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Article

Detection of association between some of nanoparticles (Zn, Ag) and rheumatoid arthritis in rats

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Abstract: Nano materials have drawn more and more interest for the treatment of rheumatoid arthritis (RA), the most prevalent complex multifactorial joint-associated autoimmune inflammatory disease, because of their special chemical and physical characteristics. Articular cartilage and bone are destroyed in RA, which is characterized by synovial inflammation and increased production of proinflammatory cytokines (IL-1, IL-6, IL-8, and IL-10). Articular cartilage and bone destruction are also linked to the development of cardiovascular disorders, including heart attack and stroke. Even though inflammatory arthritis can be monitored and diagnosed using a variety of imaging techniques, and even though efforts are being made to improve the sensitivity and precision of these techniques, accurately diagnosing RA is still challenging, especially in its early stages. Here, we aim to outline the advantages of utilizing different nanomaterials as enhanced drug delivery systems for the efficient management of the illness as well as next-generation RA imaging and detection tools employing contrast agents and nanosensors. Immune Ninja indicated that, when compared to the control group, the induction of RF arthritis (RF) in the G2 white rat resulted in a significant increase (P<0.05) in the level of concentrations of the cytokines IL-6, TNF-α, IL-1β, and IgG and IgM antibodies. Enjoyable control (G1). Additionally, after deciding on the various compounds and treating the groups with their free and hybrid forms (i.e., before and after loading) for the two treatment periods, it obtained a significant adjustment (P<0.05) to the level of IL-1β concentration in the groups. In contrast, the levels of TNF-α cytokine were significantly (P<0.05) higher in the two groups treated with MTX and NAP for both treatment periods compared to G2. Nevertheless, for training under full treatment, the IL-6 concentration was significantly (P<0.05) lower than that of (G13----G6). The results also indicated a decrease in body levels of IgM in groups (G13----G3), and this became significant (P <0.05) At university (G13----G6) for complete recovery. The results of the study recorded a significant decrease (P<0.05) in the level of the studied immune parameters Compared to when treated with MTX and NAP treatments in their free form for the entire treatment period, when treated with the aforementioned treatments after loading them on the nanocomposites under study for half the treatment period and calculating the T value, which indicates time savings. The amount of treatment was approximately halved, which indicates an improvement in the efficiency of the two treatments and a reduction in waste by 50%. Additionally, the study showed that the thickness of the right foot in the positive control group G2 was significantly higher (P<0.05) than in the negative control group after male white rats were given an arthritis induction. Following treatment with MTX and NAP in their free forms, G6 and G7, respectively, this increase decreased; this decrease persisted significantly (P<0.05) following loading. The above two treatments were applied to the prepared nanocomposites, After applying the two treatments to these compounds, the affected foot's recovery rate and swelling reduction reached roughly 50%. The two free-form nanocomposites Agnano and ZnO were used to study the inhibitory effect on the growth of Escherichia coli and Staphylococcus aureus (T1 and T2) and comparing the results with the inhibitory capacity of the two antibiotics (ER) and (GN (free) T3 and T4) with what was recorded by the synergistic effect of the two antibiotics after loading them, and the results therein indicated an increase in the average diameter The inhibition cycle was significantly (P<0.05) in groups T5 and T7 treated with two nanocomposites loaded with antibodies

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GN/ZnO (GN and GN/Agnano) (contrasting medications and substances in bacterial isolates of E. coli). The two nanocomposites loaded with the ER antibiotic in groups T6 and 8, ER/(TZnO and ER/Agnano), significantly increased the diameter of the inhibition ring ($P<0.05$) when applied to E.coli isolates as compared to T2, T1, and T4, respectively. The results showed that treating S. aureus isolates with (GN/ZnO and T5) (GN/Agnano and T7) led to a significant increase in the area of the inhibition ring. Significant ($P<0.05$) compared to the inhibitory episode at T2, T1 and T3, respectively. In contrast to what was observed in the inhibition area in treatments T2, T1, and T4, respectively, the diameter of the inhibition ring for the growth of S. aureus bacteria increased following treatment with the two nanocomposites loaded with ZnO / ER (ER and Agnano /T6) ER and T8 significantly ($P<0.05$). Treatment with the lowest MIC inhibitory concentration for the two nanocomposites (Agnano, ZnO) and the antibiotic (ER).

Keywords: Rheumatoid Arthritis Disease, Nanocomposites, TNF-A And IL-6

1. Introduction

One of the most prevalent illnesses in the world, rheumatoid arthritis (RA) is spread at rates of more than 1% according to global data. (1). Its status as an illness defines it. Autoimmune, RA targets various body joints, encompassing the tiny joints in the foot, ankle, knee, and hand. Moreover, the synovial membrane is impacted, which comprises synovial fluid and two or three layers of epithelial cells, and often affects both the right and left sides simultaneously. Due to the thicker membrane and high concentration of inflammatory cells (macrophages and T lymphocytes) in the afflicted joints. huge (causing harm) (2). Tumor necrosis factor alpha is the majority of the cytokines found in the tissue and joint fluid. The body secretes a protein called tumor necrosis factor (TNF-α) by immune system cells, also known as cell proteinuria. If secreted in excess, it can cause rheumatoid arthritis and interleukins from them. IL-1, 6, 10, 8 (1, 6, 8, 10) Interleukin-1 The condition is activated by these cytokines, particularly TNF-α. The activation of T-lymphocytes (CD4+) in response to inflammation will increase monocyte and macrophage activity. The big monocyte macrophage in joint tissue known as a fibroplast-like synoviocyte. These inflammatory mediators are stimulated by FLS, which causes cells that degrade bone and cartilage to appear. in the fluid of the joints. Furthermore, the surface expression of chemicals stimulates these cells. T cell expression of chemicals on the cell surface, followed by stimulation of T cells (CD4+). Activated B-cell lymphocytes that undergo differentiation to generate immunological proteins including rheumatoid factor (RF-)Factor rheumatoid (3).

