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Original Research Article

Therapeutic Effects of Curcumin Nanoparticles against Monosodium Iodoacetate Induced Osteoarthritis in Rats

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Abstract: The experiment involved thirty male rats divided into six groups. One group received saline as a control, another received monosodium iodoacetate (MIA) to induce OA, and the remaining four groups received MIA alongside various treatments: chitosan NPs, curcumin-loaded chitosan at two different doses (150 mg/kg and 300 mg/kg), or ibuprofen, a common pain medication. The researchers monitored changes in the knee joints by measuring their diameter and examining X-ray images throughout the experiment. The results revealed that curcumin nanoparticles, particularly at the higher dose (300 mg/kg), were significantly more effective than chitosan or the lower curcumin dose (150 mg/kg) in reducing joint swelling and preventing cartilage degeneration. Interestingly, this higher dose of curcumin nanoparticles demonstrated effectiveness comparable to ibuprofen. These findings suggest that curcumin nanoparticles have the potential to be a valuable therapeutic agent for OA. Their anti-inflammatory properties may offer relief from pain and swelling in the joints, while the nanoparticle delivery system may improve curcumin's bioavailability and effectiveness compared to traditional curcumin. The study paves the way for curcumin nanoparticles as a potential alternative to NSAIDs for OA patients who experience side effects or intolerance to these medications.

Keywords: Osteoarthritis, curcumin, nanoparticles, x-ray, knee joint diameter measurement, therapeutic agent.

INTRODUCTION

Osteoarthritis (OA), a degenerative joint disease characterized by progressive cartilage breakdown and inflammation, is a leading cause of pain and disability globally. Current treatment options primarily focus on managing symptoms such as pain and inflammation, with limited ability to halt disease progression. Therefore, there is a significant unmet need for novel therapeutic strategies that target the underlying mechanisms of OA (Neogi, 2013).

Curcumin, a natural compound derived from turmeric, has gained significant interest for its potential therapeutic benefits in various diseases, including OA. Curcumin possesses potent anti-inflammatory and antioxidant properties, suggesting its ability to modulate the inflammatory response and oxidative stress associated with OA pathogenesis. However, curcumin suffers from poor bioavailability, limiting its therapeutic efficacy when administered orally (El-Saadony *et al.*, 2022).

Nanoparticle technology offers a promising approach to enhance curcumin's therapeutic potential. By encapsulating curcumin within nanoparticles, researchers aim to improve its solubility, stability, and delivery to target tissues, thereby overcoming limitations associated with conventional curcumin administration (Chopra *et al.*, 2021).

Currently available treatment options for OA primarily focus on symptomatic relief. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to manage pain and inflammation, but their long-term use can be associated

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with gastrointestinal and cardiovascular side effects. Other treatment options include physical therapy, injections (corticosteroids, hyaluronic acid), and in severe cases, joint replacement surgery. However, there is a lack of effective therapies that can prevent disease progression or promote cartilage repair. This highlights the urgent need for novel therapeutic approaches that target the underlying mechanisms of OA and offer a more holistic solution for managing this debilitating disease (Magni *et al.*, 2021).

This study investigates the therapeutic effects of curcumin nanoparticles against monosodium iodoacetate (MIA)-induced OA in rats. We hypothesize that curcumin nanoparticles will significantly reduce joint inflammation and cartilage damage compared to control groups and other treatment options. The findings from this research will contribute valuable insights into the potential of curcumin nanoparticles as a novel therapeutic strategy for managing OA.

MATERIALS AND METHODS

Experimental Animals, Housing and Adaption:

Thirty 12-week mature male Albino rats weighing [180-210g] were supplied from Veterinary Medicine Laboratories veterinary medicine Faculty, university of Tikrit-Iraq. Rats were housed in the animal House of faculty of Science, Kufa University, 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, cloves 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.

Ethical Considerations:

All experimental procedures involving animals were carried out in accordance with the ethical guidelines approved by the relevant institutional committee.

