Preparation and diagnosis of Xerogel nanocomposites And Studying Their Effect on TNF-α Level before and after Loading Dexamethason in Male White Rats Induced Rheumatoid Arthritis

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Abstract

The current study aimed to evaluate the mechanism of reducing the effect of (RA) after it was induced in male white rat with a Complete Freund's Adjuvant (CFA) at TNF-α level by treating Xerogel Nanoparticles and dexamethasone nanocompounded (Xerogel / Dexa) . The free Xerogel nanoparticle had a surface roughness coefficient of 0.327 nm and the total percentage of volumes was 90% less than 130 nm, and the results also indicated that induced rheumatoid arthritis causes Significant increase ($p < 0.05$) in the mean concentration of neoplastic necrosis factor (TNF-α) in the blood serum of male white rats, as they reached a concentration level (10.68 ± 461.80) Pg / ml in the positive control group (G2) for six weeks of treatment compared with the negative control group G1)), as the concentration level in it reached (69.97±0.88) Pg / ml for the same period, and there was also a significant decrease ($P < 0.05$) in the level of concentration of TNF-α among members of the G5 group treated with the Xerogel / Dexa nanoparticle, as this concentration reached (182.98 ± 6.99) Pg / ml compared to the positive control groups (G2) treated with the free Xerogel (G3) and the group treated with free Dexa (G4) for a six-week treatment period in these groups as these concentrations reached (461.80± 10.68, 483.74± 3.59, 300.32± 4.03) Pg / ml, respectively.

Key Word: *Xerogel nanocomposites, TNF-α, Dexamethaon*

Introduction

Rheumatoid arthritis (RA) is one of the most common diseases in the world, with global data reporting that it distributed by more than 1% [1]. It is an autoimmune disease and affects different joint parts of the body and parallel to the right and left sides, RA affects most often in the small joints of the hand as well as foot, knee, and ankle, and thus targets the synovial membrane [2,3] . Research evidence has confirmed there are many biological, environmental, psychological, and personal factors with rheumatoid arthritis [4] There is also no cure for RA yet, but it can be lived a long and active life with rheumatoid arthritis, the treatments available at present are used to reduce the destructive inflammatory effect of the joint and prevent other complications of the disease as well as to maintain the flexibility and movement of the joint and thus reduce pain. Among the most important drugs used in the treatment of arthritis are NSAIDs and modified medicines [5]. Nanotechnology is new approach for treatment RA [6]. The pathological mechanisms were divided into three main stages, the first stage in which some cells are migrated to the tissue of the membrane Synovial membrane and this stage is characterized as non-qualitative and depends on various environmental factors related to rheumatoid arthritis (RA) in the late stages of untreated cases and in this type of infection in many cases ends full recovery in a short or long time or leads to chronic low-intensity inflammation responsible for the second stage of inflammation The tissue of the synovial membrane and cellular oscillation with the development of inflammation and its transmission to the tissue of the meniscus Synovial cartilage is similar to chronic rheumatoid arthritis (RA) but is a non-specific inflammation. The third stage includes stimulating immune proteins by cellular activation

and then developing and spreading the entire synovial membrane tissue inflammation and the destruction of joints. This stage causes the bone cartilage to be directly destroyed, all these manifestations represent the characteristic symptoms of RA [7, 8]. The current study aimed to evaluate the mechanism of reducing the effect (RA) by nanoparticles (Xerogel) after it was induced in male white rat with the material (CFA), through the following axes: 1.Preparation, diagnosis, and, study of some characteristics of the xerogel, which is made of zinc and silver.2. Induction of RA in rats .3. The oral experimentation of animals with the nanobot prepared Xerogel as a processing material.1. Download the treatment of Dexamethason Nanoboat Xerogel.2. Study the effect of xerogel nanocomposite after inducing arthritis TNF-α..

Methods

Design of the experiment : The male white rat was randomly distributed into five groups with animals per group and swallowed orally according to the weight of the rat's body for six weeks and as follows: 1. Group G1: was daily dragged by a solution of vesicle salt and promised a negative control group.2.Group G2: Induced arthritis by injecting Complete Freund's adjuvant-CFA (0.1) ml with the right foot as a positive control.3 . G3: Arthritis was induced and orally tested by Xerogel for 14 days after. 4. G4: Arthritis induced and orally tested by dexamethasone for 14 days. 5.Group G5: Arthritis induced and orally tested by Xerogel/ dex for 14 days.