Numerous biological, environmental, psychological, and individual factors have been found to have a strong correlation with rheumatoid arthritis (4). Although there is currently no known cure for arthritis, rheumatoid arthritis patients can still lead active, long lives provided they receive the proper care, which includes joint protection and lifestyle modifications. Currently available treatments, however, aim to preserve flexibility while lowering the disease's damaging inflammatory impact on the joint and averting further complications. thereby minimizing joint movement and pain. Disease-modifying drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are two of the most important medications used to treat arthritis (5).

Many bacteria have a high potential for resistance to antibiotic treatment (MDR), which leads to increased infections due to pathogens by bacteria that are resistant to current antibiotics, which are ineffective and represent a growing problem. Because it increases the risk of infection and death from these microbes, antimicrobial resistance is one of the biggest threats to human health. Resistance genes are a major factor in the increased antibiotic resistance of microorganisms. Thus, using alternative approaches, such as the use of nanocomposites, is the first step in preventing the emergence of antibiotic resistance and lowering the use of antibiotics (6). In a similar manner, for patients who take treatment with gold salts for long periods extending to years, it can be considered an effective treatment with good benefit. Also, as with gold salt and antimalarial treatments, there is little randomized trial research evidence to prove its effectiveness in delaying the

progression of the disease, so its current use is limited in the United Kingdom. Common side effects of treatment are nausea, mild rash, and a less common and more important side effect is thrombocytopenia. Proteinuria and Thrombocytopenia (7). A study loaded the Fluorocytosine-5-FC-5 treatment, which is used to inhibit the growth of Candida albicans bacteria, onto nanoparticles of layered zinc oxide (Nanoparticls / Zinc / LHs). The results led to the inhibition of the ability of bacteria to differentiate and spread, as it was noted that the highest area of inhibition was at 25 mg/L for treatment alone, and the concentration reached 5 mg/L after loading it on zinc (8).

Aim of the study

The current study aimed to evaluate the mechanism of some treatments used to reduce the effect of RA and loaded with nanoparticles (Zinc Oxide, Ag nano & Xerogel Nanoparticles) after it was induced in male white rats. Complete Freund's Adjuvant (CFA) is recommended in order to lessen the damage caused by medications, as numerous studies have demonstrated that they disrupt certain functional blood parameters and functions. Additionally, CFA is recommended due to the disease's significance in our society and the fact that it is a common and widely spread illness. The liver, kidneys, and cartilage tissue in the joints, and to know the inhibitory effect of the two nanocomposites, free Zinc Oxide and Ag nano, along with the antibacterial agents GN and ER, after loading them on the two nanocomposites, on two types of bacteria: E. coli and S. aureus

2. Materials and Methods

2.1.study groups and blood samples collection

Following a 12-hour starvation period, the animals were weighed, put under ether anesthesia, and had five milliliters of blood extracted from each heart. method of cardiac puncture. Over the course of eight weeks, blood samples were taken from them using a 5-ml medical syringe. The free drug, the free nanocomposite, the drug loaded on the nanocomposite, the induction of arthritis and its aftermath, and the positive and negative control groups. inserted into anticoagulant-free plastic tubes to ensure an adequate amount of After that, the serum was separated in a centrifuge for ten minutes at 3,000 rpm. serum free of erythrocytes using a micropipette. The serum is divided among sterile, spotless tubes and kept cold. When freezing at a temperature of -20 °C in a lab freezer, necrosis factor and other functional parameters are to be measured. immune proteins, interleukins, and oncogenes.

2.2.The principle and method of the diagnostic kit to measure the level of TNF-α, IL-1ß, IL-6:

The ELISA technique was utilized to estimate the levels of TNF-α, IL-1ß, and IL-6., which is a solid phase enzyme for microtiter plate, where monoclonal antibodies are used. Directly against specific antigenic specificity of TNF-α, IL-1ß and IL-6

2.3.Methods calculate an antibiotic's and nanocomposites' inhibitory activity against bacteria:

Testing was done on the inhibitory activity of free ZnO NP and Ag NP nanocomposites(9).

2.4. Experimental design

Male white rats were divided into groups of ten at random and given oral doses based on their body weight for two different dosage periods: one lasting six weeks (the full dosing period) and the other lasting three weeks.

2.5.Statistical analysis

An analysis of variance was performed for a factorial trial of 13 × 2 × 5 replicates in a completely randomized design to study the effect of treatment by treatment and the nanocomposite and the time period in the studied standards, and the use of analysis of variance according to the completely random design to study the effect of The treatment with the treatment and the nanocomposite in the right foot's thickness and the L.S.D. test for significant differences between the means. Revised Least Significant Differences and a T-test to test the differences between the effect of treatment for one and a half days of

free treatment duration of treatment after loading the treatment on the nanocomposite (10).

3. Results and Discussion

The findings show that injecting male rats with CFA caused rheumatoid arthritis. The average concentration of IL-1β in the G2 positive control group was significantly ($P<0.05$) higher than that of the negative control group after six weeks and three weeks of infection, respectively, with an average of 86.99 and 72.28 pg/ml. (G1) 6.85, 7.97) pg/ml for half of the period (three weeks) and for the entire period (six weeks), respectively, during which no CFA was injected, and this was consistent with the study's findings (11). The findings demonstrated that male rats' blood had more white blood cells after being induced to develop rheumatoid arthritis with CFA. Additionally, this aligns with the study's findings(12), which provided an explanation for the rise by highlighting the role white blood cells play in the inflammatory immune response and their use as an indicator of the disease's severity. It is also attributable to The substance known as interleukin-1 (IL-1) is secreted by macrophages in response to elevation because it increases white blood cell filtration. from the bloodstream to the joints in the bones, which causes an increase in their quantity and accumulation.

