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Ameliorative Effect of Curcumin and Curcumin Nanoparticles against CFA-induced Arthritis in Adult Male Wistar Rats

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Abstract

This research was accomplished to clarify the effect of curcumin (Cur) and curcumin nanoparticles (Cur-NPs) on rheumatoid arthritis (RA).48 male rats were divided randomly into group (I): control group (II): Cur group; group (III): Cur-NPs group; group IV: RA group; group V: Cur Treated (Tr) group; group VI: Cur-NPs Tr group; group VII: Cur Protective (Pr) group; group VIII: Cur-NPs Pr group; There was significant increase in serum MDA, RF, Anti-CCP, IL-6, and TNF- α levels and a greatly marked drop in levels of total antioxidant (TAC) in the rheumatoid arthritis (RA) group in comparison to control group with improvement their levels in groups(V to VIII). In conclusion, our results showed that the administration of Cur and Cur-Nps provided antioxidant and anti-arthritic effects against the toxicity of CFA. The histological and x-ray findings confirm the biochemical observations.

Keywords: Curcumin (Cur); Curcumin nanoparticles (Cur-Nps); Rheumatoid arthritis (RA).

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder resulting from a mix of genetic, epigenetic, non-genetic, and environmental influences. RA attacks cartilage and bone, causing joint damage and dysfunction **[1].**In 2020, the disease's existence in Egypt was about 5% **[2].** In recent decades, there has been an increase in the prevalence of RA and the disability-adjusted life years that go along with it**[3]**. Curcuminoids are low-molecular-weight bioactive agents that occur naturally. They have been used for a long time as food preservatives, particularly in Southeast Asian countries **[4,5]**, culinary spices, and coloring agents in beverage manufacturing **[6,7].** Three main components make up curcuminoids, which are polyphenols: Cur (77%), desmethoxycurcumin (17%), and bisdemethoxycurcumin (3%) **[8,9].**Curcuminis the most important to health and the most biologically active element **[10,11]**. It is a drug for treating RA because of its anti-inflammatory and antioxidant effects **[4,12,13].** Curcumin has been demonstrated in several trials to be effective as a medication for treating a range of illnesses **[14,15].** It is a therapeutic drug with many advantageous functions, but its most significant aspect is that it has no adverse effects, but has low bioavailability and low water solubility.Nanoparticles (Nps) are a helpful method to get over these problems and increase the bioavailability of these items because most natural compounds have poor absorption, rapid metabolism, and excretion **[16,,17,18,19].** Large surface area, regulated particle size, precise placement, stability, bioavailability, biodegradability, and controlled drug release are among the benefits of employing Nps**[20].**Cur or Cur-Nps show a wide range of physiological and pharmacological actions, such as antioxidant, antiangiogenic, anticancer, antiviral, antifungal, and antibacterial agents **[21];** neuroprotective, antidiabetic **[22];** antineoplastic, immune-modulating, and neuroprotective impact **[23-26].** The present study aimed to improve the anti-inflammatory and antioxidant effects of Curcumin and curcumin-nanoparticles against rheumatoid arthritis.

2. Materials and methods

2.1. Animal groups

Forty-eight male Wistar rats weighing from 110 to 130 g were obtained from the Faculty of Agriculture, Alexandria University (Alexandria, Egypt). Rats were housed under a 12:12 h light-dark cycle at a controlled temperature of 20–22 °C

