**How to Cite**:
Kshash, I. M., & Hussain, K. A. M. (2022). Preparation of a green nanocomposite from Cinnamomum zeylanicum and its effect on some immunological parameters in rats with rheumatoid arthritis. *International Journal of Health Sciences*, *6*(S3), 6153–6168. https://doi.org/10.53730/ijhs.v6nS3.7348

# Preparation of a green nanocomposite from Cinnamomum zeylanicum and its effect on some immunological parameters in rats with rheumatoid arthritis

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***Abstract*---**The current study aimed to evaluate the anti-arthritic effects of a nanocomposite from Cinnamomum zeylanicum, and to further demonstrate its mechanism of action in RA induced RA by Complete Freund's Adjuvant (CFA). and it was diagnosed by atomic force microscopy (AFM) and infrared spectroscopy (FT-IR) revealed the emergence of new diffraction levels for nanocomposites, which clearly indicates the success of the loading process. X-ray evaluation clearly showed the severity of joint deformity according to the extent of osteoarthritis, joint spaces, soft tissue inflammation, subchondral erosion, and articular sclerosis. The immunological results indicated that the induction of rheumatoid arthritis (RF) in male G2 white rats led to a significant ($P<0.05$) increase in the level of IL-1β and IL-6 cytokines (186.89,109.52) pg/ml both compared with the control group. Negative (G1) (29.40,32.48) pg/ml. There was also a significant decrease ($P<0.05$) for the concentration level of IL-1β, IL-6 in the G7 group dosed with cinnamon nano compound loaded with MTX pg/ml (34.33, 48.62), respectively, the present results indicate that the stable nanoparticles with Ceylon Cinnamomum extract (Z.C./NPs) have potent anti-arthritic activity that is mediated by IL-6, IL-1β inhibition and cytokine resistance. Inflammatory.

***Keywords*---**green nanocomposite, Cinnamomum zeylanicum, immunological parameters, rheumatoid arthritis.


*Manuscript submitted: 9 March 2022, Manuscript revised: 18 April 2022, Accepted for publication: 1 May 2022*

## Introduction

Nanotechnology is one of the promising technologies in the scientific fields that scientists rely on to make drug breakthroughs that change the concept of medicine and treatment for many diseases. The signs of nanomedicine have begun to appear in a new and continuous way towards better health and a longer human life (Modi *et al.* 2022) (Ventola 2012) One of the priorities of research in NATO medicine is drug delivery into tissues, which is based on the manufacture of nano-micromaterials that improve drug bioavailability. This means that the drug molecules are located in the target place of the body, where they work with maximum effectiveness, and therefore the rate of drug consumption decreases, its side effects decrease, as well as the total cost of treatment. Therefore, one of the most important duties of nanomedicine is to manufacture new drugs with greater benefits and fewer side effects. Nanotechnology can offer new drug delivery solutions in many areas (Kirtane *et al.* 2021) (Fornaguera and García-Celma 2017) particularly in Biomedical sciences, and NPs can be prepared and stabilized by physical and chemical methods, as some of them have toxic effects and lead to non-environmentally friendly by-products, which is the reason for the need for green synthesis of NPs without the use of toxic chemicals, however with the rapid development it has been proposed to use biological methods to protect the environment (Gupta and Mishra 2020) An advance in green synthesis of NPs has been the use of biological entities such as microorganisms or plant extracts to produce NPs nanoparticles in an environmentally friendly manner (Hano and Abbasi 2022) (Singh *et al.* 2018)

Rheumatoid arthritis (RA) is a chronic systemic disease of unknown etiology that affects the connective tissue and is one of the most common in the world, with a distribution of more than 1% of the population worldwide (Guo *et al.* 2018) an autoimmune disease that can Arthritis begins to damage cartilage soon after disease onset, and none of the currently available medical interventions can reverse this damage. In addition to pain and joint weakness, RA leads to a significant reduction in patients' life expectancy. For this reason, early intervention with antirheumatic agents has become the standard of care for active and modern rheumatoid arthritis. The therapeutic potential has expanded greatly in recent years to include, in addition to disease-modifying antirheumatic drug (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs). inflammatory drug (NSAIDs) ,(Muñoz-Martínez, Segura-Puertas, and Gómez-Moreno 2021) , making it possible to keep the inflammatory aspect of the disease in actual or near remission in most patients. This will likely reduce the burden of joint destruction that It is tolerated by rheumatoid arthritis patients, despite the benefits of these drugs, but they also have negative effects, so it is imperative to find new treatment methods that are more effective and less negative in terms of effects, and these methods include nanotechnology (Feng and Chen 2018)