Study Design:

The study employed a randomized controlled trial design. Thirty adult's male albino rats were randomly allocated to six groups (n=5 per group):

Group I: Negative control (normal saline)
Group II: Positive control (MIA-induced osteoarthritis)
Group III: MIA + chitosan (200 mg/kg)
Group IV: MIA + curcumin-loaded chitosan (150 mg/kg)
Group V: MIA + curcumin-loaded chitosan (300 mg/kg)
Group VI: MIA + ibuprofen (40 mg/kg)

Osteoarthritis was induced in groups II-VI via intra-articular injection of 3 mg monosodium iodoacetate (MIA). Treatments were administered orally for 21 days, starting 7 days post-induction.

Induction of Osteoarthritis by Monosodium Lodoacetate

To induce osteoarthritis, a solution of monoiodoacetate (MIA) was prepared under sterile conditions. Given the known toxicity of MIA, appropriate personal protective equipment, including gloves and a mask, was worn throughout the preparation process. Prior to injection, the animal was positioned supine, and the fur surrounding the intended injection site was carefully removed to ensure asepsis. The area was then swabbed with alcohol. With the knee flexed, the patellar tendon was visually identified. To maintain this position, the index finger was placed beneath the knee joint, and the thumb was placed above the anterior surface of the ankle joint. A 26-gauge needle, affixed to a syringe, was carefully advanced along the knee, taking care to avoid puncturing the skin prematurely. Once the needle reached the gap beneath the patella, slight pressure was applied to mark the injection site. The needle was then inserted through the patellar tendon at a perpendicular angle to the tibia. The injection proceeded smoothly, with no noticeable resistance. Care was taken to keep the injection superficial. Following the injection, the knee area was gently massaged to facilitate even distribution of the MIA solution. The animal was then returned to its cage and allowed to recover uneventfully (Bae *et al.*, 2018).

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.

X- Ray Examination:

On day 30 post-MIA injection, animals from all groups were anesthetized. To ensure consistent positioning for radiographic imaging, both hind limbs of each animal were fully extended and secured to the table using tape. Radiographs of the right knees were then captured in the anterior-posterior position. To minimize image distortion and ensure optimal image quality, a focal film distance of 60 cm was maintained. The X-RAY device was operated at 55 KV and 3mA, consistent with established radiographic techniques for small animal imaging (Hamdalla *et al.*, 2022).

Measure the Knee Diameter {Swelling}:

Changes in knee joint dimensions over time were evaluated using a manual caliper to measure the anterior-posterior diameter of the affected joint at various time points (days 0, 7, 14, 21, and 28) following MIA injection. This non-invasive method allowed for the assessment of joint swelling and inflammation, which are hallmark features of osteoarthritis. The use of a manual caliper for joint diameter measurements is a well-established technique in arthritis research, providing valuable quantitative data on disease progression and the efficacy of therapeutic interventions (Badawi *et al.*, 2013). By tracking changes in joint diameter over time, the researchers aimed to gain insights into the dynamic nature of joint inflammation and the potential therapeutic benefits of curcumin-loaded chitosan nanoparticles in mitigating these changes.

Statistical Analysis:

Data were analyzed using GraphPad Prism version 8 software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was employed to assess differences between groups. Results were expressed as mean ± standard error of the mean (SEM). A p-value < 0.05 was considered statistically significant.

RESULTS

The result of the effects of curcumin nanoparticles and ibuprofen on osteoarthritis, specifically measuring the knee joint diameter in male rats over a period of 28 days were showed in day 0 there were no statistically significantly differences ($P>0.05$) between all experimental groups in knee joint diameter measurements also in day 7 show no significantly differences ($P>0.05$) except for the control negative group, which showed a significantly difference ($p<0.05$) compared to all other groups. While after 3 weeks of curcumin treatment positive and chitosan groups were not significantly different ($p>0.05$) from each other but showed a significantly increase ($p<0.05$) in diameter when compared with other groups as shown in Figure 1.

There were no significantly differences ($p>0.05$) between the ibuprofen group and the curcumin groups (150 mg/kg/B.W. and 300 mg/kg/B.W.), but they markedly reduced swelling in the right knee joint compared to the positive and chitosan groups.