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and maintained for a 7-day acclimatization period with a pellet diet and drinking water *ad libitum.* Rats were divided into eight groups, 6 rats in each group. **Group I (control group), Group II (Cur group)**: rats were administered orally with curcumin (200 mg/kg /day) for 4 weeks **[27]**. **Group III (Cur-Nps group)**: rats were administered orally with curcumin nanoparticles (15 mg/kg /day) for 4 weeks **[28]**. **Group IV (Rheumatoid Arthritis group" RA"):** rats were injected with a single subcutaneous injection of 0.1 mL of CFA into the subplantar region of the left hind foot paw to induce arthritis **[29]**. **Group V (Curcumin treated group" Cur Tr") & Group VI (Cur-Nps treated group " Cur-NpsTr"):** rats were injected with a single subcutaneous injection of 0.1 mL of CFA into the sub-plantar region of the left hind foot paw to inducearthritis.Afterone day rats were administered orally with Cur and/or Cur-Nps for 4 weeks. **Group VII (Curcumin protective group" Cur Pr") & Group VIII (Cur-Nps protective group "Cur-NpsPr"):** rats were administered with Cur and/or Cur-Nps orally for 2 weeks and after that rats were injected with a single subcutaneous injection of 0.1 mL of CFA into the sub-plantar region of the left hind foot paw to induce arthritis then rats were administered with Cur and/or Cur-Nps during the subsequent period.

❖ **Ethical statement**

The animal study was done after receiving approval from the Animal Ethics Committee of Fayoum University, Egypt, with approval number AEC2352. The experiments were sustained by the guidelines given by the faculty of science.

2.2. Sample collection

After the experiment ended, the animals fasted before being sacrificed under anesthesia using ether (5g/Kg by inhalation). Blood samples were collected in two tubes; the first one contained EDTA to determine hematological measurements. In the second tube, blood was allowed to remain for 15 minutes at 37ºc, then centrifuged at 4000 rpm for 20 minutes for the separation of serum. The serum was removed and preserved in plastic vials at -20ºc until used for further biochemical analyses. Rat foot tissue was extracted right away, and some of the tissue was presser ved for histological examination in a 10% buffered formalin solution.

2.3. Measurement of Body Weight (BW) Changes

Change of body weight was measured from the first day to the end of the experiment every 7 days.

2.4. Hematological Analysis

Blood samples were collected from rats for the evaluation of Hb content, total RBC, total WBC count, and measurement of HCT. For the estimation of Hbcontent, blood was diluted with Drabkin's reagent and measured in a spectrophotometer. Blood cells were suspended in RBC and WBC-diluting fluid and counted in a hemocytometer under a light microscope.

2.5. Biochemical analyses

2.5.1. Liver function biomarker assays

AST and ALT were evaluated by the colorimetric method using a commercial kit from Diamond Diagnostics, Egypt, according to the method explained by **Young [30]**.

2.5.2. Kidney function biomarker assays

Urea and Creatinine were evaluated according to the method of **Young [30]& Larsen [31]**.

2.5.3. Oxidative stress biomarkers assays

Lipid peroxidation was evaluated by measuring MDA levels according to the method of **Martinez [32]**. Total antioxidant capacity was estimated according to the method of **Koracevic[33]**.

2.5.4. Anti-inflammatory biomarkers assays

Serum Rheumatoid Factor (RF), serum TNF-α, serum IL-6, and serum anti-cyclic citrullinated peptide antibody (Anti-CCP) were measured using sandwich ELISA kits and ESR assay using the Westergren method.

2.5.5. Histopathological examination

The rat ankle joints were harvested immediately after being humanely sacrificed and fixed in 10 % buffered formalin solution. Decalcified in 10% formic acid for 21 days, embedded in paraffin, sliced into solid sections of 3–5 µm thickness, and stained with hematoxylin and eosin (H&E) for general evaluation of cartilage damage. Slid pictures were captured using an Olympus Magnus microscope camera, and processed by Olympus MagVision image analysis software, samples taken from rats **[34].**

2.6. Statistical analysis

The analysis was done with Microsoft Excel (version 10) and the Statistical Package for the Social Sciences (SPSS software version 16) on a personal computer. All values are expressed as mean ± standard deviation (SD) **[35]**.

