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RESEARCH PAPER

Synthesis of Nanoscale Xerogel/MTX and Study Its Effects on the Liver and Kidney Tissue and Level of IgG in Rats with Rheumatoid Arthritis

Naseer Marza Hamza, Kiaser Abdulsajjad M. Hussain, and Alaa Hussein Al-Safy*

Department of Biology, College of Education for pure, University of Kerbala, Iraq

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ABSTRACT

The current study aims to reveal the therapeutic effect of Xerogel nanocomposite prepared and diagnosed on liver and kidney tissues and the level of rheumatoid factor(RF) concentration in rats induced by Complete Freund Adjuvant(CFA) with rheumatoid arthritis(RA). Twenty male albino rats were randomly divided into four groups, each group containing 5 rats treated for 40 days: group 1 the negative control group, group 2 in which rheumatoid arthritis(RA) was induced by(CAF) by injecting 0.5 ml of the substance into the sole of the foot. Right was a positive control group, group 3: rats dosed with methotrexate (MTX) 125 mg/250g, group 4: rats dosed Xerogle/MTX 0.125mg/250 mg, liver and kidney parts were stained with hematoxylin-eosin and the immune cytokines of rheumatoid factor(RF) immunoglobulin protein IgG were measured. The results of the current study showed that the induction of rheumatoid arthritis(RA) by CAF led to changes in the tissues of the liver and kidneys through necrosis and hemorrhage in their tissues, as well as an increase in the level of rheumatoid factor (G2) concentration, and that treatment with (MTX) 125 mg / 250 g (G3) led to restoration The damages that occurred after the induction of rheumatoid arthritis and reduced this rise significantly. Also, treatment with the nanocomposite Xerogel/ MTX 0.125 mg / 250 g (G4) also led to a significant reduction in the level of IgG concentration in albino rats by reducing damage and inflammation in liver and kidney cells. The study indicated that treatment with Xerogel nanocomposite loaded with MTX increases the protective effect against the harmful effect of CFA-induced rheumatoid arthritis(RA) in liver and kidney tissue and lowers the immune parameters represented by rheumatoid factor(RF) IgG.

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INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common diseases in the world, with global data reported to have a distribution of more than 1% [1]. It is characterized by being an autoimmune disease that affects different parts of the joints of the body in parallel on the right and left sides [2]. RA most often affects the small joints of the hand as well as the foot, knee and ankle, targeting the synovial membrane) (which is two or three layers of epithelial cells that contain inside Synovial fluid, as this membrane in the affected joints is thicker

* *Corresponding Author Email: naser.m@uokerbala.edu.iq*

with a large accumulation of inflammatory cells (T-lymphocytes, macrophages), which leads to its damage [3]. Research evidence confirms the existence of a close association of many biological, environmental, psychological and personal factors with rheumatoid arthritis [4]. However, the treatments available at the present time are used to reduce the destructive inflammatory effect of the joint and prevent other complications of the disease as well as maintain the flexibility and movement of the joint and thus reduce pain. Among the most important medications used in the treatment of arthritis are non-steroidal anti-inflammatory drugs (NSAIDs) and Disease-modifying antirheumatic drug (DMARDs) [5]. Nanotechnology small size and large area of the surface make the nanoparticles applicable to all aspects of different life applications [6]. NPs have a high effectiveness compared to the bulk scale that enables them to participate in applications in vivo and in ivtro , which can enter the bloodstream and pass the organs (including the brain, heart and kidneys), these molecules are like nanocarriers to decrease of drug toxicity, the ZnO-chitosan-folate system is an example of the nanotransmitters supplying doxorubicin (DOX), which is one of the drugs used in the treatment of rheumatoid arthritis (RA) and antineoplastic agent used in the treatment of cancer [7] Nps also have specific drug loading and an effective photoluminescence capability for selective delivery of anti-cancer drugs where DNA can theoretically be damaged by the action of Nps via single oxygen production, Nps may also induce cytotoxicity against cancer cells by means of apoptotic induction, suggesting the design of chemical modifications of CuO NPs to produce active molecules to interact with more macromolecules [8,9]. The rate of drug release from the Nanocarrier depends on many factors including pH, particle size, surface properties, rate of degradation, intensity of surface interaction between drugs and NPs [10]. Many drug (DMARDs) have been prescribed some separately and others mixed , such as Methotrexate (MTX) Amethopterin,4-amino 4-deoxy-N10-methylpteroyl-acid is an antagonist of Folate. MTX targets Dihydrofolate reductase (DHFR) or RFC-Reduced folate carrier (the RFC was observed to have a greater MTX affinity than follic acid and vice versa) [11]. MTX has an effect on cytokines, as studies have shown by study Immunoglobulin biopsies suggest that concentrations of lL-1ß and TNF-α decline, mononuclear cell production is improved in the bone marrow after the use of MTX as a therapy for rheumatoid arthritis patients [12]. In vitro studies yet there is an immediate need for more effective drugs with fewer side effects [13]. Animal models have been used to research the causes and mechanisms of rheumatoid arthritis and in the pharmaceutical industry, as animal models are characterized by the ease of developing the disease in them in short periods, as well as the presence of great similarity between them and humans, as studies confirmed that the development of the disease incidence of this disease leads to a severe effect that ends Destruction and erosion of the joint after two weeks. The disease was induced by injecting 0.1 ml of Complete Freund Adjuvant (CFA) in the right foot of the rat's Achmad region [14].