The results of the X-ray image showed narrow joint space, an irregular cartilage surface, accumulation of inflammatory fluid, and joint swollen for control positive group as shown in Figure 2A.

While the results of X-ray images of control negative group showed no radiographic changes after 28 days, the cartilages were normal, normal joint space and no inflammatory fluid. As shown in Figure 2B chitosan group showed cartilage degeneration, narrow joint space, cartilage surface was irregular, accumulation inflammatory fluid and joint swollen as shown in Figure 2C.

Ibuprofen group after 28 days showed mild decreased of joint space, accumulation inflammatory fluids, severe irregular cartilage surface and joint swollen was clear. As shown in Figure 2D.

The growp that treated with curcumin 150 mg group after 28 days showed mild inflammatory, fluid accumulation, nearby normal joint space, relative regular cartilage surface and moderate joint swollen. As shown in Figure 2E.

Finally, the group that treated with curcumin 300 mg group showed little inflammatory fluid, nearly regular cartilage surface, normal joint space and mild joint swollen. As figure 2.F. showed it.

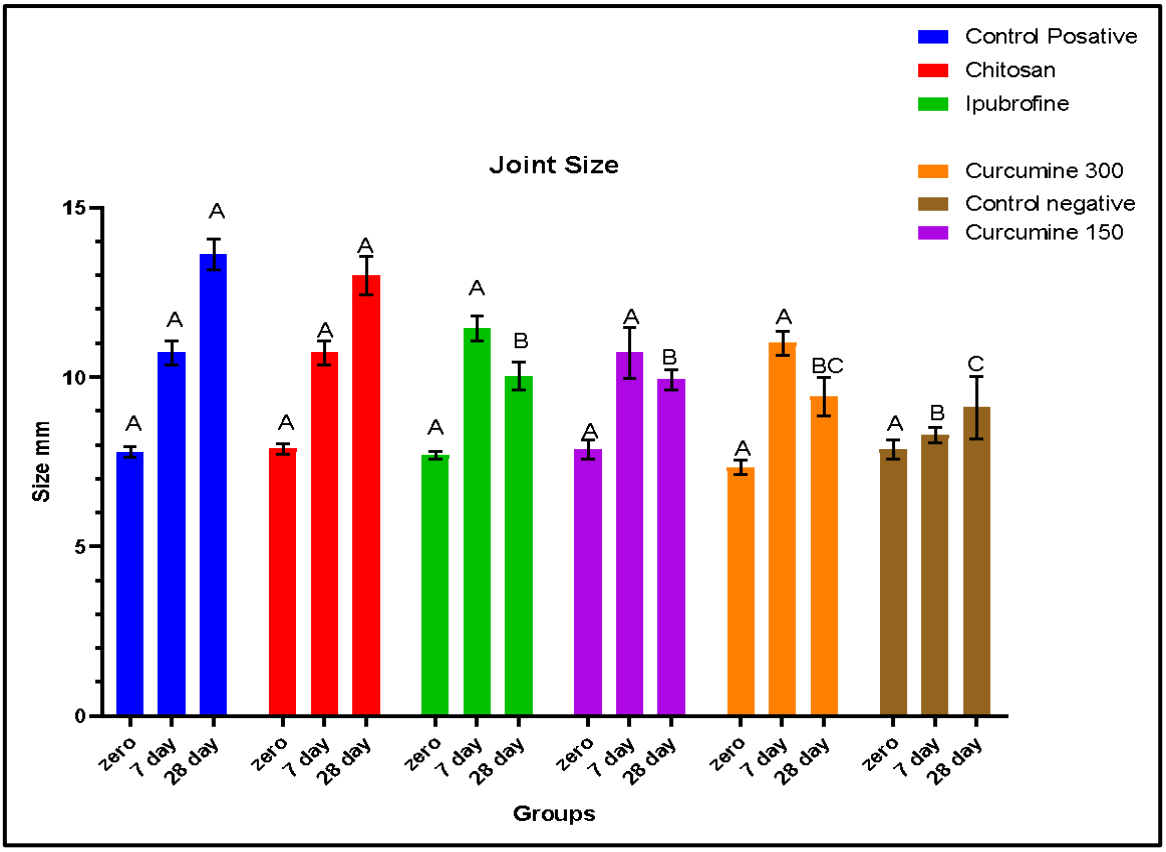
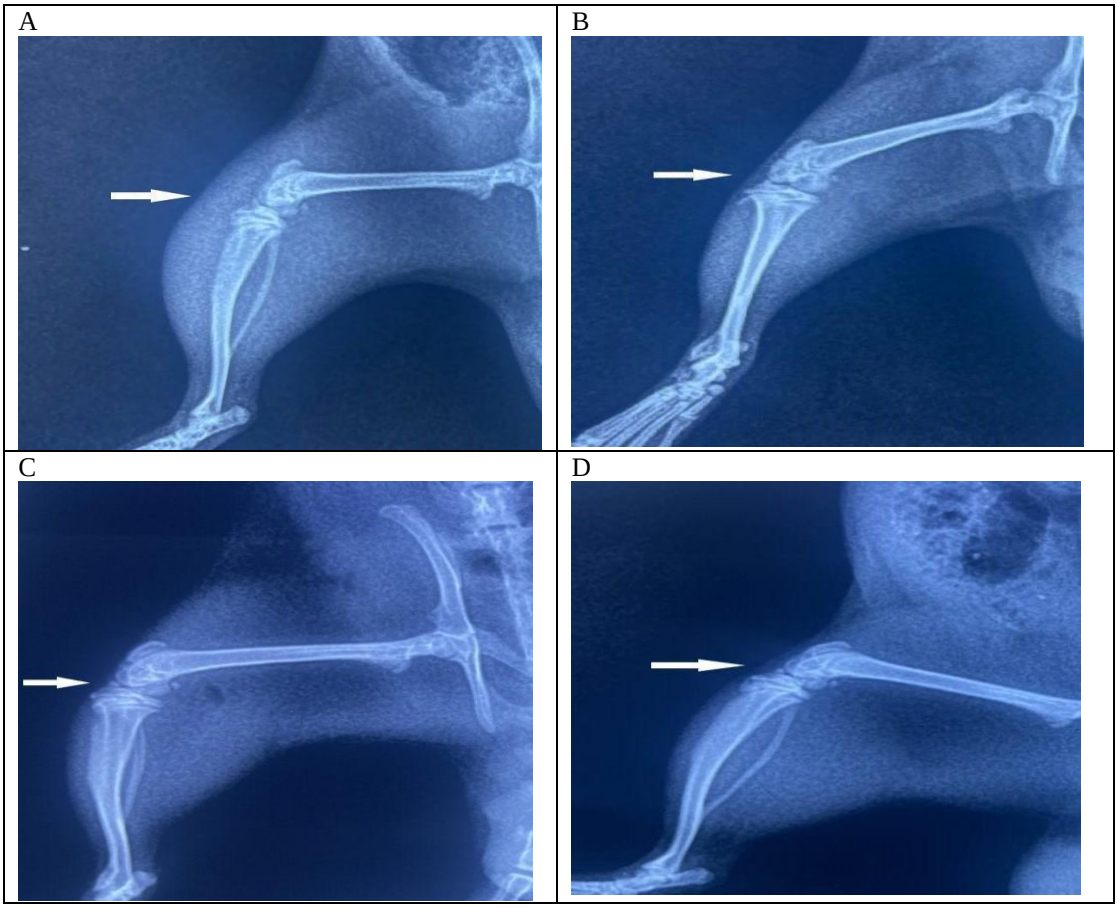


Figure 1: Effect of curcumin on swelling (right knee joint diameter measurements) (mm)
Data presented as Mean ± SE. The different small letter denoted significance between groups, $p<0.05$. n=6