3. Results

3.1.Changes in body weight

At the end of the experiment, all studied groups showed some increases in their body weights if compared tothe beginning of the experiment except the RA group which showed a highly significant decrease in body weight in comparison with the control group ($p < 0.001$) as shown in **Table (1).**

3.2.Hematological analysis

Our results of hematological analysis showed a highly significant decrease in Hb content, RBCs count, and HCT% and a highly significant increase in platelet and WBCs count in the RA group (Group IV) and all protective & treated groups (group V to VIII) when compared with the control group (P<0.001). In comparison with the RA group. Group V to group VIII showed a highly significant decrease in platelet and WBCs count and a highly significant increase in Hb, RBCs, and Hematocrit% (P<0.001) as shown in **table (2).**

3.3. Biochemical analysis

Our results showed a highly significant rise in serum ALT, AST, Urea, Creatinine activity, ESR, RF, Anti-CCP, TNF-α, and IL-6 in the RA group in comparison with the control group (P<0.001). Also, there was a highly significant increase fromgroup V to group VIIIwhen compared to the control group (P<0.001). On the other hand, there was a highly significant decrease in these parameters from group V to group VIIIwhen compared to group IV (P<0.001). The serum level of MDA was significantly increased in groupIV as compared with the control group. There was a significant decrease from group V to group VIII in comparison with the RA group.There was a highly significant decrease in serum TAC in treated and protective groups when compared with the control group (P<0.001). In treated and protective group, there was a highly significant rise when compared with the RA group, as shown in **tables (3&4).**

Table (1): Mean ± SD of body weight (g) at the start and end of the experiment in different groups**.**

	Mean± SD	Mean± SD	% of change	$P^{(a)}$ value	$P^{(b)}$ value
	At the start	At the end			
Group I (control)	136±3.22	172±1.94	26.47↑		
Group II (Cur)	133±1.72	159±1.47	19.55↑	*< 0.001*	
Group III (Cur-Nps)	135±2.31	160±1.47	18.52↑	*< 0.001*	
Group IV (RA)	133±3.25	119±1.47	10.526↓	*< 0.001*	
Group V (Cur Tr)	135±2.13	145±2.92	7.41 ↑	*< 0.001*	*< 0.001*
Group VI (Cur-Nps)	136±1.96	147±4.4	8.088 ↑	*< 0.001*	*< 0.001*
Group VII (Cur Pr)	133±2.42	151±5.19	13.53 ↑	*< 0.001*	*< 0.001*
Group VIII (Cur-Nps)	133±1.67	156±4.57	17.29↑	*< 0.001*	*< 0.001*

$P^{(a)}$ value versus control group $P^{(b)}$ value versus RA group

P> 0.05 is non-significant $P \leq 0.05$ is significant *P* < 0.001 is Highly significant.

Table (2): Mean ± S.D. of RBCs, Hb, WBCs, platelets, and HCT% in the different groups**.**

	RBCs ($x10^6$/uL)	Hb (g/dL)	WBCs ($x10^3$/uL)	Platelet ($x10^3$/uL)	HCT (%)
Group I (control)	5.81±0.14	15.41±0.36	7.66±0.38	282±9.35	43.43±1.05
Group II (Cur)	5.61±0.22	14.53±0.20	8.05±0.18	283±9.83	38.78±2.25
Group III (Cur-Nps)	5.78±0.29	14.78±0.36	8.23±0.23	275±11.58	40.05±0.65
Group IV (RA)	3.28±0.17 [a]	9.73±0.25 [a]	18.8±0.54 [a]	559±8.01 [a]	26.8±2.02 [a]
Group V (Cur Tr)	4.16±0.22 [a b]	12.55±0.16 [a b]	14.61±0.23 [a b]	431±14.37 [a b]	33.56±0.27 [a b]
Group VI (Cur-Nps)	4.4±0.16 [a b]	12.78±0.31 [a b]	13.18±0.25 [a b]	340±8.61 [a b]	34.18±0.19 [a b]
Group VII (Cur Pr)	4.5±0.20 [a b]	13.41±0.27 [a b]	11.83±0.12 [a b]	328±9.83 [a b]	36.75±0.42 [a b]
Group VIII (Cur-Nps)	4.66±0.19 [a b]	13.81±0.20 [a b]	10.25±0.18 [a b]	301±7.52 [a b]	37.38±0.32 [a b]

P [(a)] value versus control group *P* [(b)] value versus RA group

P> 0.05 is non-significant *P ≤ 0.05* is significant *P* < 0.001 is highly significant.