The results of the current investigation show that CFA-induced rheumatoid arthritis in male albino rats raises the average blood serum concentration of tumor necrosis factor (TNF-α), a finding that is morally significant ($p<0.05$). This is in line with the study's findings when compared to the negative control group G1, whose concentration level reached (72.53, 73.39) Pg/ml for the full and half-term of treatment, respectively. For the entire and half-term of treatment, the two positive control groups (G2) had levels of 460, 61, and 449 Pg/ml, respectively(13), which suggested that the immune system's stimulation against invasive microorganisms was the cause of the elevated platelet count. Pathologically evident monocyte infiltration in the joints of mice induced arthritis by causing necrosis factor secretion Neoplastic. Additionally, it demonstrated a rise in TNF-α concentration in the compound-treated groups G5, G4, and G3. Ag nano, ZnO Xerogel, and nanoparticles for (Total) rates These rates (72.572, 01.581, 50.496) Pg/ml) were reached for the full and half-term of treatment, in comparison to the average concentration in the positive control group (G2), which reached 454 pg/ml.

After arthritis is induced, the concentration of interleukin-1L-6 increases significantly ($P<005$) in male white rats in the positive control group (G2).which, after eight weeks of infection, had a concentration of 25.442 pg/ml. In contrast, its level was 36.34 when compared to animals in the negative control group (G1) that were not given arthritis. Pg/ml eight weeks after the start of the experiment and the results of this indicate that the treatment of new animals Illness with the nanocomposites under study leads to a decrease in the level of its concentration in those aggregates, as its level is in the G3 aggregates. (ZnO treatment), (G4) (Ag nano treatment), (G5) (Xerogel treatment), respectively. 440, 22. 407, 18 (385 pg/ml) for six weeks following infection in comparison to the control group (G2) and for the duration of the entire treatment period, and that's what I discovered with To study (14) to contrast the impact of zinc oxide nanoparticles' two shapes (paper and spherical). with an area of Almost identical surface. It was found that the spheroids, despite being effective, had no effects in stimulating the secretion of IL-6, and a slight increase in the secretion of TNF-α resulted from the increase in the rate of macrophages in mice treated with zinc oxide particles. spherical cells, compared to mice that were treated with zinc oxide paper particles, as it was observed that the primary cells were dendritic cells. It caused the cytokine TNF-α to be produced in higher amounts than the spheroids, Therefore, a key element in determining the immune response to nanoparticles is targeting particular cell types.

3.1. Diagnosis of hybrid nanocomposites:

3.1.1.FT-IR spectrum

The infrared spectrum was studied to diagnose the prepared nanocomposites loaded with the two arthritis treatments under study. Zinc oxide and silver nanocomposites were diagnosed, as well as the prepared xerogel compound and the methotrexate and 36aproxen treatments, respectively (ZnO, Ag nano, Xerogel MTX and NAP) before and after loading. Treatment.

3.1.1.1.FT-IR spectrum of free silver nanoparticles (Ag nano):

The results indicate the presence of a large, very sharp cross-sectional peak overlapping between 3554-3362 cm-1, which is due to the hydroxyl groups contained in the composition of the alcohol ethylene glycol involved in the preparation of the silver nanocomposite. The band at 1633.9 cm-1 is due to the transformation of the The carbonyl is gluconic acid, and the bands (1393, 1371, 1360) cm-1 are all due to gluconic acid. The two bands (1148, 1105) cm-1 are also due to the composition of the aforementioned acid that forms silver, and the large transverse band is between (619-682) cm. -1 It goes back to the Ag-O metallic bond (15).

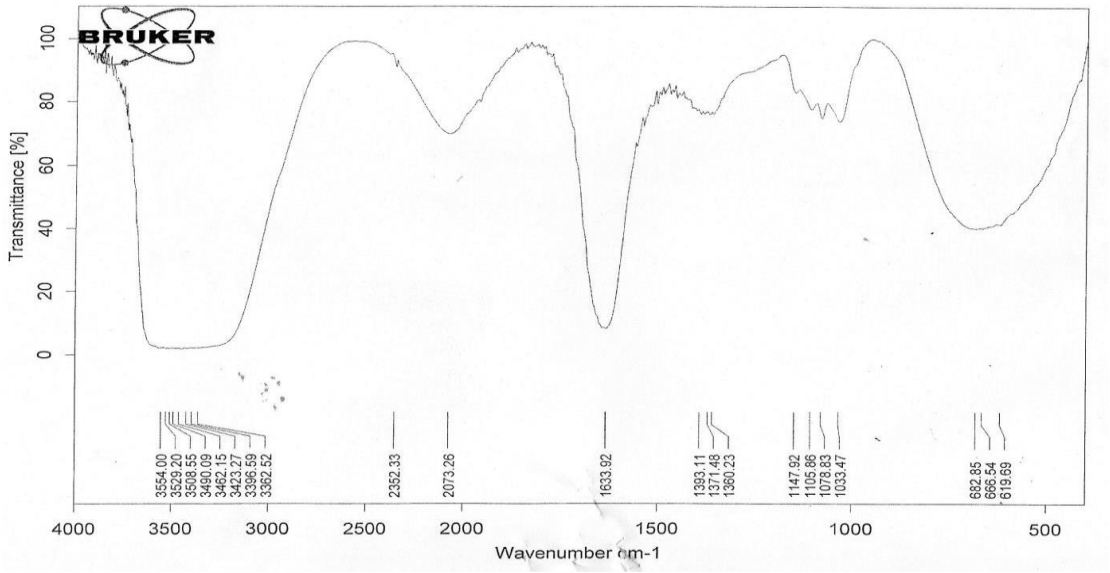


Figure (4-1) The free silver nanoparticles'FT-IR spectra (Ag nano).

FT-IR spectrum of free Xerogel: 3.1.1.2.

Infrared spectra were obtained for the xerogel solution of the nanocomposite Xerogel. The spectrum showed the transverse bands at frequencies (3000-3500) cm-1, which belong to the hydroxyl bond (O-H) in alcohol or water, H2O. Here we notice the overlap between The peaks that led to the display of the band of these aqueous molecules are from alcohol, ethylene glycol and water. The bands located between (2976-2789) cm-1 are due to the vibration of the CH3 groups, while these vibrations are between (1599-1550) cm-1. They correspond to the vibrations of the C-C bond.