Preparation of Xerogel nanocomposites: Dissolve 29.7 g of ZnO (NO3) hydro-nitrate nitrate in 167 ml of ethanol.2. Add 2 ml of ethylene clay-cool to the solution (Solution #1).3.4.2 g of silver nitrate (AgNO3) in 100 ml of distilled water 4.(Solution #2).5. Prepare a solution containing 26 ml of distilled water plus 0.6 ml of HNO3 (solution No. 3). 6. Add the ingredients of solution #3 to solution #1 and shake well (Solution #4). 7. Add the components of solution 2 to solution #4.8. Add to the last solution 2g of PVP. 9. Heat the resulting solution at a degree of heat up to 120 °C to vaporize two- thirds of the above solution . 10. The mix was centrifuged and collected after its burned in the oven with a temperature of 400 °C. 11. keep the output until use. [9]

Diagnosis of hybrid nanocomposite: The method used to diagnose hybrid nano -vehicles included the use of Atomic Force Microscope (AFM)

The induction of arthritis: RA induced by The Complete Freund Adjuvant (CFA) (CFA) (Biotechnology, Inc.).Canada Santa Cruz, according to the modus operandi [10]. The development of inflammation performed by injecting 0.1 ml of Complete Freund Adjuvant (which contains the heat-dead bacteria Mycobacterium tuberculosis) in the right foot of the rat's soles. Both weights of animals and The size of the foot are taken before the injection and after 14 days of injection as well as after six weeks of treatment. And the increase in foot thickness and stiffness. Newly developed arthritis is progressed 10-45 days from the start of the injection process [11]. The model for the development of arthritis by CFA has now proposed the development of this chronic infection, which is the characteristic of which is very similar to those resulting in the case of human rheumatoid arthritis [12,13]

After starving the animals for 12 hours, they were weighed and drugged with ether, and blood samples collected 5 ml per animal directly from the heart in a cardiac puncture. Blood samples are putting in 5ml wine syringes in 8 weeks of the development of arthritis and after treatment, the free drug, free nanocomposites, and nano-compound drug, as well as for the negative and positive control group. The samples put in anticoagulant-free tubes and centrifugation at a speed of 3000 rpm for 10 minutes, the serum was divided into clean, sterile appandorf tubes and kept in freezing at -20 m in the laboratory freezer for measuring other functional criteria including tumor necrosis, cytokines, and immune proteins.

Result and Discussion

Diagnosis of xerogel free nanocompound by Atomic Force Microscopie (AFM);

Figure (1) of the Atomic Force Microscope (AFM) indicate that the xerogel free nanoparticles have a surface roughness coefficient of nm of 0.327, but for molecular size ratios of the compound equal to 10% of molecular sizes less than 50.00 nm, and 50% for molecular sizes less than 90.00 nm, the sizes, in general, were 90% less than 130.00 nm.

