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Antioxidant effects of curcumin nanoparticles on induced osteoarthritis in rats

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Abstract
Osteoarthritis (OA) is a degenerative joint disease affecting millions worldwide, and current treatments carry risks of long-term side effects. Curcumin, a natural polyphenol, shows promise as a safer alternative due to its anti-inflammatory and antioxidant properties. However, its low bioavailability limits its clinical application. This study aimed to investigate the antioxidant effects of curcumin nanoparticles on induced osteoarthritis in rats. The research was conducted at the animal house of the Faculty of Science, Kufa University, Iraq, over a specified experimental period. The study employed a randomized controlled trial design with six groups of rats, including a negative control, a positive control (MIA-induced osteoarthritis), and four treatment groups receiving chitosan, curcumin-loaded chitosan (two doses), or ibuprofen. The treatments were administered orally for 21 days, starting 7 days' post-induction. The nanocomposites were characterized using X-ray diffraction (XRD) and Zeta potential measurements. The results demonstrated that curcumin nanoparticles, particularly at a higher dose (300 mg/kg), significantly increased glutathione (GSH) and superoxide dismutase (SOD) levels while decreasing malondialdehyde (MDA) levels compared to the positive control group. This indicates enhanced antioxidant capacity and reduced oxidative stress in rats with osteoarthritis. Ibuprofen showed less pronounced effects compared to the higher dose of curcumin nanoparticles. The study concludes that curcumin nanoparticles have the potential to be a therapeutic agent for osteoarthritis by alleviating oxidative stress and enhancing the body's antioxidant defenses. Further research is needed to explore long-term effects and clinical applications.

Keywords: Osteoarthritis, curcumin, nanoparticles, oxidative stress, therapeutic agent.

Introduction

Osteoarthritis (OA) is a widespread degenerative joint condition impacting millions globally. Current treatments, such as NSAIDs and surgery, offer pain relief but carry long-term side effect risks. This has spurred research into safer alternatives, with curcumin emerging as a promising candidate due to its anti-inflammatory and antioxidant properties [1].

Curcumin, a natural polyphenol derived from turmeric (Curcuma longa), has been used traditionally for its medicinal benefits. Modern studies support its effectiveness against chronic diseases like cancer, heart disease, and stroke, highlighting its antioxidant and anti-inflammatory capabilities [2]. However, curcumin's low bioavailability and rapid metabolism have limited its clinical application.

To overcome these limitations, researchers are exploring nanotechnology. Curcumin nanoparticles, with their ultra-small size, enhance curcumin's absorption and bioavailability, enabling targeted delivery to cells or tissues [3]. This approach holds the potential to revolutionize treatment by providing safer and more effective therapies for various chronic diseases. Curcumin's antioxidant effects are particularly noteworthy. Its chemical structure makes it a potent antioxidant, even more so than vitamins C and E. It functions as a free radical scavenger and boosts glutathione (GSH) production, an endogenous antioxidant that shields cells and tissues from oxidative damage. Additionally, curcumin elevates superoxide dismutase (SOD) activity and glutathione S-transferase (GST) levels in cells and serum, as supported by *in vitro* and animal studies [4].

Oxidative stress, caused by excessive free radical production after reperfusion, is a common occurrence in ischemic reperfused tissues, seen in conditions like stroke, heart attack, surgery, and organ transplantation. Curcumin has demonstrated the ability to scavenge free radicals, mitigate oxidative stress, and reduce tissue damage associated with ischemia-reperfusion injury. By neutralizing oxidative stress and inflammation, curcumin safeguards tissues and promotes overall health [5].

The antioxidant properties of curcumin, combined with its anti-inflammatory effects, make it a promising therapeutic agent for OA. This is particularly relevant as oxidative stress and inflammation play crucial roles in OA pathogenesis. By targeting these processes, curcumin offers a potential approach to alleviate OA symptoms and potentially modify disease progression [6].

Materials and Methods

Experimentall 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, 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.

spectrum was measured in the range of 400-4000 cm-1. The visible bands were fixed, and most of the main bands were identified [7].

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.

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.

Characterization of curcumin loaded on chitosan

The nanocomposites were further characterized using X-ray diffraction (XRD). This technique elucidates the crystallographic structure of materials by analyzing the diffraction patterns produced when X-rays interact with the sample, Powder x-ray diffraction patterns were recorded on a D8-Advance Bruker with Cu-Kα radiation (λ = 1.5406 nm). Zeta (ζ) potential measurements were determined using a Zeta Potential DLS analyzer from Particulate Systems. Data were processed with the use of a software supplied by own manufacturer.