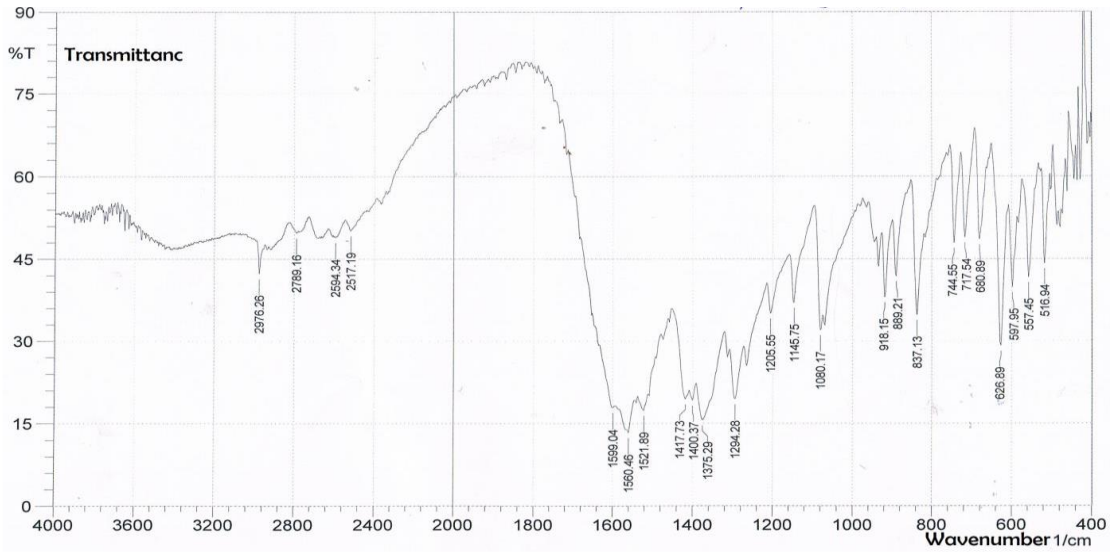


Figure (4-2) The free Xerogel nanocomposite's FT-IR spectrum

3.1.1.3.FT-IR spectrum for treating (MTX) with ZnO nanocomposite

shows the X-ray spectrum of nano-zinc loaded with methotrexate, and it is observed in it, in addition to the bands of free nano-zinc oxide prepared above, the appearance of the frequencies of the MTX bonds overlapping with the frequencies of nano-ZnO, which are (1606, 1558, 1508, 1384) cm-1, which are... To the amine and phenyl groups included in the composition of the MTX treatment, they appear overlapping with the spectrum of the ZnO nanoparticles, which clearly indicates that the loading of the MTX has actually occurred on the surface of the zinc nanoparticles via the hydrogen bonds of the amine (2NH) **groups** **(16)**

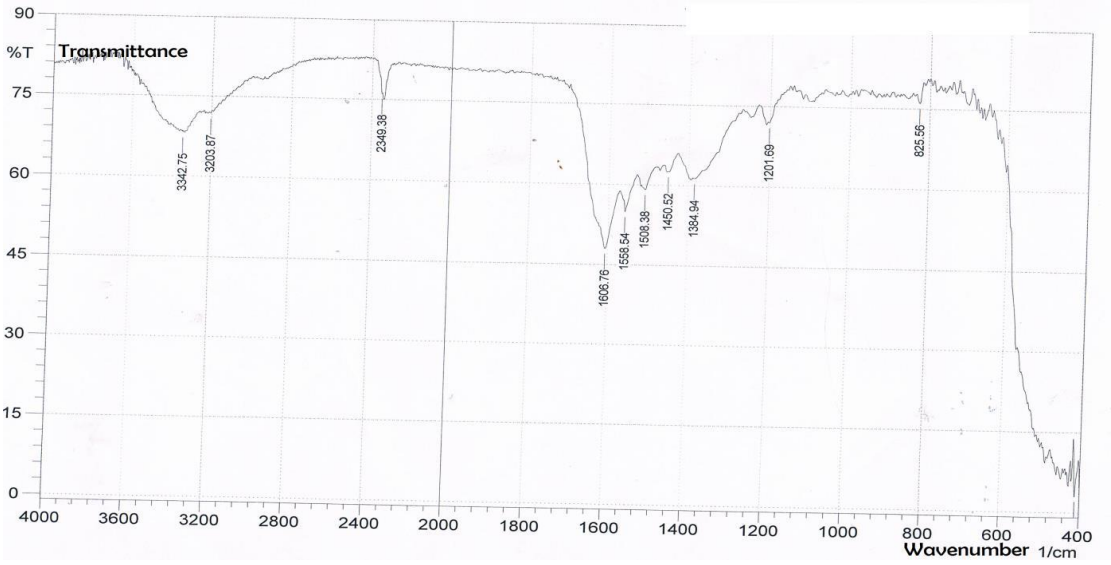


Figure (4-3) FT-IR spectrum for the treatment of (MTX) with the nanocomposite Spherical ZnO ZnO/MTX

3.1.1.4. FT-IR spectrum for treating (MTX) with the nanocomposite : (Agnano/MTX) Agnano

The FTIR spectra of nanoparticle structures loaded on nanoscale surfaces correspond to the structures of Nanoparticles with chemical properties representative of the MTX compound showed differences in the FTIR spectra between the formulations of the silver nanoparticles and the MTX loaded one. The additional peaks observed in the MTX loaded nanocomposite formulations were the presence of bands at frequencies. 1459-1638 (cm-1), representing the amine group, as well as the vinyl group belonging to the chemical structure of MTX, which appeared at frequencies (1633) cm-1. These spectra conclusively

prove that the MTX was loaded on the surface of the silver nanoparticles by means of hydrogen bonds and H-groups. The amine 2NH from (17) (MTX).