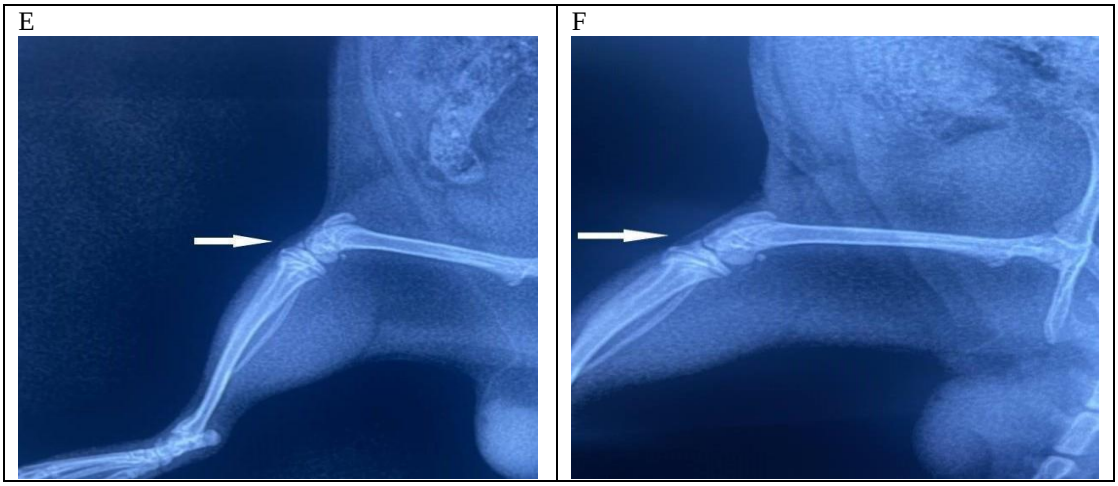


Figure 2: Anteroposterior radiograph of the right knee joints effect of curcumin and ibuprofen on knee joint, A. control positive group, B. control negative group, C. chitosan group, D. ibuprofen 40 mg/kg group, E. curcumin 150 mg/kg group, F. curcumin 300 mg/kg group

DISCUSSION

The results showed the effect of curcumin nanoparticles and ibuprofen on osteoarthritis by monitoring swelling, specifically measuring the diameter of the right knee joint in male rats over a period of 28 days lnitially, on day 0, no statistically significantly differences ($P > 0.05$) were observed in knee joint diameter measurements in all experimental groups. On the seventh day, there was a significantly increase in all groups over the negative control, which showed a significantly difference ($P<0.05$) compared to all other groups. After three weeks of treatment with curcumin, the positive groups and chitosan did not show any statistically significantly differences ($P > 0.05$) between them, but they showed a significantly increase ($P < 0.05$) in diameter compared to the other groups. This is evidence of the success of the disease stimulation trial and negates the possibility of a therapeutic effect of chitosan (See Figure 1).

The observed results can be attributed to the anti-inflammatory properties of curcumin (Menon and Sudheer, 2007), which has been reported to relieve symptoms of osteoarthritis by reducing inflammation and swelling. ln addition, curcumin nanoparticles may have enhanced bioavailability and therapeutic efficacy compared to conventional curcumin due to its improved solubility and absorption(Chopra *et al.*, 2021). lbuprofen, a commonly used nonsteroidal anti-inflammatory drug, has shown similar efficacy to curcumin in reducing swelling, suggesting its potential as an alternative treatment option for osteoarthritis.

A meta-analysis of 23 studies conducted across 7 countries investigated the efficacy and safety of curcumin in treating knee osteoarthritis (KOA) (Zhao *et al.*, 2024). The analysis revealed a significant reduction in the use of rescue medications among patients receiving curcumin, either as a standalone therapy or in combination with other treatments. This finding suggests that curcumin effectively manages KOA symptoms, potentially reducing the need for additional pain relief. Moreover, the study concluded that curcumin exhibits good clinical efficacy and safety in treating KOA. While the potential for curcumin to enhance treatment efficacy and reduce adverse reactions when used in combination with other drugs is promising, further clinical and basic research is warranted to validate this synergistic effect (Zhao *et al.*, 2024).

Also, another study, this study aimed to evaluate the arthritic activity of PLGA nanoparticles loaded with curcumin and meloxicam in rats with arthritis caused by adjuvant substances. The results of the aforementioned study showed that curcumin reduced nCur/Mlx significantly ($p > 0.05$) from swelling. Claw and arthritis, body weight recovery. In addition, the mono- and di-complex loaded nanoparticles significantly ($p > 0.05$) down-regulated pro-inflammatory cytokines (Aslam *et al.*, 2023).