MATERIALS AND METHODS

Nanoparticles prepared

Analyzed NPs were obtained via sol-gel processes using the oxidation reduction of cations (Ag and Zn). During formulation of NPs the functionalization can be implemented using Ethylene glycol(EG). Through activating functional groups on NP surfaces and subsequent conjugation with ligands (MTX), as a reducer and stabilizer agent in 70 % ethanol after NP formation. At the resulting Xerogel surface [14].

Chemical Materials

Silver nitrate(41mg / ml) and zinc anitrate hexahydrate (70mg / ml), 25ml ethanol 95%, and 5ml ethylene glycol(from BDH)(England), sodium carbonate(from merk germany). BDH-Methotrexate (MTX).

Diagnosis of nanoparticle

Nanocomposite Xerogel /MTX test approaches include the Atomic Force Microscope (AFM).

Experimental and animal design

Twenty- five healthy young male Wistar Albino rat used in this study, weighing between 200-250 g and their age (10-12 weeks), the infection was developed by injecting 0.1 ml of Complete Freund Adjuvant (CFA) containing Mycobacterium tuberculosis in the right foot of the rat's Achmad region. The white rat males are assigned randomly to each group and are fed orally according to the rat body weight for the first six weeks after 14 days of injection for each study group [14].

Group 1 (G1): Is daily dose with a saline physiological solution and is considered as a negative control group.

Group 2 (G2): Is CFA injection and represents a positive control group for six week .

Group 3 (G3): Is CFA injection and represents a positive control group and treated with MTX 125 mg /250 g in body weight/ 2 time in 0.5 ml for six week .

Group 4 (G4): Arthritis develops and is orally

treated after 14 days of development by 3. 2 mg / Nanoparticular Xerogel 0.125 mg MTX(Xerogel/ MTX) /250 g in body weight/ in 0.5 ml for 6 week.

RESULTS AND DISCUSSION

Atomic Force Microscope (AFM)

The results of image of the atomic force microscopy (AFM) (Fig. 1) indicate that the free nanocomposite Xerogel has a surface roughness modulus of 0.327 nm, but in the case of loading the MTX 0.488 nm treatment and the difference between the surface roughness of the Xerogel before and after loading the MTX treatment. (Fig. 2) nm equals 0.161. As for the ratios of molecular sizes, a table 1 for the compound, it equals 10% for molecular sizes less than 50.00 nm, and 50% was for molecular sizes less than 90.00 nm, while the sizes in general were 90% less than 130.00 nm, as The proportions of molecular sizes after loading the MTX treatment in Table 2 were 10% for less than 60.00 nm and 50% for less than 90.00 nm, and the sizes were 90% under 140.00 nm.