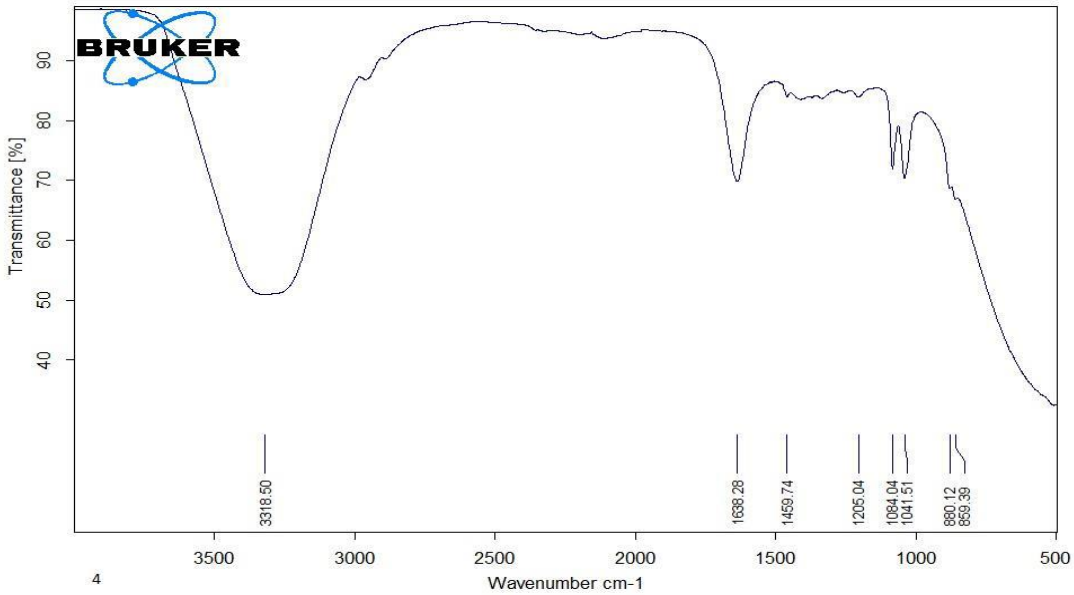


Figure (4-4) FT-IR spectrum for the treatment of (MTX) with the nanocomposite Agnano (Agnano/MTX)

3.1.1.5. FT-IR spectrum for treating (MTX) with the nanocomposite : (Xerogel/MTX) Xerogel

The results of the infrared spectrum of the Xerogel compound loaded with M TX treatment show the presence of a large transverse band at (3333 cm-1) attributed to the hydroxyl groups of the Xerogel compound and the alcohols used in its preparation. This band is interspersed with frequencies dating back to the adsorption (adsorption) molecule. Water is physically present as it overlaps in this region. The band (1634 cm-1) is due to the amine groups (NH) as well as to the stretching of the hydroxyl bond (OH). As for the transverse band at (1341 cm-1), (are for the vibrations of the carbonyl bond present within the structure of MTX, the bands) 1083, 1043 (cm-1 respectively are attributed to the (C=C) bond, the frequencies (800-400 (cm-1) are due to the vibration of the metallic bond) (18).(

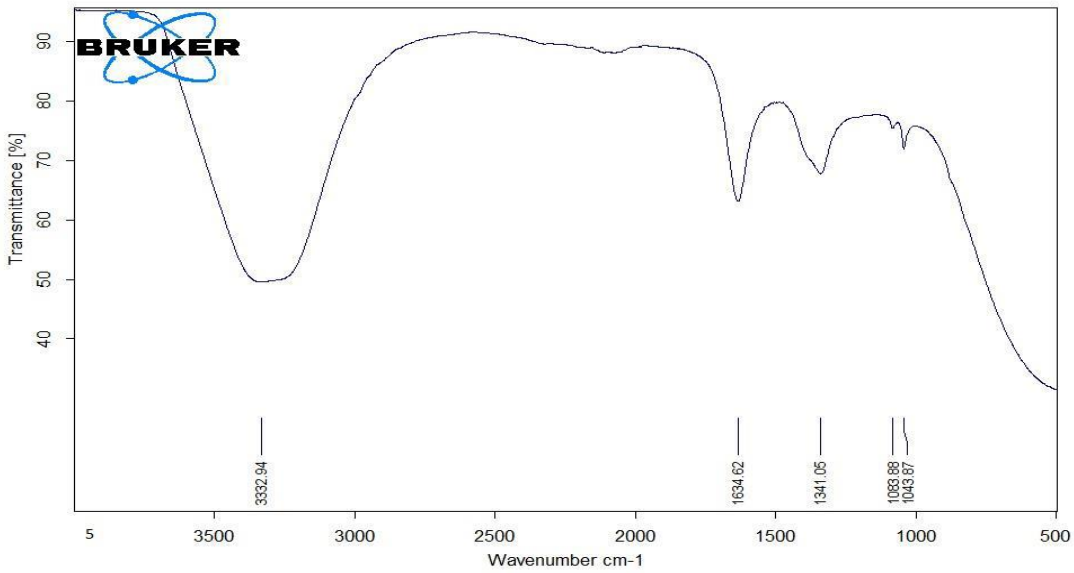


Figure (4-5) displays the FT-IR spectrum of the nanocomposite Xerogel treatment with (MTX). (Xerogel/MTX)

3.1.1.6.FT-IR spectrum (NAP) with ZnO nanocomposite
): Zinc Oxide/NAP)

The loading of the ZnO nanocomposite with the treatment of nproxen certainly appears through the presence of the new transverse shape at 3331 cm-1, which is due to the participation of hydroxyl groups and the combination of 3CH,CH groups that belong to the nproxene. We find that the nproxen bands have been displaced from their normal positions, which appears in the spectrum. Compatible has been shifted to 1604 cm-1, 1458 cm-1, has been shifted to 1538 cm-1, 1390 shares are approximately in place, These displacements of the aforementioned bands are due to the presence of interaction between the nproxen groups and the surface of the zinc nanoparticles through the hydrogen bonding groups present on the oxygen and hydrogen atoms, while the bands 1267 and 1226 cm-1, which belong to the nproxen, remained at approximately the same frequencies on the surface of the zinc compound (ZnO). (Nano.

The band at 1082 cm-1 has been shifted to the new position (1076 cm-1), and the frequencies between (400-923 cm-1) represent the vibrations of the carboxyl groups as well as groups belonging to the zinc nano-metallic bond (19) .