The X-ray images provide a comprehensive visual representation of the structural changes that occur in the knee joints of mice subjected to osteoarthritis and subsequent treatment with various agents.

ln the positive control group, severe cartilage degeneration, narrow joint space, irregular cartilage surface, inflammatory fluid accumulation, and joint swelling were evident after 28 days, indicating the success of the OA induction experiment (Figure 1a). X-ray imaging was employed to visualize the structural changes in the knee joints of rats following the induction of osteoarthritis and subsequent treatment with various agents. This technique is consistent with previous studies that have utilized radiographic analysis to assess joint damage in animal models of osteoarthritis. For instance, Belkhodja *et al*., (2017) investigated the radiographic and histopathological changes in an osteoarthritis rat model treated

with essential oils of Rosmarinus officinalis and Populus alba. In their study, osteoarthritis was induced by unilateral intra-articular injection of mono-iodoacetic acid, and X-ray examination was performed to evaluate the resulting changes in the knee joint. Similarly, in the present study, X-ray imaging was used to monitor the progression of osteoarthritis and assess the therapeutic efficacy of curcumin-loaded chitosan nanoparticles and ibuprofen in mitigating joint damage (Belkhodja *et al.*, 2017).

On the contrary, the negative control group showed no radiographic changes, with normal cartilage, joint space, and absence of inflammatory fluid indicating that the mice were healthy (Figure 1b).

Treatment with chitosan began on the seventh day and continued until the 28th day, which led to cartilage degeneration, decreased joint space, irregular cartilage surface, inflammatory fluid accumulation, and joint swelling as well, which indicates the lack of pharmacological effectiveness of chitosan (Figure 1C). That is agreed with (Wang *et al.*, 2022) Who found long-term effects, and retention in the joint cavity.

ln the ibuprofen group, treatment starting on day 7 resulted in a slight reduction in joint space, inflammatory fluid accumulation, severe cartilage surface irregularity, and obvious joint swelling after 28 days, indicating partial relief of symptoms, which is normal and scientifically proven also recent study investigated about curcuma longa l. extract exhibits anti-inflammatory and cytoprotective functions in the articular cartilage of monoiodoacetate-injected rats found ats treated with ibuprofen or CL exhibited suppressed morphological alterations and mineralisation parameters, including bone mineral density, bone volume/total tissue volume, trabecular number, and trabecular thickness, and increased trabecular separation (Kim *et al.*, 2024) (Figure 1D).

Administration of curcumin at a dose of 150 mg/kg/day, which began on day 7 and continued until day 28, resulted in mild inflammatory changes, fluid accumulation, a relatively uniform cartilage surface, and moderate joint swelling, indicating partial relief of the disease manifestations (Figure 1E). Also this agreed with the previous study (Asif *et al.*, 2023) who found Curcumin-loaded NPs showed anti-inflammatory activity (59%) by HRB membrane stabilization method. Anti-arthritic activity (66%) was comparable to standard drug (Asif *et al.*, 2023).

ln particular, in the 300 mg curcumin group, treatment initiated on day 7 resulted in minimal inflammatory fluid, nearly regular cartilage surface, normal joint space, and mild joint swelling after 28 days, suggesting significantly attenuation of osteoarthritis-specific changes (Figure 1F). In previous study aimed to evaluate effects of low- and high-dose curcuminoids on pain and functional improvement in patients with knee OA found significantly difference in pain relief or AEs between curcumininoids versus NSAIDs was found (Hsiao *et al.*, 2021).

We believe that Curcumin very important clinical application value in the treat of osteoarthritis and may become a replacement for NSAIDs. In patient's intolerant to NSAID and their side effects.

CONCLUSIONS

The present study investigated the therapeutic potential of curcumin nanoparticles against monosodium iodoacetate (MIA)-induced osteoarthritis (OA) in rats. Our findings demonstrate promising results, suggesting that curcumin nanoparticles hold significant promise as a novel therapeutic strategy for OA management.

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