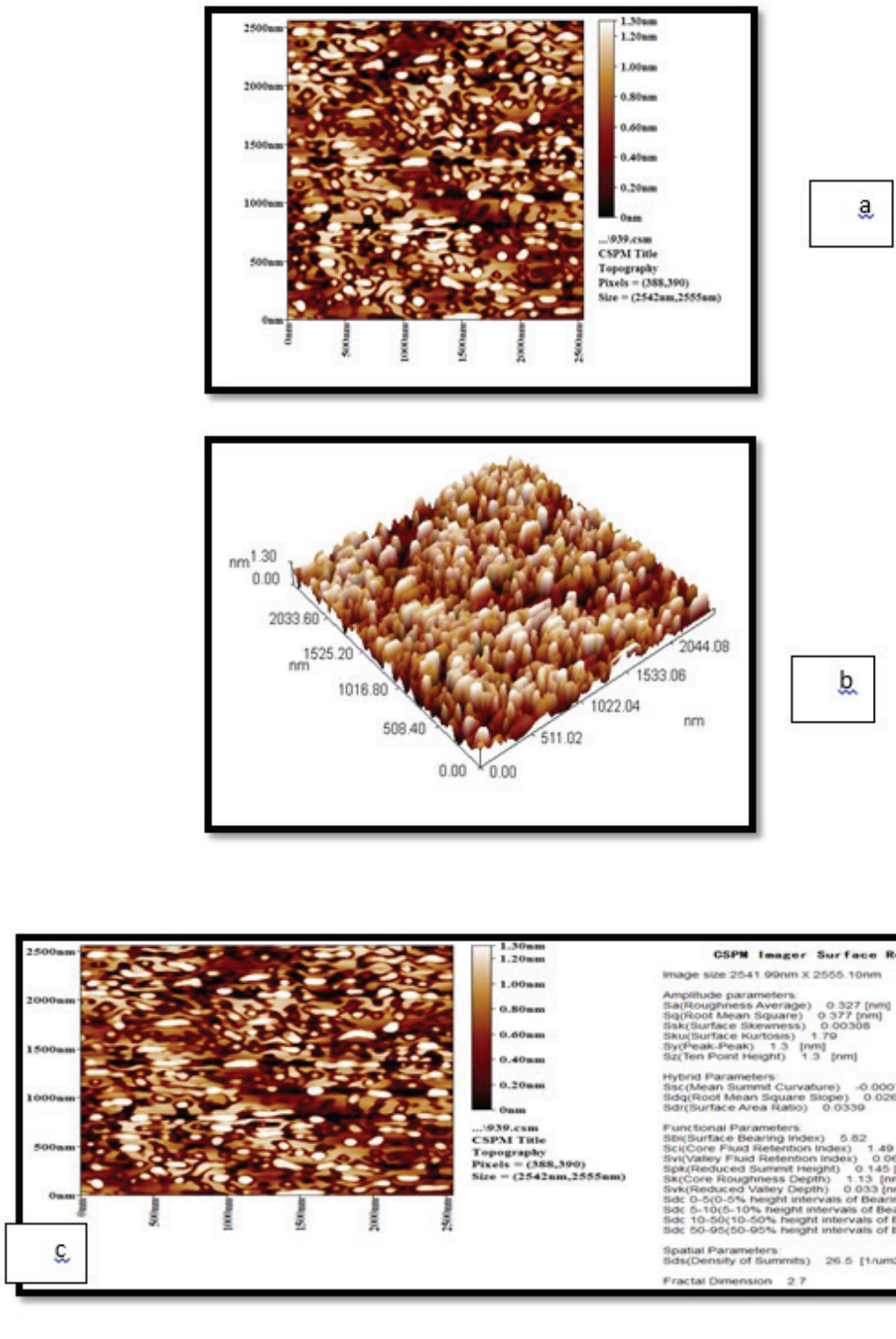

Figure (1) Atomic power microscope images of Xerogel free nanocomposite a/ 3D image, b/ 2D image, c/ 2D image showing all the details of the molecules:

Table (1) Total rate of particle volumes of the Xerogel free nanocompound and the different ratios of those volumes

Avg. Diameter:94.14 nm			<=10% Diameter:50.00 nm					
<=50% Diameter:90.00 nm			<=90% Diameter:130.00 nm					
Diameter(nm)<	**Volum e(%)**	**Cumulati on(%)**	**Diameter(nm)<**	**Volum e(%)**	**Cumulati on(%)**	**Diameter(nm)<**	**Volum e(%)**	**Cumulati on(%)**
25.00	0.38	0.38	80.00	4.51	35.71	135.00	4.14	90.98
30.00	1.13	1.50	85.00	5.64	41.35	140.00	1.88	92.86
35.00	0.75	2.26	90.00	7.52	48.87	145.00	0.38	93.23
40.00	1.13	3.38	95.00	3.76	52.63	150.00	2.26	95.49
45.00	1.50	4.89	100.00	7.14	59.77	155.00	1.13	96.62
50.00	1.50	6.39	105.00	1.50	61.28	160.00	0.38	96.99
55.00	5.26	11.65	110.00	5.64	66.92	165.00	1.13	98.12
60.00	4.14	15.79	115.00	5.26	72.18	175.00	0.75	98.87
65.00	4.89	20.68	120.00	4.89	77.07	180.00	0.38	99.25
70.00	6.02	26.69	125.00	4.89	81.95	185.00	0.38	99.62
75.00	4.51	31.20	130.00	4.89	86.84	200.00	0.38	100.00

Effect of treatment BDexa is free and the free nanocompound charged with Dexa treatment in the concentration levels of tNF-α tumor necrosis factor

The results of the current study table (3-2) show that the induction of reproductive arthritis by CFA causes a moral increase ($p<0.05$) in the concentration of tnf-α in the blood serum of white rat males as they reached a concentration level of 10.05. 68 ±461.80) Pg/ml in positive control group (G2) for six weeks of treatment compared to g1 negative control group with a concentration level (0.88 ±69.97) pg/ml for the same period, this is consistentWith the findings of a study [14] which attributed the reason for the high platelet count is due to the stimulation of the immune system against the invasion of pathogenic microorganisms and it is clear that the infiltration of single cells into the joints of mice induced by arthritis causes the secretion of tumor necrosis factor.

A similar study indicated [15] that opsonization is one of the most important determinants of the arrival and distribution of nanoparticles in the body of the organism and that the stimulation of the most common protein components to surround the nanoparticles and the formation of the protein halo are proteins of the complementary, albumin, and Lipoproteins, fibrinogen, the surfaces of nanoparticles with antigen markers make them vulnerable to attack by the Mononuclear phagocytic system (MPS) blood cells are exposed to certain nanoparticles that may produce cytokines, but the exact mechanisms of cytokine production are not yet known.Unlike traditional antigens lipoproteins polysaccharides, DNA, RNA and which stimulate cytokines after induction of TLRs, it is not known whether nanoparticles are identified by immune cells by immune cells SPECIFIC TLRs, or by other receptors.