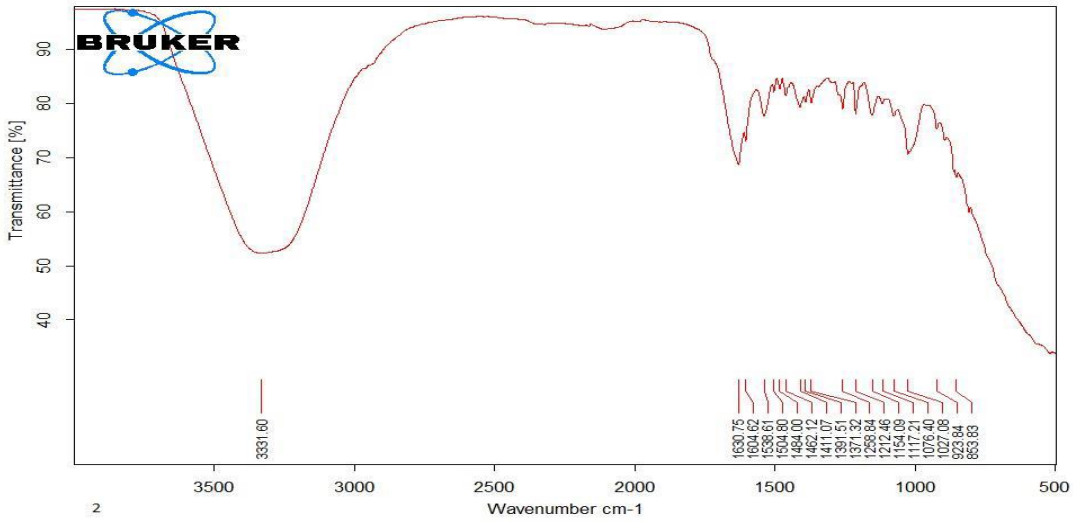


Figure (4-6) shows the FT-IR spectrum of (NAP) treatment with ZnO nanocomposite (ZnO/NAP)

3.1.1.7.FT-IR spectrum for treating (NAP) with the nanocomposite
: (Agnano/NAP) Xerogel

The two bands (3213, 3194) cm-1 belong to nanosilver (Agnano) stabilized with glucose with the spectrum of naproxen treatment, and the bands (3001, 2972, 2939) cm-1, respectively, belong to the vibrations of the bonds of naproxen (NAP), as these bands were distinguished with the spectrum Nano-silver with some displacements. Also, the large band present in the silver spectrum at (1633 cm-1) has been shifted to a new location (1728 cm-1) due to the surface bonding of nano-silver with naproxen (NAP-Agnano), and the vibrations resulting from the superposition of naproxen also appeared. The temperature range is between (600-1502) cm-1 with the nanosilver after loading it with the treatment. Also, the transverse band (682 cm-1) in the spectrum of the nanosilver shows the start of clear vibrations of the naproxen ligands, and this conclusively confirms the loading of the treatment on the prepared nanocomposite.

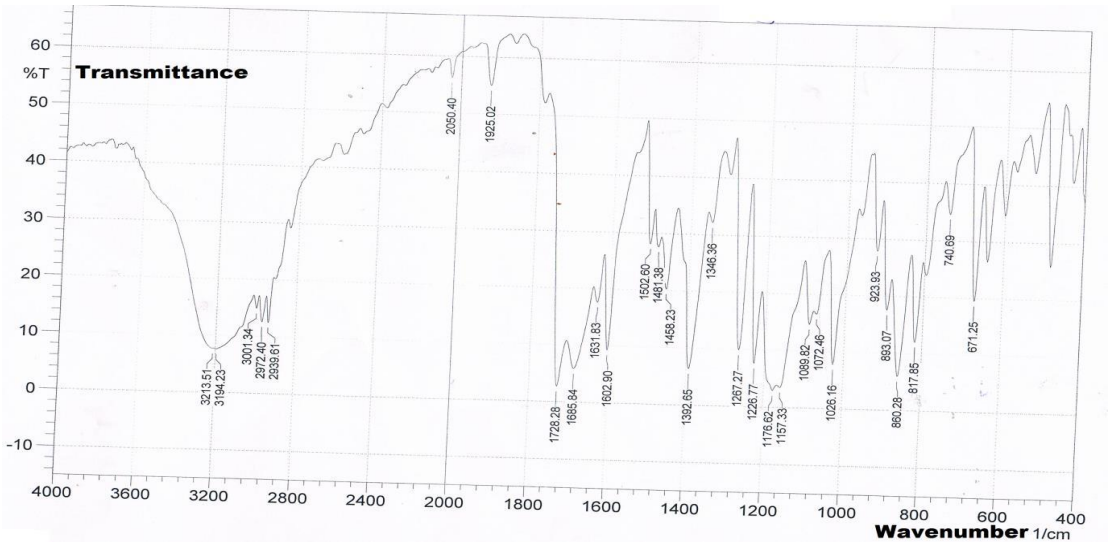


Figure (4-7) displays the FT-IR spectrum of the Ag nanocomposite treatment using (NAP) (Agnano/NAP)

3.1.1.8.FT-IR spectrum for treating (NAP) with nanocomposite : (Xerogel/NAP) Xerogel

The appearance of the large transverse band at (3319 - 3258) cm-1 represents the vibrations of the hydroxyl group, the physically adsorbed water groups, and the hydrogen bond groups, and the band (1603 cm-1) is due to the stretching of the hydroxyl bond. It appeared in the infrared spectrum of the Xerogel nanocomposite loaded with naproxen treatment. At) 1633 cm - The sharp and symmetrical beam (1390) cm-1 overlapped with the Xerogel beams and was shifted to 1342 cm-1. The beam (1226.77 cm-1) remained almost in place as it appeared at (1227 cm-1). As for the beam (1267.27 cm-1). 1) has overlapped and shifted to a new location at (1193 cm-1), while the 1168.9 cm-1 band has shifted to the location (1193.9 cm-1). This is due to the presence of bonding between the nproxen groups and the surface of the nano-Xerogel compound, and the bands between (400-900) cm-1, which belongs to MO groups and these are natural bands)(20) (21)