## Material and Method

### Experimental and animal design

Thirty-five male laboratory white rats were used, weighing 180-220 g and aged (10-11 weeks), the infection appeared by injection of 0.1 ml of (CFA) in the sole of the right foot in the first six weeks and 14 days after injection for each group (Adeneye et *al*. 2014):

- The first group G1: A daily dose of plain water is given, and the negative control group is considered to be the negative control
- The second group G2: (0.1) ml of CFA was injected into the sole of the right foot on the first day of the experiment to induce arthritis, and it was considered a positive control group.
- The third group G3: Induced arthritis and taken orally 14 days after induction of arthritis with free methotrexate.
- Group four G4: Arthritis induced and administered orally 14 days after induction of arthritis with cold aqueous cinnamon extract at a concentration of 350 mg/kg.
- Group five G5: Induced arthritis and administered orally 14 days after induction of arthritis with nanocomposite (cinnamon extract) at a concentration of 350 mg/kg.
- Group six G6: Induced arthritis and administered orally 14 days after induction of arthritis with cold aqueous cinnamon extract dissolved in methotrexate treatment at a concentration of 350 mg/kg.
- Group seven G7: Induced arthritis and administered orally 14 days after induction of arthritis with the nanocomposite loaded with MTX methotrexate treatment at a concentration of 350 mg/kg.

**Preparation of the cold aqueous extract of *Cinnamomum zeylanicum***

It was followed (Parekh and Chanda 2007) in preparing it by taking 20 gm of the vegetable powder in a 500 ml glass beaker, adding 200 ml of distilled water, and placing it in a vibrating incubator for 24 hours at a temperature of 37 °C. The mixture was filtered by a medical gauze consisting of several layers. Then it was placed in glass tubes. The tubes were centrifuged at a speed of 5000 rpm for 10 minutes, then the filtrate was filtered with 0.22 µm perforated filter papers. Powder. The powder was placed in a sealed and opaque tube and kept in the freezer at a temperature of 18°C until use. The process was repeated several times to obtain a sufficient amount of extract.

**Preparation of *Cinnamomum zeylanicum* bark extract**

The method of (Gauthami *et al*. 2015) was followed by (Sathishkumar *et al*. 2009) Where 2.5 gm of Ceylon cinnamon powder was taken and 100 ml of distilled water was added to it and boiled for 5 minutes in a 500 ml beaker and after filtering by using filter paper No. 1 . The extract was kept at 4 °C.

**Prepare the cinnamon nanoparticles**

I followed the method (Sathishkumar et al. 2009) as follows: One (1 ml) of Ceylon cinnamon bark extract was added to 50 ml of 1 mM silver nitrate (AgNO3) solution and kept at room temperature for 8 hours to produce silver nanoparticles. The solution initially appeared yellowish in color and when silver nitrate was reduced Ag+ changed the dilute form to a dark color. The color change of the solution was measured each time for 1 hour. The color intensity changed after silver was reduced to nanoparticles from Cinnamon zeylanicum. The reaction and color change with time was recorded.

### Diagnostics of nanoparticles

Examination of the free nanocomposite loaded with MTX treatment by FT-IR (Fourier transforminfrared spectroscopy) and atomic force microscopy (AFM)

### X-ray evaluation

Mice were anesthetized by injecting Ketamine Injection, xylazine Injection at a concentration of 0.5 ml each. Radiographs were taken with an X-ray machine (Siemens Mobilett Pluse X-ray, ALWISAM RADIOLOGY CLINC, KARBALA). The focal distance of the film was 60 cm at 55 kV and 3 mA. The severity of joint deformity was clearly scored according to the extent of osteoarthritis, joint spaces, soft tissue inflammation, subchondral erosion, and articular sclerosis (A *et al.* 2015) X-ray images were analyzed and recorded in detail by two certified radiologists who were blinded on treatment groups.

## Results and Discussion

### Atomic force microscopy images of *Cinnamomum zeylanicum* extract and cinnamon nanoparticle free

Figure (1) shows the outer surface of the cinnamon extract molecules, where the surface roughness modulus of the cinnamon extract was 6,120nm. When cinnamon extract was converted to the cinnamon nanocomposite, Figure (2), the roughness modulus of this compound became 9,330nm, where the difference before and after the conversion of cinnamon to the nanocomposite was nm. 3,21 This is evidence that the roughness of the outer surface has increased after the transformation of cinnamon into a nanocomposite. As for the square root rate of Ceylon cinnamon extract, it was equal to 7,977 nm, while the complex of cinnamon nanoparticles was 12.74 nm. And the difference in the square root rate is 4,763 nm, and this indicates an increase in crystallinity in addition to the crystal homogeneity of the nanocomposite after the transformation from what is extracted. The surface area of the free cinnamon was 12.05%, while the average surface area ratio was 10.72%, which is conclusive evidence of the formation of nanoparticles from *Cinnamomum zeylanicum* extract.