The same table also shows a slight increase in tNF-α concentration rates in the G3 group treated with xerogel nanocomposite for six weeks of treatment with rates of 3.59 ±483.74 pg/ml compared to a concentration rate in the Positive Control Group (G2). It was (10.68 ±461.80)

pg/ml, while this increase was moral (p<0.05) compared to the negative control group (G1) at (0.88 ±69.97) Pg/ml.

The results of the current study were in agreement with the findings of(16) noting that nanoparticles that enter the body by The experimentation is subjected to complex reactions of blood cells and proteins and as soon as they enter the condensation of blood proteins on their surfaces and adsorption and proteins on the surfaces of nanoparticles equine corona (protein corona) these interactions determine the biological distribution and therapeutic effectiveness of nanoparticles and contribute to the type of immune resp

The results of the current study in Table (2) indicate that animals induced by rheumatoid arthritis and dexa therapy (G4) will have lower tnf-α concentrationrates (P<0.05) with a concentration rate of 4.03 ±300.32 pg/ml compared to the positive control group (G2) and negative control (G2) for the treatment lukewarm rate of treatment, which is consistent with the rate of tetanus. With The [7] which indicated that the use of cortisone treatments including Dexamethasone has a significant role in inhibiting the production of tnf-α tumor necrosis through its effect on cyclooxygenase and cyclooxygenase enzymes. As a result, the production of TNF-α, which is mainly manufactured by pharyngeal cells during COX-1 activity, inhibits the aggregation of these cells and produces quantities of this compound as well as reduces the production of tumor necrosis through synergistic action with other treatments such as Infliximab (IFX) by reducing the composition of Antibody production of this treatment antibody towards sifx (ATI).

The results of the table (3) show a moral decrease (P <0.05) in the concentration of TNF-α in members of the G5 group treated with the Xerogel/Dexa nanoboat, which amounted to 6.99 ±182.98 pg/ml compared With positive control totals (G2) and free compound therapy xerogel (G3) and the group treated with free Dexa treatment (G4) for a six-week treatment period in these compounds as these concentrations (10.68 ±461.80), 3.59 ±483.74, 4.03 ±300.32) PG/ml respectively.

The results of the study showed an agreement with the study conducted by [17] which indicated that the preparation of a nanocompound of chitosan gel and non-ioncompound (niosomal) pregnant for the treatment of arthritis cortzoni /niosomal) and testing Its effectiveness on healthy volunteers in the treatment of localized psoriasis has shown a low level of tnf and no sensitization or irritation compared to the use of niosomal alone and free treatment.The results of the current study were in agreement with the findings of [18] which indicated that the load of MTX treatment on the nanoboat Theranostic gold (Au) half-shell NPs or Polysialic acid (PSA)-trimethyl chitosan (TMC) NPs (xerogel) stimulates the formation of antibodies to the direction of specific receptors of the pharyngeal cells CD46, and that the load of MTX therapy is much more effective than free treatment in inhibiting the production of TNF-α tumor necrosis factor and eliminating the progress of arthritis significantly in experimental animals.

The results of the current study coincided with the findings of [19], which confirmed that the effectiveness of Hydrogel nano -compounds and their consideration as smart therapeutic carriers for arthritis treatments to targeted biological sites due to their absorption capacity and high control over the release of treatment in areas Inflammation which protects the joint from the action of inflammatory factors and tumor necrosis factor TNF-α due to several factors, compatibility High because it contains large amounts of water help to accept it from natural tissues and this reduces the immune response trend, the ease of cracking it by the body making it vulnerable to the extrusion devices and thereby reducing its toxicity, as well as its ability to carry and maintain treatments until they reach the target tissues, and also the ability to escape from the ventricle retina and reach the target tissue and organize the liberalization of treatment in them.

Table (3): TNF.& pg/ml cytokine concentrations before and after b treatmentDexa Free and Xerogel Free Nanoboat and Loaded With Xerogel/Dexa Treatment

Average concentration ±TNF.& Standard Line (pg/ml)	Treatment
0.88 ± 69.97 a	Nagtive control(G1)
10.68 ± 461.80 b	Positive control (G2)
3.59 ± 483.74 b	(G3) Xerogel
4.03 ± 300.32 c	Dexa (G4)
6.99 ± 182.98 d	Xerogel /Dexa (G5)

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Conflict of interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kerbala University and all experiments were carried out in accordance with approved guidelines.

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