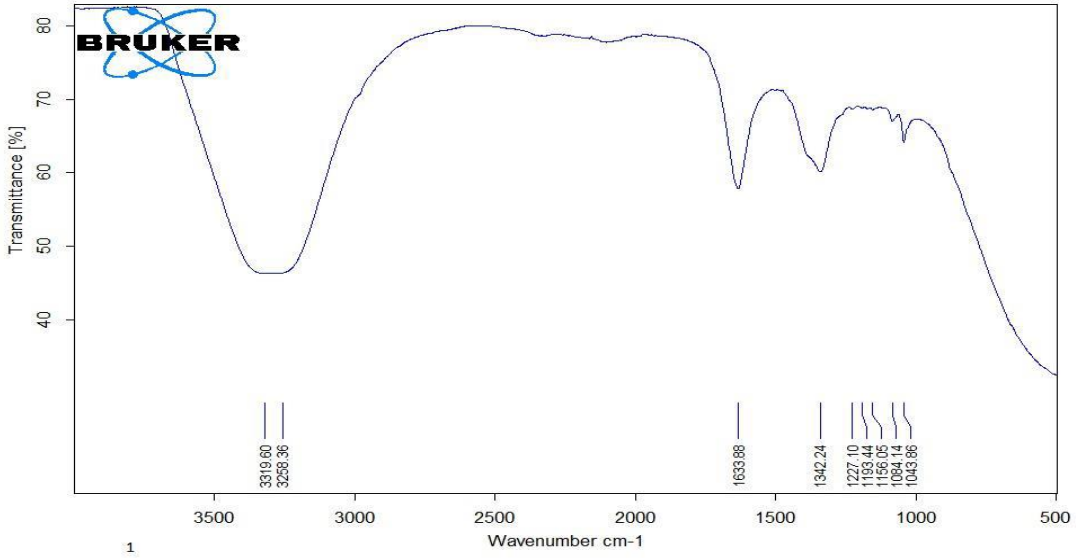


Figure (4-8) displays the NAP treatment's FT-IR spectrum using the nanocomposite. Xerogel (Xerogel/NAP)

3.2. Effect of treatment on immune variables

3.2.1. Effect of treatment with MTX, NAP free and free nanocomposites loaded with NAP, MTX treatment on the concentration levels of the cytokine IL-1β:

15The results of the table (in CFA) indicate that inducing rheumatoid arthritis in male rats by injecting them with substance 4 (at a concentration rate of $P<0.05$) led to a

significant significant increase in (G2 positive control group) 72.28, 86.99, reaching Comparing the average concentration of IL-1β for the full period (six weeks) and half the period (three weeks) of infection in the negative control group, which was 6.85 pg/ml (G1), which was not injected with the substance pg/ml (pg/ml) 7.97, and this was in agreement. With the findings of CFA studies (22). It was shown that treatment for a full period at a concentration rate of $P<0.05$, it led to a significant decrease in (G3) free Zn with the nanocomposite IL-1β (50.15) The rate of ZnO IL-1β (06.56)pg/ml is significantly reduced when G2 G3 is treated with the nanocomposite for half the period, as opposed to the positive control group (pg/ml infected and untreated for the entire period). The findings presented above differed from the conclusions drawn by the studies (23)(24). According to these studies, the immune system's primary job is to identify and detect foreign substances in order to defend the host. Nanoparticles have the ability to obstruct this process or to self-identify as exogenous antigens, which triggers the ZnO immune response (25)(26)(27). The current study's results also show that, throughout the course of treatment, there was a slight increase in the rate of IL-1β concentration when compared to the positive control group (G2). This increase was significant ($P<0.05$) in the half of the treatment period, when the concentration of pg/ml of IL-1β reached (131.53 and 91.27), respectively. The results of the current study are consistent with the findings of studies (28) (29) which indicated that particles. Silver nanoparticles larger than 100 nm tend to aggregate relatively quickly in the laboratory compared to nanoparticles smaller than 100 nm.

When mice are treated with the nanocomposite poly-hydroxylated metallofullerenol, the balance of cytokines is polarized towards TH1 cytokines, with TH1 production in the serum increasing IFN-γ & TNF-α cytokines and TH2 production and cytokines (IL-6, IL-5, and IL) decreasing. Treatment with this compound (30) is thought to increase the production of large amounts of pro-inflammatory cytokines, such as TNF-α, IL1-β, IL-12, and IFN-γ. To clone the DNA for the production of these cytokines in the serum of mice as a result of their treatment with these modified nanocomposites. In addition, the findings of research (31) indicated that the management of (Celecoxib-hyaluronate-Cxb) loaded on liposomal nanoparticles. It is more effective in controlling pain and protecting cartilage from erosion than treating Cxb alone, or free liposomes. Although liposomes are stable, effective, and biocompatible, their residence time in the body is relatively short compared to silver nanocomposites used as DDSs. A study (32) indicated that zinc oxide nanoparticles (ZnO nano) that are used to deliver non-steroidal arthritis treatments to the cells collected in the joint are better than nanocomposites (liposomes) and those that contain polymers, as liposomes It can be used to deliver Lactoferrin treatment for arthritis resulting from injection of collagen-induced arthritis in mice, but its efficiency is much lower compared to The compound used to deliver the same treatment, as these compounds were used as DDSs containing nano-zinc oxide, and this may be the reason for improving the effectiveness of treatment with the NAP/ZnO compound for animals after they were infected with CFA, which was shown by the results of the current study.