Table (3): Mean ± S.D. of ALT, AST, Urea, creatinine, MDA, and TAC in the different groups**.**

	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)	MDA (nmol/ml)	TAC (Mm/L)
Group I (control)	33.5±0.83	15.5±1.04	16.15±0.71	0.573±0.013	4.15±0.09	0.44±0.01
Group II (Cur)	34.5±0.83	15.1±0.75	18.87±0.51 [a]	0.581±0.046	3.5±0.09	0.45±0.02
Group III (Cur-Nps)	34±0.63	15.1±0.75	17.76±1.78	0.588±0.066	3.38±0.07 [a]	0.46±0.03
Group IV (RA)	52.83±0.98 [a]	33.5±1.04 [a]	43.9±2.16 [a]	1.27±0.03 [a]	14.16±0.66 [a]	0.20±0.01 [a]
Group V (Cur Tr)	45.33±1.03 [a b]	27.16±0.72 [a b]	34.58±1.28 [a b]	0.980±0.008 [a b]	10.38±0.23 [a b]	0.30±0.01 [a b]
Group VI (Cur-Nps)	43±0.63 [a b]	25±0.63 [a b]	31.92±0.80 [a b]	0.818±0.011 [a b]	9.45±0.18 [a b]	0.33±0.01 [a b]
Group VII (Cur Pr)	40.5±0.83 [a b]	23±0.63 [a b]	29.44±1.05 [a b]	0.788±0.007 [a b]	8.47±0.15 [a b]	0.35±0.01 [a b]
Group VIII (Cur-Nps)	38.83±0.75 [a b]	20.83±0.75 [a b]	26.6±0.40 [a b]	0.758±0.019 [a b]	7.15±0.11 [a b]	0.38±0.01 [a b]

P [(a)] value versus control group *P* [(b)] value versus RA group

P> 0.05 is non-significant *P ≤ 0.05* is significant *P* < 0.001 is highly significant.

Table (4): Mean ± S.D. of RF, Anti-CCP, ESR,TNF-α, and IL-6 in the different groups**.**

	RF (IU/ml)	Anti-CCP (IU/ml)	ESR (mm/hr)	TNF-α (ng/L)	IL-6 (ng/L)
Group I (control)	8.33±0.88	2.59±0.30	3.45±0.17	56.36±0.30	3.33±0.05
Group II (Cur)	8.44±0.09	3.54±0.10 [a]	2.73±0.18 [a]	62.45±2.23 [a]	3.24±0.05
Group III (Cur-Nps)	8.27±0.04	3.21±0.08	2.65±0.18 [a]	51.03±0.84 [a]	3.15±0.03 [a]
Group IV (RA)	19.57±0.18 [a]	19.2±0.47 [a]	10.03±0.16 [a]	91.43±0.95 [a]	9.23±0.175 [a]
Group V (Cur Tr)	16.28±0.20 [a b]	7.67±0.09 [a b]	8.16±0.10 [a b]	84.2±0.53 [a b]	7.52±0.07 [a b]
Group VI (Cur-Nps)	14.28±0.16 [a b]	7.22±0.09 [a b]	7.83±0.08 [a b]	72.4±0.18 [a b]	7.33±0.03 [a b]
Group VII (Cur Pr)	12.21±0.17 [a b]	6.7±0.18 [a b]	7.48±0.07 [a b]	68±0.40 [a b]	6.59±0.09 [a b]
Group VIII (Cur-Nps)	11.45±0.12 [a b]	6.53±0.09 [a b]	7.03±0.16 [a b]	61.12±0.74 [a b]	6.24±0.02 [a b]

P [(a)] value versus control group P [(b)] value versus RA group

$P > 0.05$ is non-significant $P \leq 0.05$ is significant $P < 0.001$ is highly significant.