Procedures for Antioxidant Parameter Assessment

Blood Collection: Blood samples were collected from the hearts of anesthetized rats using a 5 ml syringe and centrifuged to obtain serum.

MDA Assessment: Malondialdehyde (MDA) levels, indicative of lipid peroxidation, were measured using a commercially available kit based on the reaction between MDA and thiobarbituric acid. Absorbance was read at 532 nm.

GSH Assessment: Glutathione (GSH) levels were determined using a kit based on GSH's reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Absorbance was measured at 412 nm.

SOD Assessment: Superoxide dismutase (SOD) activity was evaluated using a kit based on the inhibition of superoxide radical-mediated reduction of tetrazolium nitro blue. Absorbance was read at 560 nm.

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.

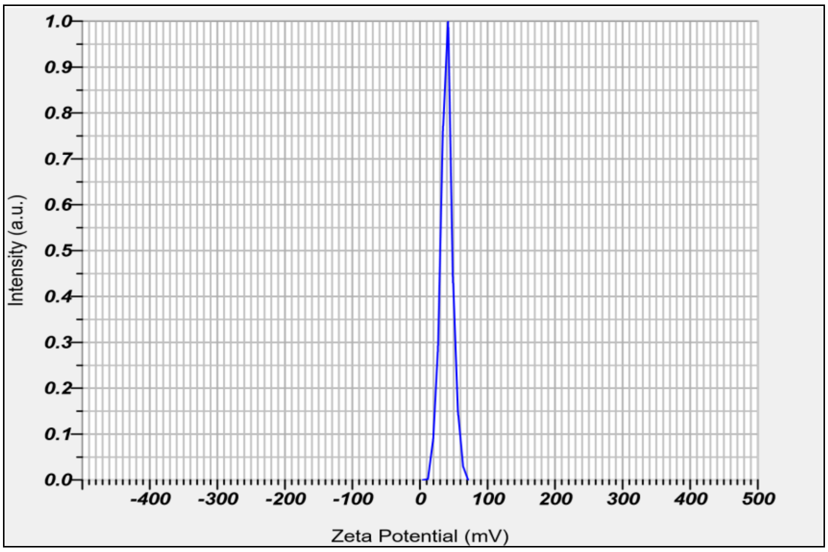
Results

The results of Zeta potential curves for three different nanoparticles: Chitosan nanoparticles (CNP), curcumin, and curcumin loaded on chitosan NPs. Zeta potential is a measure of the electric charge on the surface of nanoparticles. It is an important parameter for characterizing nanoparticles as it influences their stability, behavior in solution, and interactions with biological systems as shown in Figure 1.

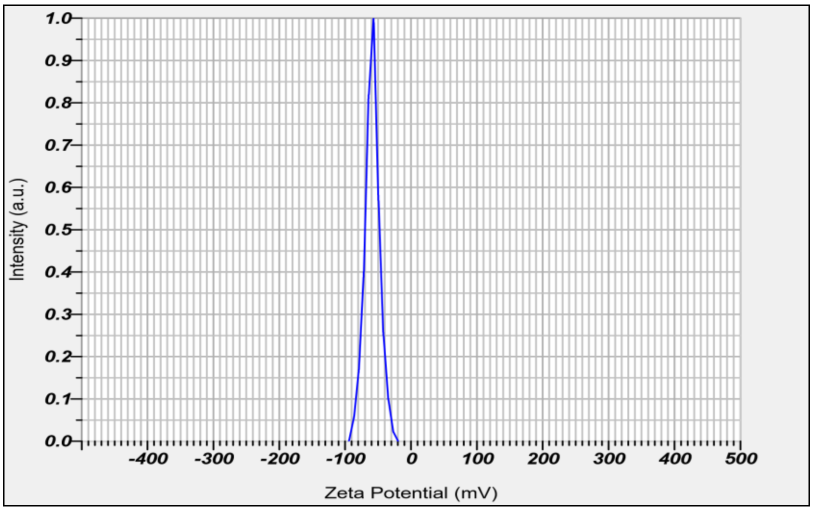
Chitosan nanoparticles (CNP): The zeta potential curve for CNP (A) shows a net positive charge on the surface of the nanoparticles was 39.04 mV. This is expected as chitosan is a cationic polymer with positively charged amino groups.