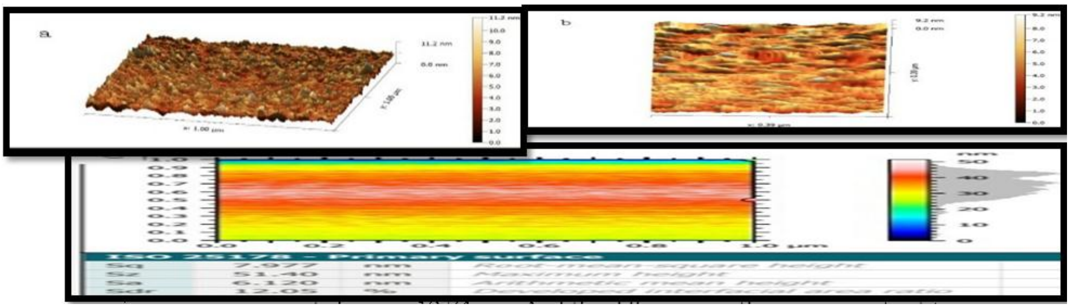


Figure 1. Atomic force microscopy images of free silane cinnamon extract (a) three-dimensional image. (b) two-dimensional image (c) two-dimensional image showing details of the molecules

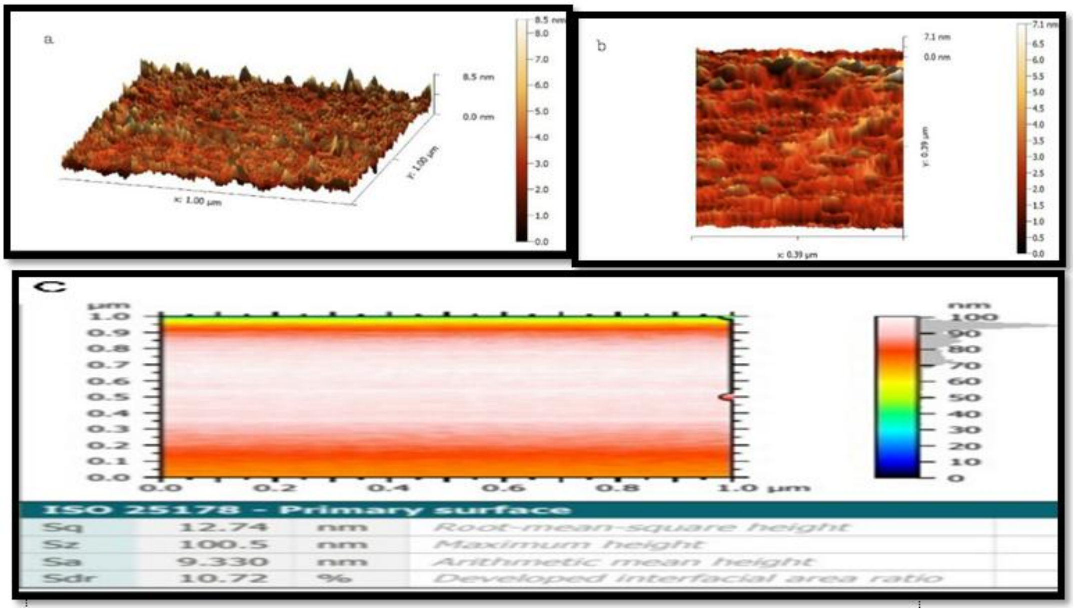


Figure 2. Atomic force microscopy images of cinnamon nanocomposite (a) three-dimensional image (b), two-dimensional image (c) two-dimensional image showing the details of the molecules

**Atomic force microscopy images of cinnamon nanocomposite before and after loading MTX treatment**

The results, as shown in Figure (2), (3) related to the atomic force microscope (AFM), showed that the free Cinnamon nanocomposite, as explained previously, has a roughness modulus of 9,330 nm. When suppository with MTX treatment, it became 6.502 nm, and the difference was in the roughness modulus before and after The loading is 2,282 nm, and this is clear evidence that the size of the particle that was loaded on the surface of the nanocomposite has an important role in the surface roughness and its crystal system in addition to the surface homogeneity. As for the root square rate of the cinnamon nanocomposite loaded with MTX treatment, it was 8,317nm to be the difference before and after loading. 4,423 nm, this indicates that the greater the difference, the evidence of the increase in the crystal structure produced after loading than before loading. As for the height rate of the Cinnamon nanocomposite loaded with MTX treatment, it was, 37,77 nm, while the average surface area is 7.610 nm