Effect of rheumatoid arthritis treatment on liver tissue

The results of the study show an Fig. 3 a cross-section of the liver tissue of white male rats, the negative control group, showing that it is composed of lobules, each containing a central vein surrounded by bands of cubic-shaped cells, hepatocytes, and between these bands there

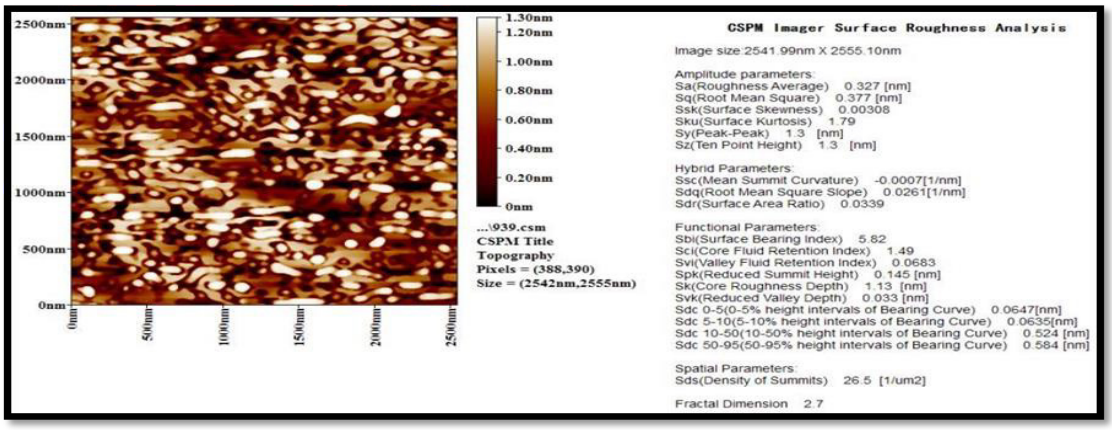


Fig. 1. Atomic force microscopy images of the free two-dimensional Xerogel nanocomposite showing all the details of the molecules.

Table 1. The average total particle sizes of the free Xerogel nanocomposite and the different proportions of those sizes.

Avg. Diameter:94.14 nm			<=10% Diameter:50.00 nm					
<=50% Diameter:90.00 nm			<=90% Diameter:130.00 nm					
Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
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

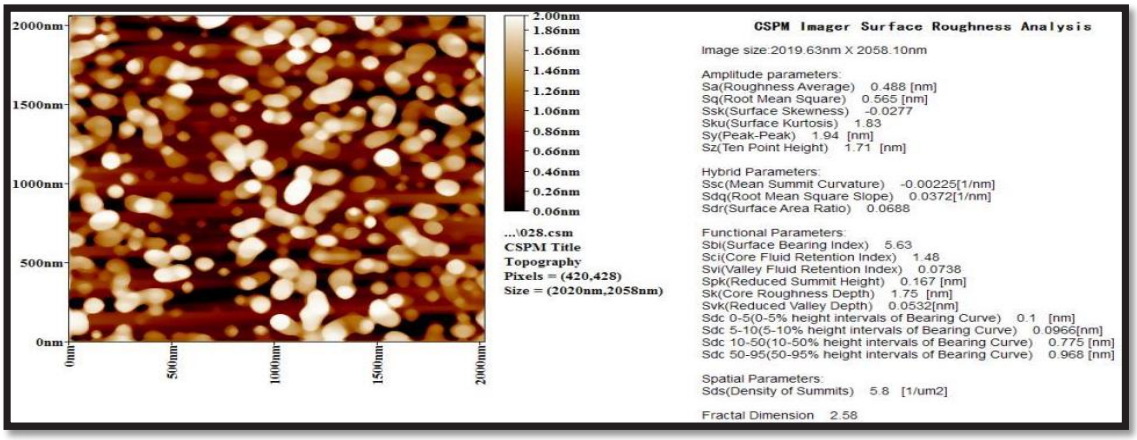
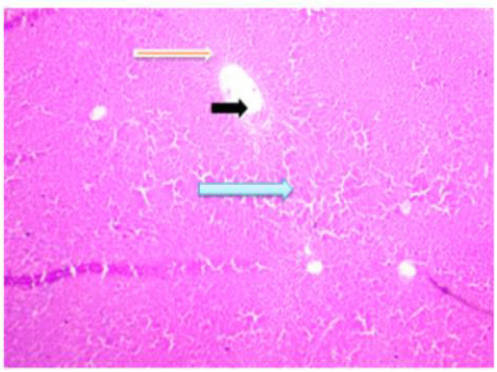


Fig. 2. Atomic force microscopy images of the Xerogel/MTX nanocomposite A two-dimensional image showing all the details of the molecules.