Curcumin: The zeta potential curve for curcumin (B) shows a net negative charge on the surface of the molecules was-58.8 mV. This is likely due to the presence of phenolic and enolic groups in curcumin, which can ionize in solution and contribute to a negative charge.

Curcumin loaded on chitosan NPs: The zeta potential curve for curcumin-loaded chitosan NPs (C) shows a shift towards a less positive or even slightly negative zeta potential compared to CNP alone was 82.5 mV. This suggests that the loading of curcumin onto chitosan NPs alters the surface charge of the nanoparticles, likely due to interactions between the negatively charged curcumin molecules and the positively charged chitosan polymer.



A



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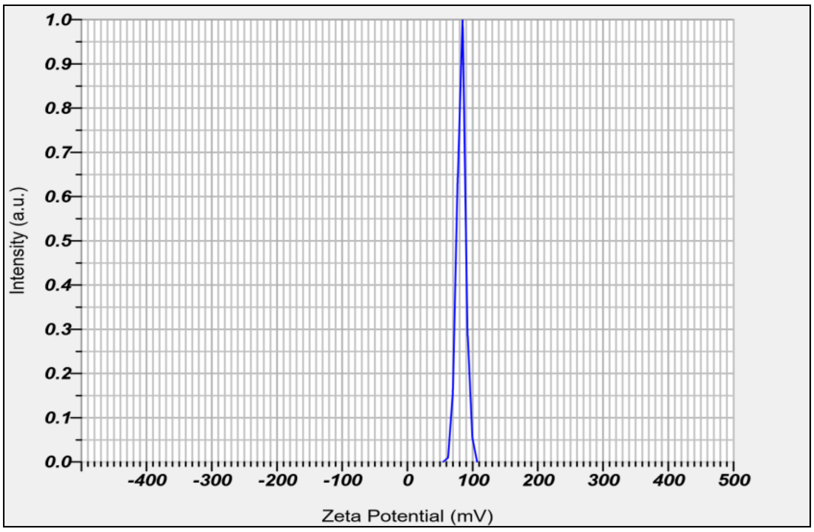

C

Fig 1: The Zeta potential curve in the current study measured A. for chitosan nanoparticles (CNP), B. Curcumin. C. curcumin loaded on chitosan NPs.

Figure 2 displays the X-ray diffraction (XRD) results for curcumin loaded onto chitosan NPs. The broad peaks seen in Figure 2's XRD pattern suggest that the structure of curcumin-loaded chitosan nanoparticles is predominantly amorphous. This indicates that nanoparticles do not have a structured alignment of atoms over a large distance. The reduced crystallinity of curcumin in nanoparticles suggests that curcumin is evenly distributed in the chitosan framework. Interactions between curcumin and chitosan could be the reason why curcumin molecules do not form a tightly organized crystalline structure.

