosan Loaded with Curcumin**

Different concentrations (4, 1, 2 mg/ml) were prepared by dissolving chitosan powder in deionized distilled water with 1% acetic acid. The mixture was left for 24 hours, stirred, and pH adjusted to 4.6. Sodium tripolyphosphate powder was dissolved in deionized distilled water to prepare a 0.25% W/V solution. Curcumin extract was dissolved in distilled water and added to the chitosan solution. This mixture underwent stirring, sonication, and TPP addition to facilitate curcumin adsorption onto

chitosan. The final solution was sonicated, filtered, and centrifuged to obtain curcumin-loaded on chitosan nanoparticles, which was done according to [13], [14], [15].

### **Characterization Of Curcumin Loaded on Chitosan**

The transmittance curves for infrared frequencies were examined for the synthetic materials used in the current study to verify the presence of different peaks representing the functional groups of each material according to the absorption capacity of infrared frequencies. Transmission Electron Microscopy (TEM) images of the nanoparticles was taken in JEOL's JEM 2200 FS transmission electron microscope operating at 200 kV. Atomic force microscopy (AFM) has also been validated, providing two- and three-dimensional images of molecular assemblies that show geometric shapes. FTIR spectroscopy is a frequently used technique for the chemical and biological analysis of samples, providing information on the vibrational and structural characteristics of a compound. Plant extracts exhibit distinctive peaks in the FTIR spectrum, indicating the presence or absence of functional groups. The infrared spectrum of compounds from zinc oxide and Chitosan, as well as the therapeutic substance (curcumin), and the nano-composites after treatment were studied. Disks of each compound were made with potassium bromide (KBr) after thorough grinding, and the infrared spectrum was measured in the range of 400-4000  $\text{cm}^{-1}$ . The visible bands were fixed, and most of the main bands were identified [16].

### **Animal Sacrificing**

All animals were anesthetized by intraperitoneal of Ketamine and xylazin (9mg/kg/B.W. and 10mg/kg/B.W.) respectively scarified, then blood samples were collected from the heart

### **ELISA Competitive Enzyme Immunoassay Technique Test (IL-6, IL-10, TNF-a)**

All immunoassays (IL-6, IL-10, TNF-a) in the current study were performed according to the instructions of the manufacturer of the kits used.

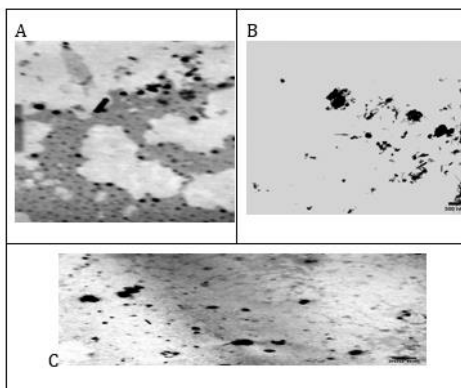
### **Statistic analysis**

Statistical evaluations were performed using One-way ANOVA test to find the differences between groups followed by least significant difference (LSD) multiple comparisons post hoc. Values of  $P < 0.05$  were considered significant.

## **RESULTS**

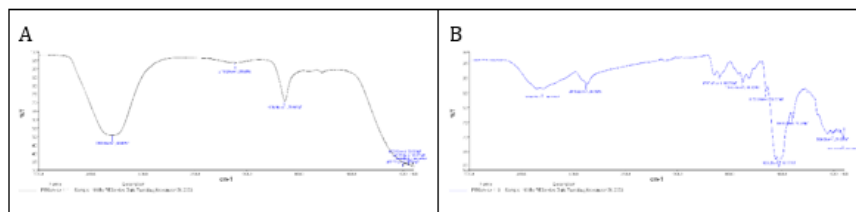
### **Characterization of curcumin loaded on chitosan**

The TEM images (Curcumin loaded on chitosan) as shown in fig 2 demonstrated the formation of aggregated particles within the chitosan as a drug carrier, The TEM analysis showed solid dense semi-spherical particles with little shape variation due to the aggregation of curcumin particles. The sample exhibited a more clearly regular semi-spherical shape of curcumin particles, with good compatibility within chitosan as carrier.