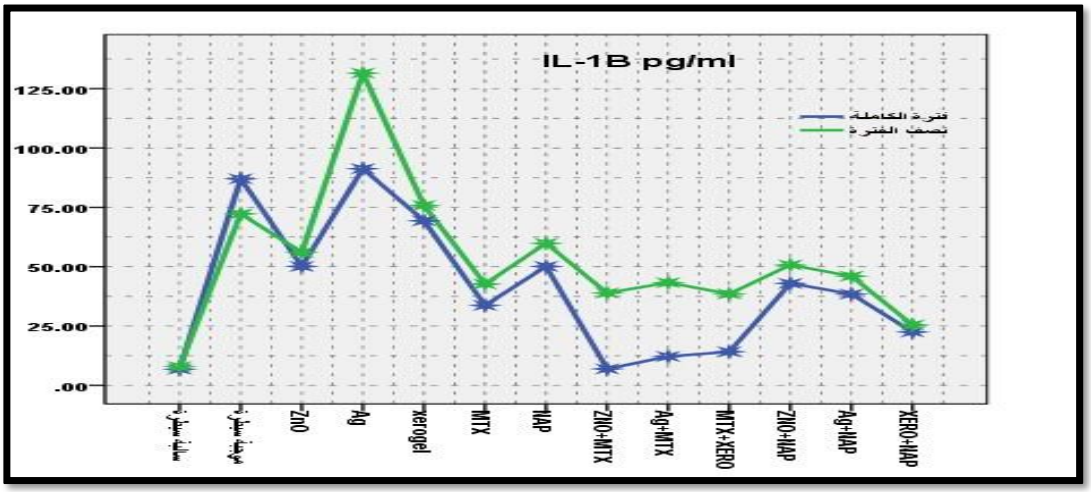
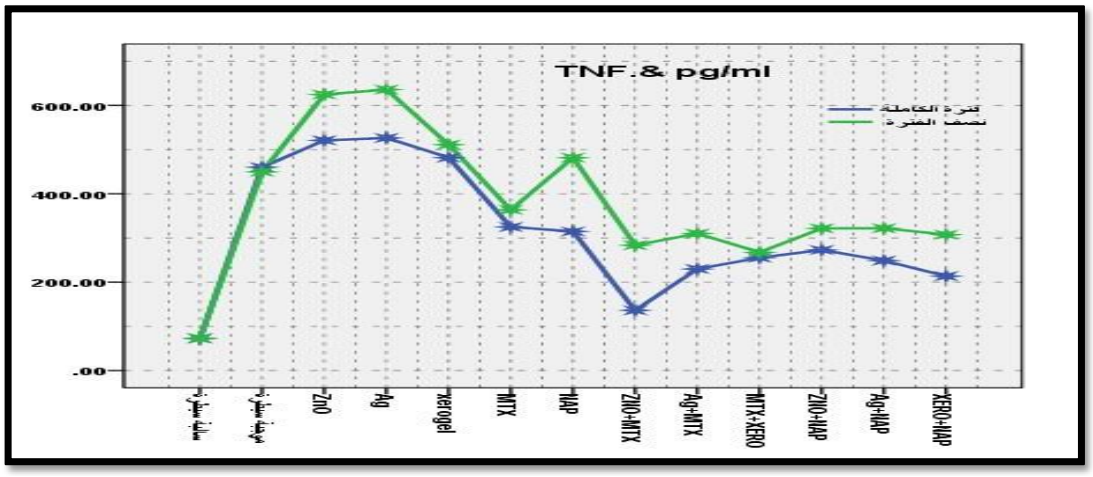


Figure (4-9)The effect of the duration of treatment with nanocomposites loaded with the treatment on the concentration rate of IL-1ß

3.3.2.The effect of treatment with free MTX, NAP and free nanocomposites loaded with therapeutic MTX and NAP on the concentration levels of tumor necrosis factor TNF-α. Based on the current study's findings, white male rats' blood serum concentrations of tumor necrosis factor TNF-α increase significantly (P<0.05) when rheumatoid arthritis is induced by CFA, with sputum concentrations reaching levels of (61.449, 17.460). (Pg/ml in the two positive control groups (G2) for the full and half-duration of the treatment, respectively, in contrast to the concentration level of 72.53, 73.39) Pg/ml in the negative control group G1, for the full and half-duration of the treatment, respectively. The present study's outcomes corroborated those of (33), who reported that complex interactions between blood cells and proteins are encountered by nanoparticles that enter the body through dosing. Blood proteins condense on their surfaces as soon as they enter, and the proteins adsorb to the nanoparticle surfaces to form the protein corona. The biological distribution and therapeutic efficacy of the nanoparticles are determined by these interactions, which also influence the corona type. The immune response here resulted in an enhancement of the secretion of tumor necrosis factor TNF-α and IL-1ß as well, as was seen in the treatment of rats with the Ag nano silver compound. The current study's findings also suggest that TNF-α concentrations will significantly drop in animals given G7 (NAP (G6) MTX) after they have been given a rheumatoid arthritis injection (P<0.05). In comparison to the positive control group (G2), where the average TNF-α concentration reached (89.454) Pg/ml for the average of the two treatment periods as well, the average of these two concentrations for both groups (G7, G6) reached (344.23, 398.15) pg/ml, and this is consistent with the study (34). The present investigation's outcomes aligned with those of (35), who verified the efficacy of hydrogel compounds. Because of their high absorption capacity and excellent control over the release of the medication in inflammatory areas, nanoparticles are regarded as smart therapeutic carriers for naproxen to target biological sites, protecting the joint from the action of inflammatory factors and tumor necrosis factor TNF-α. This is caused by a number of things, one of which is its high compatibility from the large amounts it contains. of water facilitates its acceptance by healthy tissues, which lowers immunological response and facilitates easy bodily breakdown. This vulnerability to the excretory systems lowers the substance's toxicity. A related study (36) found that one of the most significant factors influencing the arrival and distribution of nanoparticles in an organism's body is their palatability, or "Opsonization." This process also attracts protein components such as complement proteins, albumin, lipoproteins, and fibrinogen, which surround the nanoparticles and form the protein corona. This is because the surfaces of the nanoparticles bear antigenic marks that make the Mononuclear Phagocytic System (MPS) vulnerable to attack.



Figure(4-10) The relationship of the duration of treatment with nanocomposites loaded with the treatment on the concentration rate of TNF-α

4. Conclusion

1. Successful loading of MTX and NAP, as well as GN and ER antibodies, and new compounds were obtained,Through the emergence of new diffraction levels for hybrid nanocomposites.
2. A gradual release process occurs within the different media, which indicates the start of treatment with good quantities of compounds
   Agnano and Xerogel nanoparticles due to high and regular release over shorter periods of time, and this is proven by kinetics equations.
   The reaction, the application of the pseudo-second order model, and the percentage of liberation compared to the hybrid compound ZnO, where the liberation was
   Slow down.

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