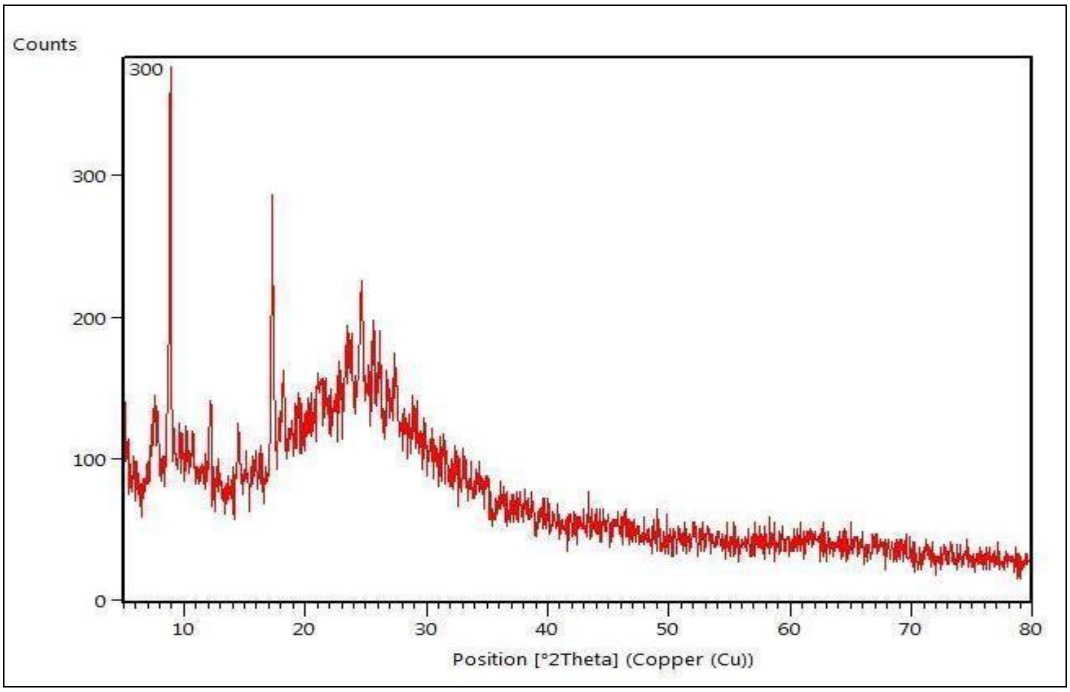

Fig 2: X-Ray Diffraction (XRD) value of curcumin loaded on chitosan NPs.

Figure 3 illustrates the effects of curcumin nanoparticles and ibuprofen on serum malondialdehyde (MDA) concentration in rats with osteoarthritis. The rats were divided into six groups: negative control (healthy rats), positive control (osteoarthritis induced by MIA), MIA + chitosan, MIA + curcumin-loaded chitosan (150 mg/kg), MIA + curcumin-loaded chitosan (300 mg/kg), and MIA + ibuprofen. The results show that the positive control group had significantly higher MDA levels compared to the negative control group, indicating that osteoarthritis induction increased oxidative stress. Treatment with curcumin nanoparticles, especially at the higher dose (300 mg/kg), significantly reduced MDA levels compared to the positive control group. This suggests that curcumin nanoparticles effectively decreased oxidative stress and lipid peroxidation in rats with osteoarthritis. Ibuprofen also showed some reduction in MDA levels, but the effect was less pronounced than that of the higher dose of curcumin nanoparticles.

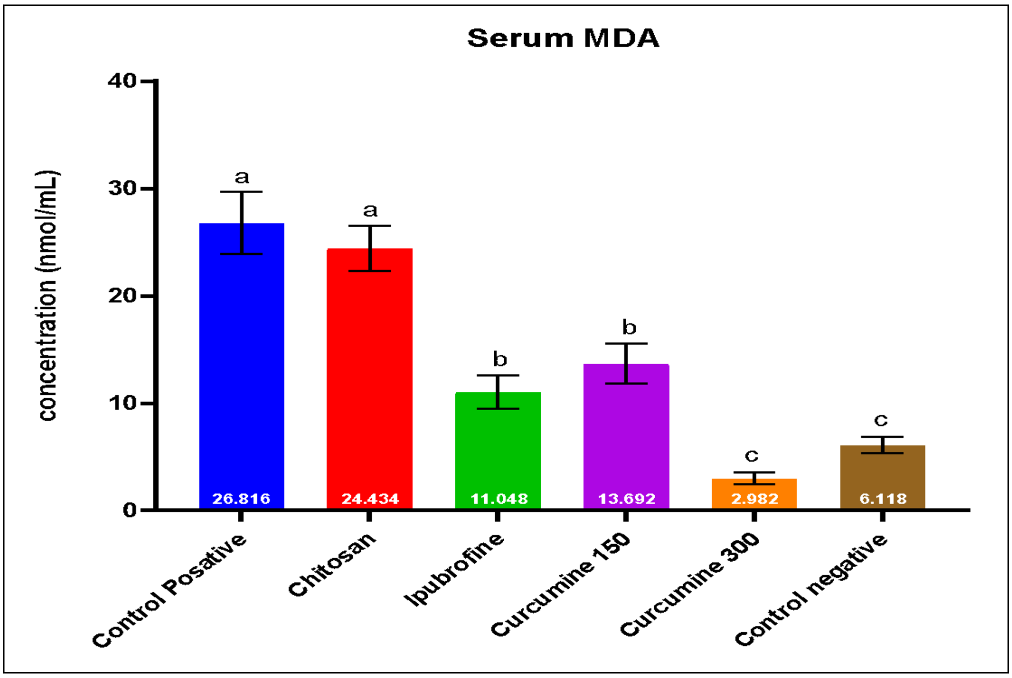

Fig 3: The effects of curcumin nanoparticles and ibuprofen on serum malondialdehyde (MDA) concentration (nmo/ml) of osteoarthritis male rats.-Data presented as Mean ± SE. The different small letter denoted significance between groups, *p*<0.05. N=6

Discussion

They showed the results of the effects of curcumin nanoparticles and ibuprofen on the concentration of glutathione (GSH) in blood serum (µg/ml) in male rats with osteoarthritis. As shown in Figure 4, the effect of different treatments on the concentration of glutathione (GSH) in the serum of male rats with osteoporosis. GSH represents one of the main *Antioxidants* that helps protect cells from oxidative damage.