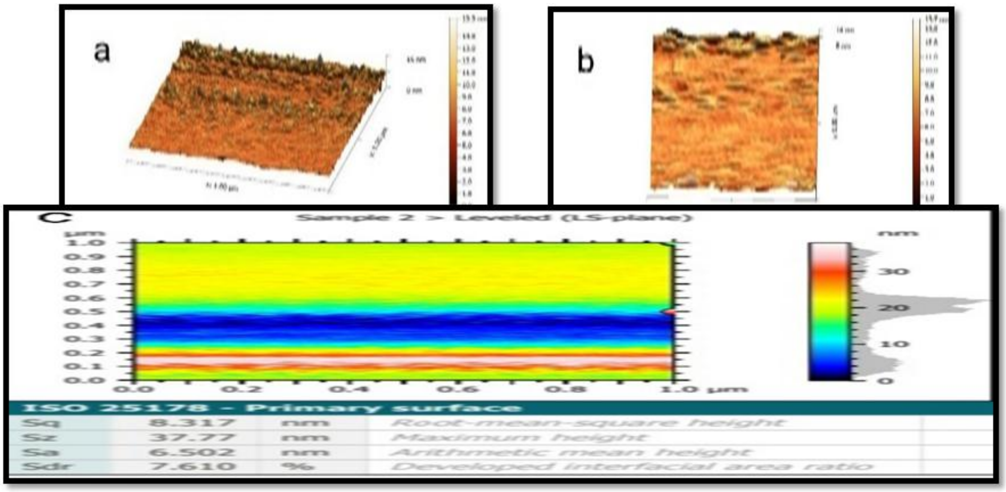


Figure 3. Atomic force microscopy images of cinnamon nanocomposite loaded with MTX treatment. (a) Three-dimensional image (b), two-dimensional image (c) two-dimensional image showing the details of the molecules

**FTIR infrared spectrum of Cinnamon nano-solution before and after loading of MTX treatment**

The results (Fig. 4) showed the presence of a sharp peak superimposed between 3897.99-3747.04 $cm^{-1}$, which is due to the extension of the O-H hydroxyl groups contained in the alcohol synthesis, while the peak at a frequency of 2.63435 $cm^{-1}$ is due to the expansion of the N-H amine group. At frequencies (2356,51,2327,97,2072,33) $cm^{-1}$ resulting from the vibration of the C=O=C bond and it has a strong extension, while the vibrations between (1842.74-1732,54) $cm^{-1}$ It corresponds to the vibrations of the C=O bonds of (vinyl/phenyl ester). The band represented at frequency 1652.73 $cm^{-1}$ expresses the C-H bond of aromatic compounds, 1635.50 $cm^{-1}$. It goes back to the C=C bond extension of the alkene. As for the frequencies between (1558,50 – 1505.71 ($cm^{-1}$) goes back to the extension of the N-O bond. As for the bands between (1495.07 – 1393,57) they are caused by the vibration of the C-H bond. The peak at frequency 667.64 $cm^{-1}$ is caused by the C-I bond. The presence of rings aromatic and alkene bonds, respectively. These bands indicate extended vibrational bands responsible for compounds such as flavonoids and terpenoids and thus can be considered responsible for the effective coverage and stabilization of the obtained AgNPs. That the presence of organic functional groups, such as alkanes, aromatic compounds and amine bonds played a major role in the production and stability of AgNPs. (Huang *et al.* 2007) (Banerjee et al. 2014) The appearance of the transverse band at the top 3850.92 $cm^{-1}$ is evidence of the stretching of the hydroxyl group O-H bond as in Figure (5), while we notice the disappearance of the frequencies located within (3867.28-3741.49) cm-1 that were present in compound Cinnamon nano, and this confirms the treatment of the treatment on the compound Cinnamon nanoparticles, the band 273435.27 cm-1 is due to the vibrations of the N-H bond of the amine groups, while the frequency between (2359.84-2330.36 $cm^{-1}$ is attributed to the presence of an O=C=O bond, The band is 1634.74 cm-1 due to the presence of the C=C bond and the N-O bond, which is found at the frequency of 1505.10 cm-1. As for the bands (1471.29 – 1455.86) cm-1, respectively, they belong to the C-H bond, band 1386. 05 cm-1 has been shifted to 1384.12 cm-1 The band 1089.39 cm-1 has been shifted to the new position 1112.87 cm-1, while the band 667.49 cm-1, which belongs to the C=C bond, remains at almost the same frequencies on the surface of the cinnamon complex nanoparticles, which clearly confirms the loading of MTX treatment on the surface of the Cinnamon nanocomposite (Sadoon and Ghareeb 2020) .