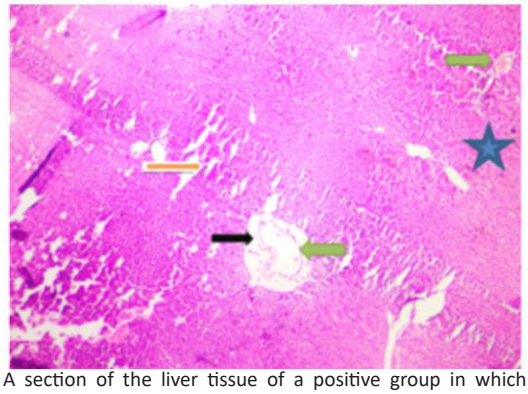
Table 2. The average total particle sizes of the free Xerogel/MTX nanocomposite and the different proportions of those sizes.

Avg. Diameter:100.38 nm					<=10% Diameter:60.00 nm			
<=50% Diameter:90.00 nm					<=90% Diameter:140.00 nm			
Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
60.00	1.21	1.21	120.00	10.12	76.52	180.00	0.40	98.38
70.00	16.19	17.41	130.00	6.88	83.40	190.00	0.40	98.79
80.00	14.57	31.98	140.00	4.05	87.45	200.00	0.40	99.19
90.00	12.15	44.13	150.00	6.07	93.52	210.00	0.40	99.60
100.00	13.36	57.49	160.00	2.43	95.95	270.00	0.40	100.00
110.00	8.91	66.40	170.00	2.02	97.98			

are blood spaces called sinusoids. Fig. 4 shows a cross-section of the liver tissue of white male rats induced by arthritis for a period of (14 days), and treated with MTX treatment, as it was noted in



A section of the liver tissue of the negative control group, in which it is noted that the central vein (black arrow) is surrounded by bands of hepatocytes (white arrow), between which there are blood spaces (sinusoids) (blue arrow) X 10.



A section of the liver tissue of a positive group in which arthritis developed for 14 days and was treated with MTX treatment, in which congestion of the expansion of the central vein (black and green arrows) with areas of bleeding (blue star) and the presence of necrosis in the hepatocytes (red arrow), between which there are blood spaces (sinusoids) is observed (blue arrow) X 4 .

several areas of the liver lobules blood congestion in the central veins and irregular sinusoids as well as necrosis of some hepatocytes and thickening of their nuclei. Pyknosis compared with the s-negative control group as Fig. 3. Fig. 5 shows a cross-section of the liver of male white rats induced with arthritis, as it was observed in several areas after treatment with Xerogle/MTX. It is noted that the tissue is little damaged with some areas of bleeding and the disappearance of central vein congestion with the presence of some damaged cells compared to the group Individuals treated with free MTX (Fig. 4). The results of the current study showed that the induction of rheumatoid arthritis in the white male rats led to changes in the liver tissue of the white male rats induced by rheumatoid arthritis compared with the negative control group. This agreed with the results of the study [15,16]. It is due to the arrival of the inducible substance to the liver through the bloodstream after being injected under the skin, and the pathological changes in the liver tissues have also been attributed to the role of lysosomal enzymes in animals induced with rheumatoid arthritis. The administration of Xerogle/MTX compound to the infected rats, the treated groups showed slight pathological changes in them and this is due to the presence of antioxidants, as Xerogle has a therapeutic effect against oxidative damage and apoptosis through its antioxidant properties as it contains

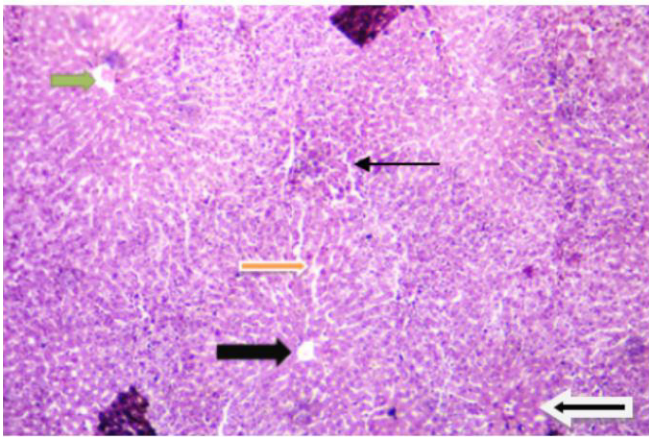


Fig. 5. Cross-section of the liver tissue of the group treated with MTX/ Xerogel compound, as it is noted that the tissue is closer to normal (red arrow) with the presence of some areas of bleeding (white arrow) and the disappearance of central vein congestion (green and black arrows) with the presence of some damaged cells (low black arrow) X 4.