The graph shows the GSH levels in each group. Higher GSH levels indicate greater antioxidant capacity. The positive control group had significantly lower levels of GSH than the negative control group, suggesting that induction of osteoarthritis reduces antioxidant capacity. Treatment with curcumin nanoparticles, especially at the higher dose (300 mg/kg), resulted in a significant increase in GSH levels compared to the positive control group. This indicates that curcumin nanoparticles effectively enhanced antioxidant levels in mice with osteoporosis. Ibuprofen also showed some increase in GSH levels, but the effect was less pronounced than that of the higher dose of curcumin nanoparticles.

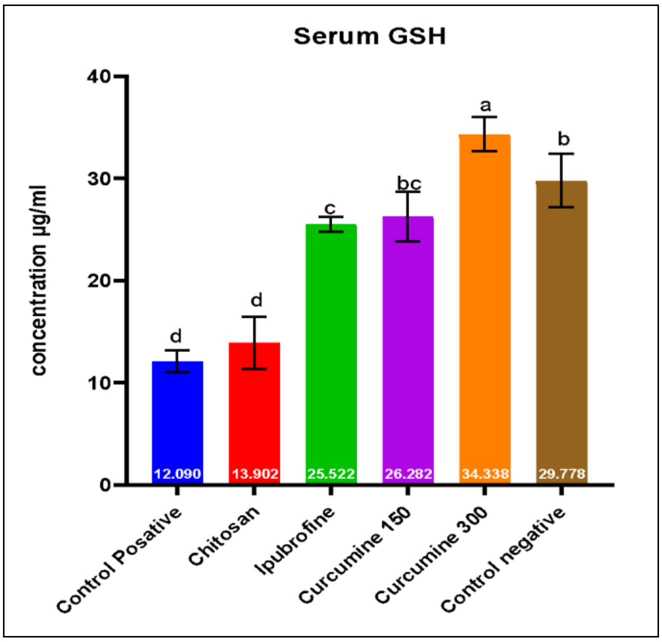

Fig 4: The effects of curcumin nanoparticles and ibuprofen on serum Glutathione (GSH) concentration(µg/ml) of osteoarthritis male rats.-Data presented as Mean ± SE. The different small letter denoted significance between groups, *p*<0.05. N=6

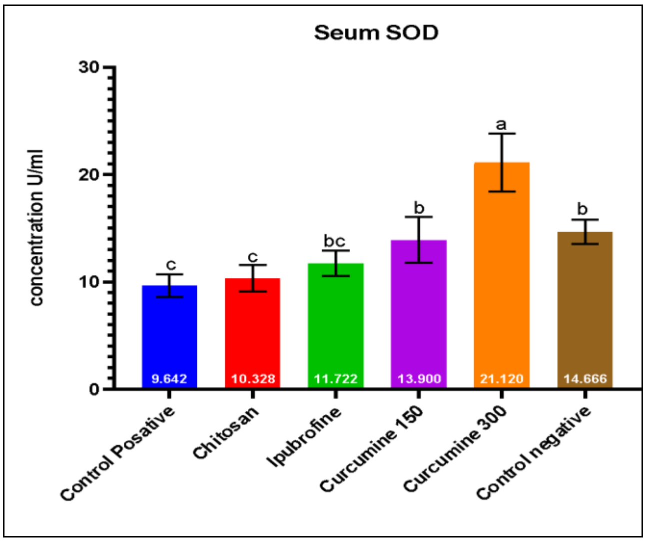

Fig 5: The effects of curcumin nanoparticles and ibuprofen on serum Superoxide dismutase (SOD) concentration (U/ml) of osteoarthritis male rats.-Data presented as Mean ± SE. The different small letter denoted significance between groups, *p*<0.05. N=6

As for the results of the effects of curcumin nanoparticles and ibuprofen on the concentration of superoxide dismutase (SOD) in blood serum (U/ml) in male rats with osteoarthritis. As shown in Figure 5, the SOD levels in each group were also shown in the graph. Higher SOD levels indicate a greater ability to neutralize superoxide radicals and protect against oxidative stress. The positive control group showed significantly lower SOD levels compared to the negative control group, indicating that the induction of osteoporosis impairs the antioxidant defense system.