3.4. *Live animal imaging and X-ray*

Our results showed that all control groups exhibited no significant change in joint tissue. In rats induced with RA, X-ray images typically reveal significant abnormalities, As the cartilage wears down due to inflammation, the space between bones becomes noticeably reduced in comparison with the control group. Moreover, reduces soft tissue swelling, improving the overall appearance of the joints with either Cur and Cur-Nps alone treated and protected groups in comparison with the untreated RA group as shown in **(Fig 1).**

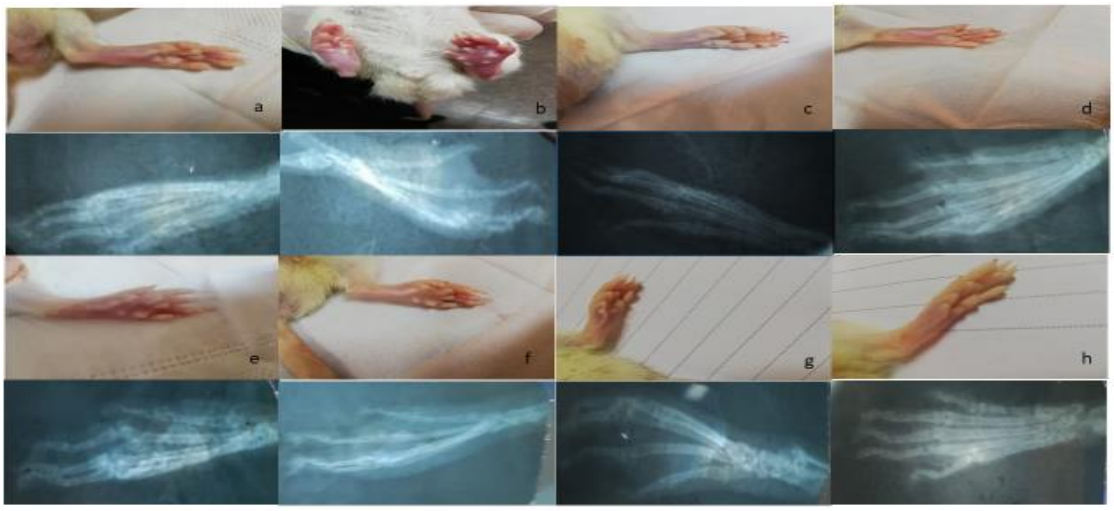

Figure (1): Live animal imaging and x-ray a control group, **b** RA control group, **c** Cur control group, **d** Cur-Nps control group, **e** Cur Tr group, **f** Cur-NpsTr group, **g** Cur Pr group, **h** Cur-NpsPr group.