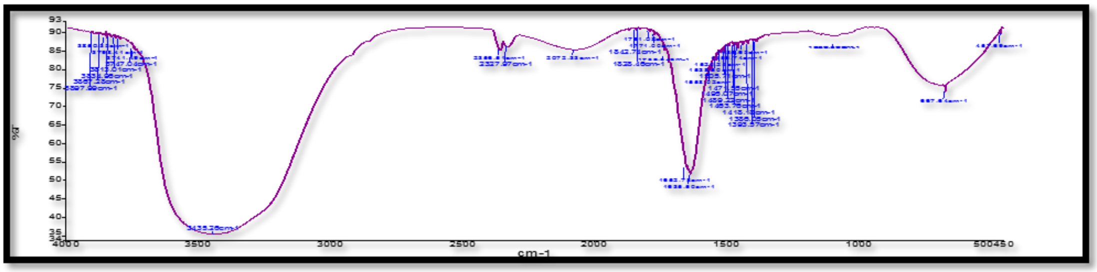


Figure 4. Shows the FT-IR spectrum of the free Cinnamon nanocomposite

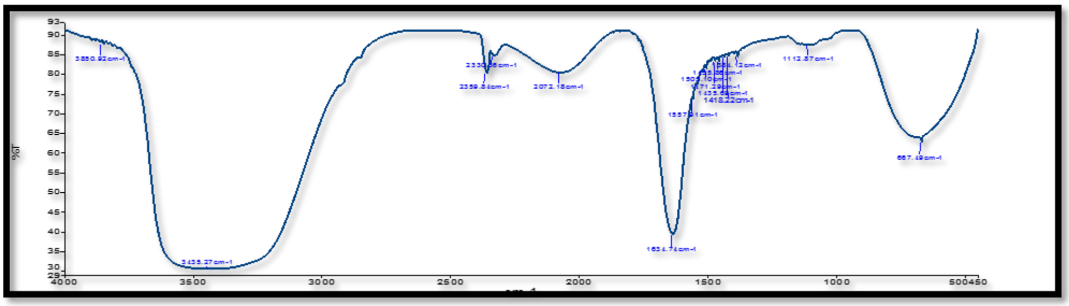


Figure 5. Shows the FT-IR spectrum of cinnamon nanocomposite loaded with MTX treatment

**X-ray evaluation**

Radiographs of the rat ankle and hind limb joints indicated that the CFA-injected rats and the G2 positive control group developed severe soft tissue swelling, excessive bone erosion with irregular and reduced joint space in the bone structure and articular calcifications with no differentiation of intervertebral discs due to changes that caused by RA disease (Figure 6a) compared to the negative control group G1 (Figure 6b), in contrast group G3, G5, G4, and G6 showed slight degenerative changes in the ankle joint and moderate swelling in the surrounding tissues with the presence of joint calcifications but less than what is found in Positive control Fig. (7) However, the MTX treatment loaded with the G7 nanocomposite cinnamon was effective as these problems were resolved. Cinnamon nanocomplex loaded with MTX significantly prevented joint destruction and soft tissue damage and the results are in agreement with (Banerjee *et al.* 2014) (Mahdi *et al.* 2018) that using natural sources such as medicinal plants can be easily used in combination with other anti-inflammatory drugs. It will be less toxic and at the same time effective in preventing joint destruction as well as synovitis, thus increasing the effectiveness of treatment for rheumatoid arthritis patients. (Mary *et al.* 2019) (Kumatia *et al.* 2019) When methotrexate was loaded with cinnamon nanoparticles, it had a strong inhibitory effect on arthritis in rats. It synergistically suppressed the increase in hind paw size and joint destruction and produced better efficacy than it was shown from this study and can be a beneficial and better agent for the treatment of rheumatoid arthritis when used in combination therapy.