Treatment with curcumin nanoparticles, especially at the higher dose (300 mg/kg), significantly increased SOD levels compared to the positive control group. This indicates that curcumin nanoparticles effectively enhanced the SOD activity and improved the antioxidant capacity of osteoporosis mice. Ibuprofen also showed some increase in SOD levels, but the effect was less pronounced than that observed with the higher dose of curcumin nanoparticles.

Discussion

Analysis of glutathione (GSH) levels (µg/ml) in serum revealed results. Specifically, results showed a significant increase (*p*<0.05) with curcumin 300 mg/kg/body weight as shown in figure 4. group compared to all experimental groups. On the contrary, the negative control group showed non-significant differences (*p*>0.05) compared to curcumin 150 mg/kg/bw. group, as well as ibuprofen.

The most likely mechanism may be curcumin's ability to activate the expression of genes that encode antioxidant proteins, including glutathione S-transferase and glutathione peroxidase. This activation promotes the synthesis and accumulation of GSH in cells [8].

Moreover, the positive control and chitosan group showed a significant decrease (*p*<0.05) in GSH levels compared to all experimental groups. These results highlight the potential of curcumin, especially at a dose of 300 mg/kg/body weight, in enhancing GSH levels, demonstrating its antioxidant properties.

Possible explanations for these results could include curcumin's antioxidant mechanisms, which may contribute to the observed increase in GSH levels, and this is what many previous studies have agreed upon, such as what [9] found Curcumin has important anti-inflammatory and antioxidant properties that allow it to be applied as treatment for several emerging pathologies.

Comparing these findings with previous research, our results support the antioxidant properties of curcumin and its potential therapeutic role in alleviating oxidative stress [10], [11].

Analysis of superoxide dismutase (SOD) levels (U/ml) in serum yielded remarkable results regarding the effects of curcumin nanoparticles and ibuprofen on osteoarthritis in male rats. Specifically, administering curcumin at a dose of 300 mg/kg/body weight as shown in figure 5. It led to a significant increase (*p*<0.05) in SOD levels compared to the other experimental groups. In contrast, curcumin at a dose of 150 mg/kg/body weight. It showed non-significant differences (*p*>0.05) compared to the negative control and ibuprofen group.

Privuse study investigated about curcumin-supplemented diets increase superoxide dismutase activity and mean lifespan in Drosophila found Lifespan extension of curcumin in Drosophila was associated with the up-regulation of Mn-SOD and CuZn-SOD genes, and the down-regulation of dInR, ATTD, Def, CecB, and DptB genes [12].

Furthermore, there were non-significant differences (*p*>0.05) between the ibuprofen-chitosan-positive and control groups when compared with each other. These results confirm the ability of curcumin, especially at a higher dose of 300 mg/kg/body weight, to enhance SOD activity, indicating its antioxidant properties.

These findings may include curcumin's antioxidant mechanisms, which may contribute to the observed elevation in SOD levels that is agreed with [13].

Comparing these findings with previous research, these findings support the antioxidant properties of curcumin [14].

Examination of malondialdehyde (MDA) levels (NMO/ml) in serum provided insight into the effect of curcumin nanoparticles and ibuprofen on osteoporosis in male rats. The results showed a significant increase (p<0.05) in MDA levels in the positive control and chitosan groups compared to all experimentall groups as shown in figure 3. Conversely, ibuprofen and curcumin at a dose of 150 mg/kg/body weight. They showed non-significant differences (p>0.05) when compared to each other and showed significant differences (p<0.05) with all experimental groups.

However, curcumin is taken at a dose of 300 mg/kg/body weight. lt showed a significant decrease (p<0.05) in MDA levels compared to the other groups that is agreed with [15]. this reduction was not significant (p>0.05) compared to the negative control group.

These results suggest that curcumin, especially at a higher dose of 300 mg/kg/body weight, may have a protective effect against lipid peroxidation induced by oxidative stress [16], as evidenced by decreased MDA levels. The contrasting effects observed between the positive and experimental control groups underscore the potential therapeutic efficacy of curcumin in alleviating oxidative damage associated with osteoporosis.

Conclusions

These results suggest that curcumin nanoparticles have the potential to be a therapeutic agent for osteoarthritis by alleviating oxidative stress and enhancing the body's antioxidant defenses. Further research is warranted to explore the long-term effects and clinical applications of curcumin nanoparticles in the treatment of osteoarthritis.

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