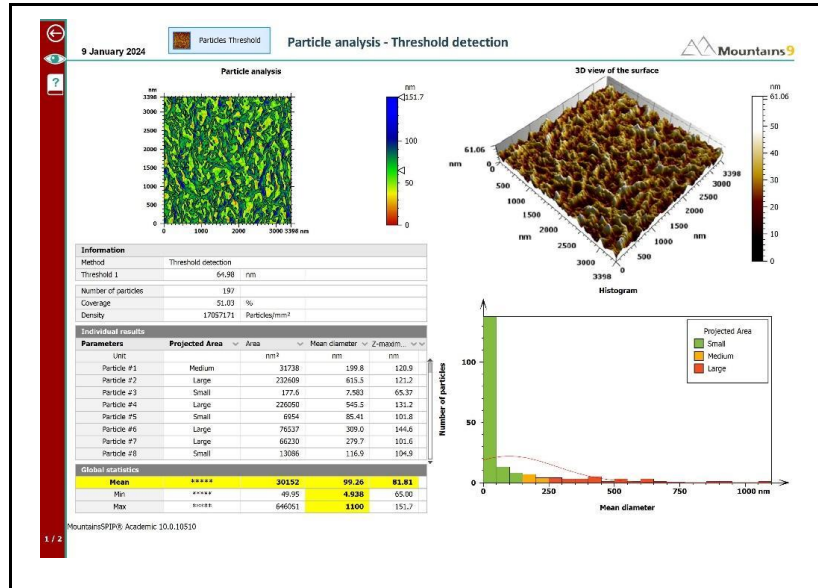
**Fig 2 TEM of A: Curcumin NPs , B: Chitosan NPs and C: Curcumin loaded on chitosan NPs 300 mg.**

The FTIR of synthetic NPs exhibited the presence of many different peaks representing the functional groups for each material according to the absorption capacity of the IR frequencies. The infrared spectrum of curcumin in fig 3A indicates the presence of several peaks located at many wavenumbers. The peak at 1634.99  $\text{cm}^{-1}$  is due to the stretched vibration of the C=C bond. The peaks 3847.42, 3791.26, and 3683.20  $\text{cm}^{-1}$  due to the stretching vibration of the OH group in the alcohol, while 3307.30, 2315.10 and 2873.94  $\text{cm}^{-1}$  are the result of the stretching of the bonds of the amine group NH. The stretching vibration of strong - N=C=S stretching isothiocyanate in curcumin was confirmed at 2121.00  $\text{cm}^{-1}$ . The peak at 1708.93  $\text{cm}^{-1}$  indicated the presence of stretching vibration of the bond strong] C-H stretching alkyne at 3289.75 and 2870.22. where the peaks at wavenumbers of 1590.46, 1026.25, 1150.94 and 2879.72  $\text{cm}^{-1}$  represent the functional groups C=C, C=O and C-O of the chitosan fig 3B. While the presence of curcumin extract in the chitosan was observed through the appearance of the functional groups CH at 894.12  $\text{cm}^{-1}$ .



**Fig 3 FTIR spectroscopy of A: curcumin loaded on chitosan NPs, B: chitosan.**

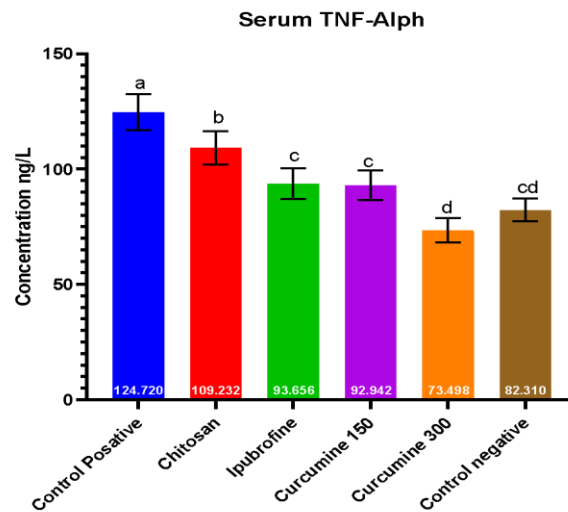
AFM shows the pictographs of the morphology and size of three-dimensional analysis graphs gained from AFM. The obtained average size from the AFM pictographs in the contact mode from the line analysis measurement by utilizing the SPMLab programmed Veeco diInnova software was around 100 nm with average value: 65 nm and Maximum: 151.7 nm and the mean was 81.81 nm. AFM was employed to characterize chitosan-loaded drugs or active compounds. AFM was used in conjunction with DLS measurement to confirm that chitosan is desired for producing well-organized, that appear Oval or elongated oval, with a smooth, regular surface, distributed in a regular manner shape appearance with less than 100 nm size. AFM has been utilized to investigate the efficacy of ultrasound-mediated drug delivery, as shown in fig. 6 .