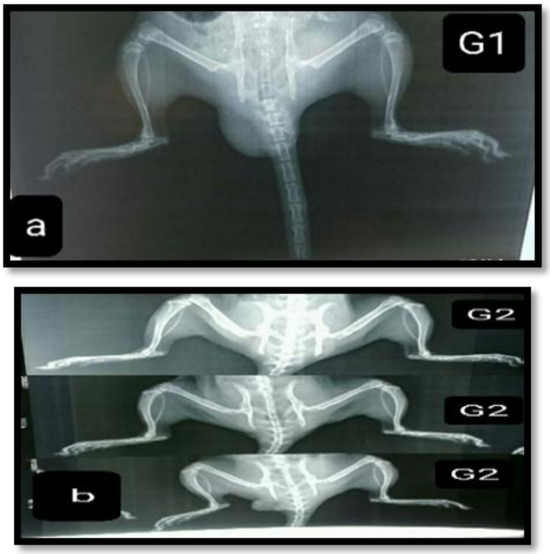


Figure 6. a shows the (healthy) negative control group (G1) b/ the positive control group (G2) in which CFA disease was induced

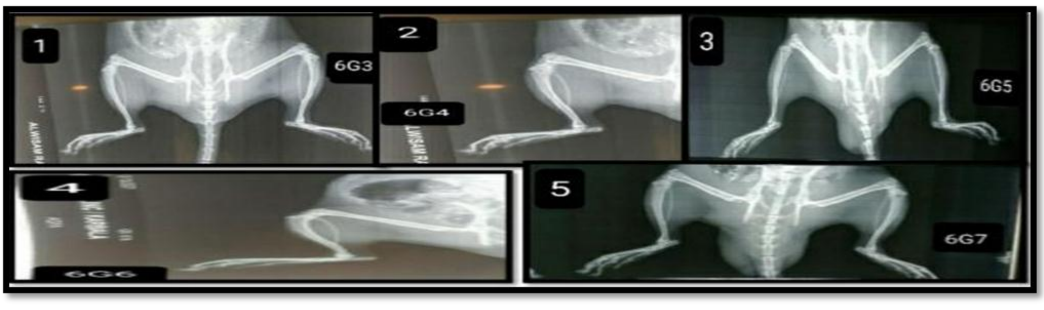


Figure (7) shows the groups that were treated during six weeks, as they represented: 1/ they were dosed with MTX (G3) treatment 2/ they were dosed with free cinnamon extract at a concentration of 350 mg/kg ((G4) 3 / they were dosed with a concentration of Cinnamon nanocomb at a concentration of 350 mg/kg (G5) 4/ Cinnamon extract was supplemented with MTX treatment at a concentration of 350 mg/kg (G6) 5/ it was dosed with cinnamon nano compound loaded with MTX treatment at a concentration of 350 mg/kg (G7).

**Effect of treatment on immunological variables**

**Effect of treatment with free MTX, free cinnamon extract, free cinnamon nanocomposite and cinnamon extract with MTX treatment and cinnamon nanocomposite loaded with MTX treatment on IL-6 concentration and IL-1β concentration**.

The results showed in Table (1) that the injection of CFA males of white rats and the development of arthritis disease in them in the positive control group G2 led to a significant increase ($p<0.05$) with an average concentration of LI-6, as its concentration level reached (186.86,140.13) pg/ml for a period of time. Three weeks and six weeks, respectively, compared to its average concentration with the negative control group G1, where its concentration level was (29.40, 25.04) pg/ml for six and three weeks, respectively, when it was not injected with CFA, and this is consistent with the results of (Takano *et al.* 2014) Disease development RA is a heterogeneous disease condition associated with elevation of certain pro-inflammatory and pro-inflammatory mediators including IL-6, which, if not suppressed, will increase macrophage infiltration into the inflamed site with increased production of autoantibodies (Kumar *et al.* 2016) but when treated with MTX treatment for a full period (G3), there was a significant decrease ($p<0.05$) as the level of its IL-6 concentration in her blood serum reached (61.67,72.80) pg/ml during six weeks (full period) and three weeks (half). Duration) respectively, and this drug is an immunosuppressive drug by inhibiting the Intracellular variability makes the immune system less effective (Bedoui *et al.* 2019) as for the group that was dosed with free cinnamon extract (G4), there was a significant decrease ($p<0.05$)) in the concentration of IL-6, as its concentration level reached (119.78, 110.26) pg/ml during three weeks (half period) and six weeks (duration complete) respectively compared to the positive control group

The results of Table (1) showed a significant decrease ($p<0.05$) in the concentration of IL-6 in the (G5) group. These animals were dosed with the free nano-compound cinnamon, as its concentration level reached (95.77, 97.89) pg/ml for three weeks and a period of six weeks, respectively, compared to the positive control group. The results of Table (1) showed a significant decrease ($p<0.05$) in the concentration of IL-6 in the (G6) group that was dosed with cinnamon extract with MTX treatment (50.80) pg/ml for a full time compared with the positive control group. The combined treatment also led to a decrease in Significant in the symptoms of arthritis, lymphocyte proliferation, improvement of liver function, neutralization of free radicals (Bradūnaitė *et al.* 2021) as if group (G6) was combined half-term (three weeks) with group (G3) that was treated with MTX for full duration ( Six weeks) there was a decrease in the level of IL-6 concentration close to its concentration in the group treated with free MTX treatment.