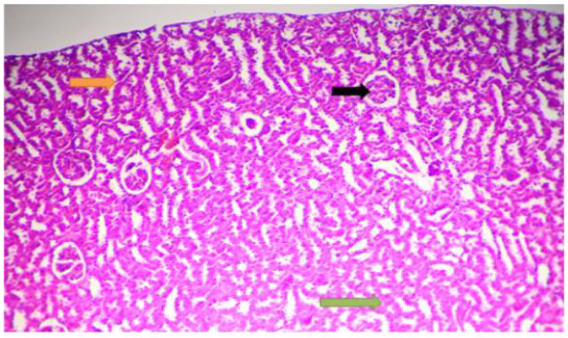


Fig. 6. Cross-section of the kidney tissue of the negative control group, showing the cortex (red arrow) region containing the renal glomeruli (black arrow) and the medulla region (green arrow) 10 X

in its composition ZnO and Ag compounds that It prevents the generation of free radicals and thus has an effect in protecting the liver from damage, or the reason for the decrease may be due to the effect of the active substances in Xerogle on the mitochondria [17], and treatment with Xerogle/MTX has caused minor changes and that the tissue is closer to normal in the liver cells, as it showed an improvement In the liver cells of animals after causing necrosis of hepatocytes, expansion of the sinusoids and infiltration of inflammatory cells due to the increase in the effectiveness of controlling the release of MTX treatment and its arrival in sufficient concentrations to the target and without affecting the other organs because they are not in large concentrations and the liver and kidneys do not have to get rid of them through metabolism in the liver and excretion by the kidney [14].

Effect of rheumatoid arthritis treatment on kidney tissue

It is noted from the (Fig. 6) a cross-section of the kidney tissue of the white male rats in the negative control group, in which a number of nephrons containing a Malpighian globule consisting of Bowman's capsule and glomerulus are observed. (Fig. 7) shows a cross-section of the kidney tissue of white male rats induced by arthritis for a period of (14 days) in which renal glomeruli enlargement due to inflammation and destruction of the epithelium of the renal tubules appears despite treatment with MTX compared to the negative control group shown in the (Fig. 6). Fig. 8 shows a cross-section of the kidney tissue of white male rats induced by arthritis. It was observed in multiple areas after treatment with MTX loaded on Xerogle in the Xerogle/MTX compound for a period of six weeks, degenerative changes with necrosis of some tubule cells and simple hemorrhage compared with the control group (Fig. 6). The results of the current study showed that the induction of rheumatoid arthritis in white male rats (the second group) led to changes in the tissues of the kidneys of white male

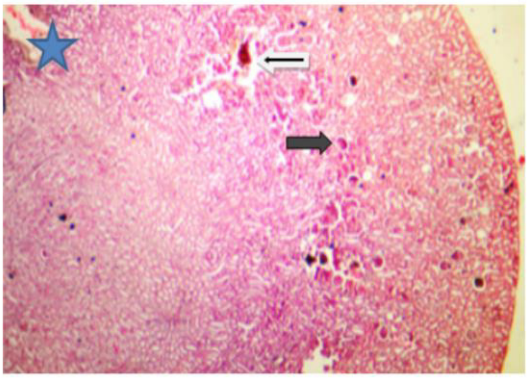


Fig. 7. Cross-section of the kidney tissue of a positive group treated with MTX, showing enlargement of the renal glomeruli (black arrow) and destruction of the epithelium of the renal tubules (white arrow) with bleeding (blue star) 4X.

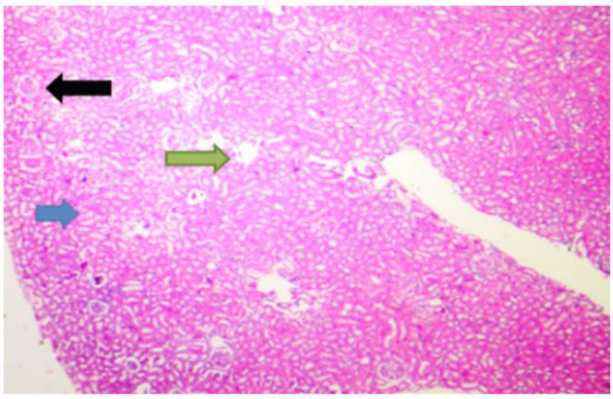


Fig. 8. Cross-section of the kidney tissue of the group treated with Xerogle/MTX, in which glomerulonephritis appears slightly (black arrow), slight damage to the epithelium of the renal tubules (green arrow) and the disappearance of bleeding (blue arrow) X4