**Fig 6 Atomic Force Microscopy (AFM)**

**The levels of tumor necrotic factor TNFa (ng/L) in the serum**

The results as shown in fig 7 illustrated the levels of tumor necrotic factor TNFa (ng/L) in six groups of rats, that showed a significant increase (P<0.05) in control positive compared with all experimental group. The result also showed a significant increase (P<0.05) in chitosan compared with all experimental groups. While non-significant (P<0.05) between Ibuprofen, curcumin 150 mg and control negative when compared with each other. and there was non-significant(P<0.05) between curcumin 300 mg with control negative.



**Fig 7 The effects of curcumin nanoparticles and ibuprofen on serum TNF alpha concentration of osteoarthritis male rats - Mean ± SEM-The different small letter denoted significance between groups, p<0.05.**

**The levels of interleukin IL-6 (ng/L) in the serum:**

The levels of interleukin IL-6 (ng/L) in the serum:

The results as shown in fig 8 illustrated a significant increase ( $P < 0.05$ ) in the control positive and chitosan groups compared with all experimental groups. While curcumin 150 mg was non-significant, with control negative ( $P < 0.05$ ). Also ibuprofen was non-significant with a negative control ( $P < 0.05$ ). Curcumin 300 mg and control negative there non-significant ( $P < 0.05$ ).

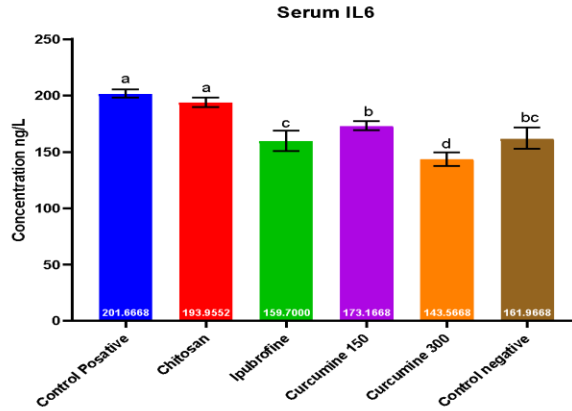
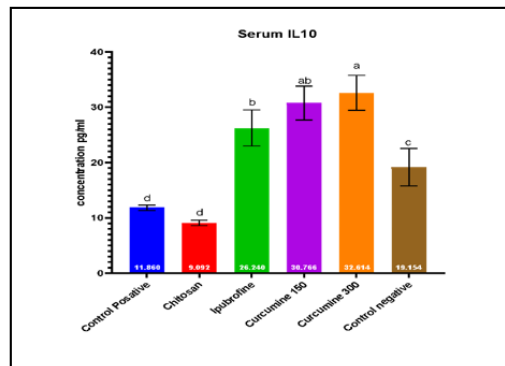


Fig . 8 The serum of IL-6 concentration (ng/L).

**The levels of interleukin-10 (pg/L) in the serum:**

Determination of the concentration of IL-10 serum samples in this study showed a significant reduction ( $P < 0.05$ ) in control positive and chitosan groups compared with all experimental groups, but no significant ( $p > 0.05$ ) difference between them groups. Ibuprofen is also non-significant ( $P > 0.05$ ) with curcumin 150 mg. control negative significance ( $P < 0.05$ ) different with all experimental groups. On the other hand, curcumin 300 mg /kg/B.W., curcumin 150mg/kg/B.W. and ibuprofen treated rats showed a significant ( $p < 0.05$ ) elevated in serum (IL-10) as compared with other groups. The results in figure(4.8) showed the effects of curcumin nanoparticles and ibuprofen on osteoarthritis in male rats.. Conversely, there was no significant ( $p > 0.05$ ) between 300mg and 150mg /kg/B.W. groups. There was significant ( $p < 0.05$ ) superiority in the improvement of inflammatory markers (IL-10) in 300mg/kg/B.W. as compared with the ibuprofen group.



Figure(4.8)The effects of curcumin nanoparticles and ibuprofen on serum interleukin-10 concentration (pg/L) of osteoarthritis male rats.

- Mean ± SEM

-The different small letter denoted significance between groups,  $p < 0.05$ .

## DISCUSSION

Curcumin is safe for most people when taken in amounts found in food. When taken as a supplement, doses up to 8 grams per day have been used without significant side effects. However, high doses can cause digestive discomfort in some individuals [17], [18]

The bioavailability of curcumin is relatively low, but can be significantly increased when encapsulated in chitosan nanoparticles. This encapsulation enhances its stability and solubility and prolongs its release, which may lead to increased efficacy in its therapeutic applications, such as anti-inflammatory, antioxidant, and anti-osteoporosis effects [19].