Whereas, the results in the table indicated a significant decrease ($p<0.05$), as the decrease in the concentration of IL-6 in the (G7) group that was treated by giving it the Cinnamon nano compound loaded with MTX treatment reached (41.5,34.91) pg/ml in six and three weeks at a time. Respectively compared to the positive control group

And there were no significant differences ($p<0.05$) with the negative control group, but when compared with the group G3 full-term with the group G7 half-term, there is a decrease in the concentration of IL-6 close to its concentration in the group treated with free MTX treatment G3 for a full treatment period (six weeks), the combination Combine drug therapy with physical therapy, to reduce inflammatory signals and/or fibrosis while enhancing skeletal muscle efficiency. Therefore, to improve the lives of RA patients and this is in agreement with the study (Huffman *et al.* 2017) the action of methotrexate-loaded nanoparticles to improve the experimental model of autoimmune arthritis by regulating T-cell homeostasis and concentrations of proinflammatory cytokines such as IL-6 concentration is consistent with The study(Park *et al.* 2022) The physical properties of the nanoformula, including size, shape, and surface charge, can alter immunotoxicity .(Wu *et al.* 2018)

Table (1) showed a significant increase (05.P<0) in the average concentration of IL-1β in the positive control group, as its concentration level reached (109.52,130.49) pg/ml for six weeks and for three weeks of infection, respectively, compared to the average of IL-1β in the positive control group. Its concentration in the negative control group (G1) (30.12,32.48 pg/ml for full (six weeks) and half-term (three weeks), respectively, which was not injected with CFA, stimulates multiple types of cells, including monocytes, fibroblasts, and cells T, as well as cytokines secreted by these cells, to induction and maintenance of the immune reaction responsible for the development of RA, and this came in agreement with the findings of studies,(Ollewagen *et al.* 2021) (Qasim *et al.* 2021) (Ahmed *et al.* 2021) (Wang *et al.* 2021) .

And treatment for the whole period with free MTX treatment (G3) led to a significant decrease (05.P<0) in the average concentration of IL-1β (56.35) pg/ml compared with the positive control group (G2 infected and untreated for the whole period, while the treatment was MTX (G3) treatment (half-term) also led to a significant decrease in the level of IL-1β (65.03) pg/ml. The results of the current study also indicate that treatment with C.Z. (G4) led to a decrease in the average concentration of IL-1β compared to the positive control group (G2) during the entire period of treatment, and this decrease was significant ($p<0.05$)) in the half period of treatment, as its concentration level reached IL-1β (88.00,78.02). pg/ml, respectively, the results of the current study are in agreement with the findings of (Kandhare *et al.* 2017) which showed that CZ Ceylon cinnamon bark has a range of treatments and applications for the treatment of immunodeficiency, and with the study (Vetal *et al.* 2013) indicated that the extract of Cinnamon bark has complementary and immunosuppressive activity.

The results of this table also indicate a significant decrease in the average concentration of IL-1β in the G5 group treated with the free Cinnamon nanocomplex for the full period of treatment compared to the positive control group G2, as it recorded an average concentration of 68.25 Pg/ml, while its concentration for half the treatment period was almost equal to the level of its concentration in The G4 group after six weeks of injury reached 75.46 pg/ml. The treatment of CFA-induced white male rats with CFA, MTX supplemented with cinnamon extract leads to a significant ($p<0.05$) decrease in the level of IL-1β concentration, compared to the G2-positive control group for the full duration of treatment, when levels reached Its concentration after treatment was (55.55) Pg/ml, and for the three-week treatment period (half the period), the results of the treatment led to a significant decrease in the level of IL-1β, reaching (59.71) Pg/ml compared with the positive control group G2, and this is in agreement with what the study indicated. (Ross, Devitt, and Johnson 2021) which confirmed that the injection of CFA into white rats in the soles of the feet leads to an increase in the level of inflammatory cytokines, and continuous inflammation leads to synovial hyperplasia, an increase in immune cells and the accumulation of macrophages, while treatment with C.Z.nano/MTX compound (G7).