rats induced by arthritis despite being treated with MTX treatment and the damage continued when comparing its members with the members of the negative control group and that the reason for these changes In the kidney tissue, it results from the arrival of CFA to the kidney through the bloodstream or it may be the result of arthritis, as it leads to glomerulonephritis [18]. The treatment with Xerogle/MTX caused an improvement in the kidney tissue of the affected animals, as the Xerogle/MTX started an anti-toxic action. This therapeutic effect is probably due to the fact that the Xerogle contains quantities of substances that protect the kidneys from toxic substances and regulate its work [19]. The results agreed with the findings of the study which showed that zinc oxide compound treatment increased kidney parameters (uric acid, creatinine, and urea) in male mice after 8, 15, and 30 days post injection, including accumulation of inflammation cells in glomerular capillaries and degeneration of proximal and distal tubules. It appears that ZnO NPs have a short-term effect on renal function, which disappears after a month due to the gradual elimination of nanoparticle uptake into the kidney.

Effect of rheumatoid arthritis treatment on rheumatoid factor (RF)

The results of Table (3) show that the animals induced with arthritis by CFA substance (G2) had a significant increase in serum IgG ($P<0.05$) after infection for a period of six weeks, where the average concentration was recorded at 3027.000 mg/dl compared with the group Negative control animals (G1) in which the average concentration of IgG was recorded (700.250) ml/dl. While treatment with Xerogel nanocomposite loaded with MTX treatment, the subject of the study, led to a significant decrease ($P<0.05$) in the level of IgG concentration in the serum, as it reached its concentration level in the G3 groups for 6 weeks (1732.000) ml/dl. The results of the current study agreed with the findings of [20] who indicated that nanoparticles that enter the body through dosing are subjected to complex interactions of blood cells and proteins, and upon entering the blood proteins condense on their surfaces, and adsorption of proteins on the surfaces of nanoparticles is Corona (protein corona) and these interactions determine the biological distribution and therapeutic efficacy of the nanoparticles and also contribute to the type of immune response to the secretion of immune proteins [21], and partial modification (15%) of the positively charged envelope of the crosslinked knedel-like nanoparticles by its binding with histamine (instead of the primary amine Group) significantly reduces the immunotoxicity of these nanoparticles, probably due to the charge reduction (due to reduced amino content) shown by a relatively low zeta potential (12-15%) [20]. The results of the current study are in agreement with the findings of [14] which confirmed that the effectiveness of nanohydrogel compounds and their consideration as smart therapeutic carriers for arthritis treatments to the targeted biological sites due to their absorption capacity and their high control of the release of treatment in areas of inflammation, which protects the joint from the action of immune proteins and necrosis factor. The reason for this is due to several factors, including high compatibility because it contains large amounts of water that helps to accept it from normal tissues and this reduces the immune response in a direction, ease of breaking it by the body, which makes it vulnerable to excretory systems and thus reducing its toxicity, as well as

Table 3. Concentrations of immunoglobulin IgG mg/dl before and after treatment with the nanocomposite Xerogel / MTX.

Avg. Diameter:100.38 nm			<=10% Diameter:60.00 nm					
<=50% Diameter:90.00 nm			<=90% Diameter:140.00 nm					
Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
60.00	1.21	1.21	120.00	10.12	76.52	180.00	0.40	98.38
70.00	16.19	17.41	130.00	6.88	83.40	190.00	0.40	98.79
80.00	14.57	31.98	140.00	4.05	87.45	200.00	0.40	99.19
90.00	12.15	44.13	150.00	6.07	93.52	210.00	0.40	99.60
100.00	13.36	57.49	160.00	2.43	95.95	270.00	0.40	100.00
110.00	8.91	66.40	170.00	2.02	97.98			

its ability to Carrying treatments and maintaining them until they reach the target tissues, and also their ability to escape from the retinal endothelial system and reach the target tissues and organize the release of treatment in them [22].

CONCLUSION

According to the findings of the present study, treatment with Xerogel nanocomposite loaded with MTX increases the protective effect against the harmful effect of CFA-induced rheumatoid arthritis(RA) in liver and kidney tissue and lowers the immune parameters represented by rheumatoid factor(RF) IgG.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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