The ability of turmeric to reduce TNF- $\alpha$  levels may be due to a number of possible mechanisms, such as Inhibiting NF- $\kappa$ B activation Curcumin can prevent the activation of the NF- $\kappa$ B pathway in chondrocytes, which is known to upregulate the expression of pro-inflammatory cytokines such as TNF- $\alpha$ . By inhibiting this pathway, curcumin helps reduce inflammation and associated cytokine levels [20].

Moreover, the chitosan group showed a significant increase ( $P < 0.05$ ) in serum TNF- $\alpha$  levels compared to all experimental groups, and this is evidence that chitosan has no therapeutic effect that disagreed with previous study [21], [22]. However, the current study is consistent with many previous studies that used chitosan as a drug carrier due to its unique properties that make it only a drug carrier [23], [24]. However, there was no significant difference ( $P > 0.05$ ) between the ibuprofen and curcumin 150 mg/kg/body weight group and the negative control group when compared to each other that is agreed with [25], [26]. This is evidence of the effectiveness of the nanocomposite, which is equivalent to the dose of ibuprofen.

Remarkably, a significant decrease in serum TNF- $\alpha$  levels was observed in the group treated with curcumin at a dose of 300 mg/kg/body weight this is consistent with many previous studies that found an inhibitory effect of curcumin on several inflammatory indicators [25], [27]. As represented in figure 4.6

Comparing the results of the current study with previous research, our findings align with studies that have demonstrated the anti-inflammatory properties of curcumin. However, the specific dose-dependent effects observed in this study provide valuable insights into optimizing curcumin dosage for maximum therapeutic benefit in osteoarthritis treatment [28].

According to a previous study, the most likely mechanism for curcumin's ability to reduce the level of immune response in a group of patients under study may be that curcumin affects the expression of genes associated with inflammatory responses, including those that regulate IL-6 production. This suggests that curcumin can lead to decreased interleukin 6 (IL-6) levels by altering IL-1 gene expression patterns [29].

Possible explanations for these findings could include different mechanisms of action and bioavailability of different treatments. Curcumin, known for its anti-inflammatory properties, may have shown dose-dependent effects, with higher doses showing greater effectiveness in modulating IL-6 levels. This is consistent with a previous studies [25], [30] that found results similar to the results of the current study, while a study by [31] disagreed with the results of the current study as it found Curcumin can suppress expression of CD80, CD86, and class II antigens by dendritic cells and blocks the release of inflammatory cytokines like IL1 $\beta$ , IL-6, and TNF- $\alpha$  from LPS-stimulated dendritic cells, also study by [32] found a significant effect of curcumin on IL-6 .

Analysis of (IL-10) levels (pg/L) yielded results indicated a significant decrease ( $P < 0.05$ ) in both the positive groups and the chitosan group compared to all experimental groups, with no significant differences between these groups. This is a good indicator because the lower the IL-10, the higher the inflammatory response [33], and this means the success of the disease stimulation experiment as

well. Evidence of the ineffectiveness of chitosan. Ibuprofen also showed non-significant differences ( $P > 0.05$ ) when compared to curcumin at a dose of 150 mg/kg. The negative control group showed significant differences ( $P < 0.05$ ) compared to all experimental groups.

The mechanism responsible for this, according to a recent study, may be the possibility of curcumin acting on cell signaling pathways, such as downregulating c-jun NH2-terminal kinase (JNK), which is involved in the inflammatory response. This modification can lead to increased expression of IL-10 [34].

Interestingly, mice treated with curcumin at doses of 300 mg/kg/body weight, 150 mg/kg/body weight, and ibuprofen showed significantly higher serum interleukin-10 levels compared to the other groups. However, there was no significant difference ( $P > 0.05$ ) between 300 mg/kg/bw. And 150 mg/kg/body weight. groups. It is worth noting that 300 mg/kg/body weight. The group showed superior improvement in markers of inflammation (IL-10) compared to the ibuprofen group.

## CONCLUSIONS

In conclusion, curcumin-loaded on chitosan nanoparticles offer a promising therapeutic approach for osteoarthritis management by effectively reducing inflammation and mitigating cartilage degeneration. The study's findings suggest that curcumin, especially at a dosage of 300mg, exhibits potent anti-inflammatory effects, as evidenced by decreased TNF- $\alpha$  and IL-6 levels and IL-10.

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