The cinnamon nanocombine loaded with MTX treatment led to a significant decrease ($p<0.05$) in the level of IL-1β concentration, as its concentration level reached (48.62) pg/ml compared with the positive control group G2 during the entire treatment period, as this decrease reaches a level that does not exist. There is a difference in the level of concentration compared to the negative control group (G1), as its concentration level reached 32.48pg/ml. Whereas, the duration of treatment with this compound has a significant effect, as the results of the mentioned table indicate that treatment with cinnamon nano compound 52.33 pg/ml for a period of three weeks (half period) leads to a significant decrease in its concentration level compared to half the treatment period with free MTX treatment as well as treatment with free cinnamon extract with cinnamon compound The free nanoparticles were added to the MTX treatment, whose percentages reached (65.03, 88.00, 75.46, 56.71) pg/ml, respectively. The results also indicate that treatment with cinnamon nanocomplex loaded with MTX (G7) treatment for half the period leads to a decrease in the level of IL-1β concentration close to its concentration in the group treated with free MTX (G3) treatment (56.35) Pg/ml for a full treatment period (six weeks).

Plant extracts showed good anti-inflammatory through their effect on cell cycle by reducing NO synthase expression Excess production of NO can lead to functional destruction of normal tissues during acute and chronic inflammation as immune cells can stimulate macrophages to release inflammatory cytokines. Among them, nitric oxide, reactive free radicals, overproduction of these inflammatory mediators can be dangerous to healthy tissues(Rao, Fang, and Tzeng 2007) Studies indicate that cinnamon affects the modulation of immune responses (Zhao *et al.* 2021) Because it contains some chemical compounds that act as immunosuppressants, such as saponins, terpenoids, phenols/polyphenols, flavonoids (Mishra *et al.* 2009) , when cinnamon extract was converted into a compound of cinnamon nanoparticles, it improved the action of cinnamon in treating inflammation. Contain. Cinnamon nanocomplex on polyphenols works to reduce inflammation by a greater percentage (Cao and Anderson 2011) , Flavonoids as an antioxidant and anti-inflammatory factor. The NO and (COX-2) Cyclooxygenase-2 (Huffman *et al.* 2017) plant material containing properties could be one of the therapeutic approaches to inflammatory disorders. It has been used as anti-diabetic, anti-pain, and diuretic. Its aqueous extract contains an antioxidant. Fits and could be a potential therapeutic approach for diseases associated with damage from free radicals (Joshi *et al.* 2010) An interesting factor about cinnamon is that it can act as an immunostimulant and inhibitor depending on species and doses which makes it a good candidate as an anti-arthritic agent (Vetal *et al.* 2013) .

Table 1
Shows the concentrations of c cytokines IL-1$\beta$ and IL-6 pg/ml before and after treatment with free MTX, free cinnamon extract, free cinnamon nanocomposite, cinnamon extract with MTX treatment and cinnamon nanocomposite loaded with MTX treatment

| Transaction groups | | Mean± Standard Error IL-6 pg/ml | Mean± Standard Error IL-1$\beta$Pg/ml |
|---|---|---|---|
| Negative control (G1) | Treatment (three weeks) | 25.04±1.05 | 30.12±7.01 |
| | Treatment (six weeks) | 29.40±0.29 | 32.48±6.08 |
| | Total | 27.22±1.43 | 31.30±5.91 |
| Positive control (G2) | Treatment (three weeks) | 140.13±1.19 | 130.49±0.46 |
| | Treatment (six weeks) | 186.89±7.55 | 109.52±9.44 |
| | Total | 163.51±14.32 | 120.00±8.74 |
| MTX (G3) | Treatment (three weeks) | 72.80±2.04 | 65.03±3.96 |
| | Treatment (six weeks) | 61.67±3.98 | 56.35±3.19 |
| | Total | 67.24±4.27 | 60.69±4.16 |
| C.Z. (G4) | Treatment (three weeks) | 119.78±0.40 | 88.00±1.86 |
| | Treatment (six weeks) | 110.26±1.86 | 78.02±3.00 |
| | Total | 115.02±2.99 | 83.01±3.76 |
| C.Z.Nano. (G5) | Treatment (three weeks) | 95.77±3.25 | 75.46±0.60 |
| | Treatment (six weeks) | 97.89±2.45 | 68.25±3.35 |
| | Total | 96.83±2.65 | 71.85±3.07 |
| C.Z. MTX+ (G6 | Treatment (three weeks) | 53.70±0.79 | 59.71±4.20 |
| | Treatment (six weeks) | 50.80±2.09 | 55.55±3.73 |
| | Total | 52.25±1.64 | 57.63±3.77 |
| C.Z.Nano +MTX (G7) | Treatment (three weeks) | 41.5±0.50 | 52.33±3.45 |
| | Treatment (six weeks) | 34.33±0.57 | 48.62±3.03 |
| | Total | 37.91±2.12 | 50.48±3.11 |
| LSD/Total | | 21.37 | 19.22 |

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