3.5. Histopathological examinations

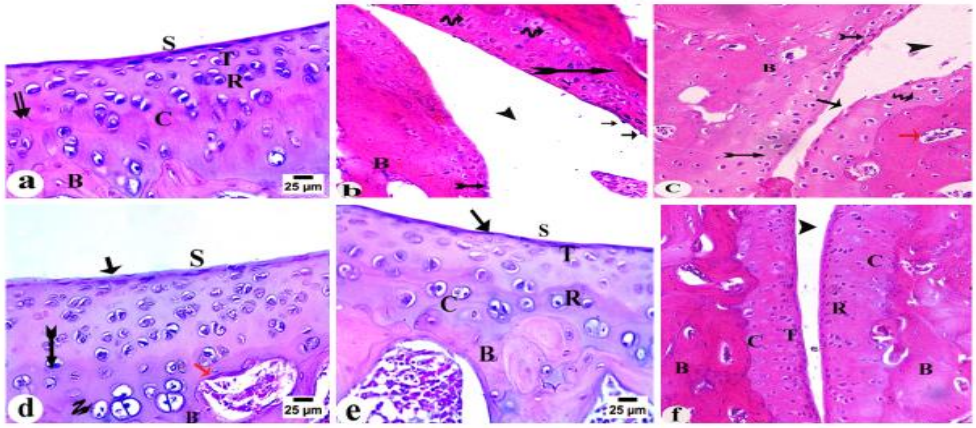

Figure (2) : **(a)** Photomicrograph of experimental groups (The control group, Cur- gp and Cur-Nps group) showing normal histological structure of articular surface The articular cartilage was formed of 4 zones: 1st a superficial tangential zone(S), 2nd transitional zone(T), a 3rd radial zone (R) and 4th zone calcified zone (C) with scattered chondrocytes bounded superiorly by tidemark line (double arrows), then underneath the calcified zone there was the subchondral bone (B).**(b)** Rheumatoid Arthritis (RA) group showing articular cartilage with irregular surface and loss of its smooth contour with pannus formation (↑), focal areas, and highly decreased calcified cartilage (➙) and degenerative changes in articular cartilage with widening articular space (▲) , Empty chondrocyte lacuna, and chondrocytes with pyknotic nuclei (↗), destruction of the adjacent bone (B). **(c)** Cur -Tr group shows little improvement, but the articular cartilage with irregular surface and loss of its smooth contour (↑), focal areas, and a decreasein calcified cartilage remain in some positions (➙) and degenerative changes in articular cartilage with widening articular space (▲), chondrocytes with pyknotic nuclei (↗), destruction of the adjacent bone (B)(red arrow) can also be noticed. **(d)** Cur-NpsTr group showing minimally an ameliorating effect on the joint structure after induction of CFA, the articular cartilage with a regular surface with nearly smooth contour (↑), decreasing chondrocyte lacuna and chondrocytes with pyknotic nuclei (↗and contain inflammation, destruction of the adjacent bone (B)(red arrow) can also be noticed. The articular cartilage appeared with a basophilic matrix containing chondrocytes inside their lacunae, with an apparent increased thickness (➙), Nevertheless, few areas of the articular cartilage were still seen with slight irregularity of its surface**. (e)& (f)** Cur and Cur-Nps protective groups respectively rats were minimally affected by the joint structure before induction of CFA, the articular cartilage with the regular surface a superficial tangential zone(S), 2nd transitional zone(T), a 3rd radial zone (R); and 4th zone calcified zone (C) with chondrocytes, but subchondral bone contain inflammation (B). (H & E Stain-Scale Bar: 25 µm).

4. Discussion

Chronic arthromeningitis, ongoing systemic inflammation, and the generation of autoantibodies, particularly against cyclic citrullinated peptides and rheumatoid factors, are the hallmarks of RA. Intraarticular cartilage damage, joint dysfunction, respiratory and cardiovascular issues, and disability are all possible outcomes of RA **[36]**. It is believed that the pathophysiology of RA involves intricate interactions between genes and the environment; smoking is the main environmental risk factor for RA development, while genetic factors account for 50% of the risk **[11,37-39].** Curcumin, a yellow hydrophobic polyphenol obtained from the herb turmeric, is inexpensive and abundantly available. Numerous chronic diseases, such as cancer **[40,41]**, type II diabetes **[42,43]**, multiple sclerosis **[44,45]**, Alzheimer's disease **[46]**, atherosclerosis and RA **[47,48]**, and more, can be prevented by curcumin's diverse pharmacological actions.

In a rat CFA model, we discovered that curcumin successfully suppressed RA. Our results showed that the Induction of arthritis resulted in a significant decrease in RBCs count and Hb in RA group. On the other hand, treated and protective groups of Cur and Cur-Nps showed a highly significant increase in Hb and RBC count in comparison with RA group **[49].**

Our study was reliable to those of **Mercan R *et al*[50]**showed a highly significant increase in WBCs count in theRA group and all treated & protective groups of Cur and/or Cur-Nps when compared to the control group. On the other hand, there was a highly significant decrease in WBCs count in all treated and protective groups of Cur and/or Cur-Nps when compared to the RA group, these results support the conclusions of **Almarzany, Z. S. [49]**.

In comparison to the control group, a marked elevation of serum AST and ALT levels was observed in the RA group.This finding is in agreement with ***Aloke et al* [51].** Whereastreated and protective groups with Cur and Cur-Nps show a reduction in serum AST and ALT in comparison with the RA group.

According to **Mohapatra, T. K.*et al* [52].** The levels of urea and creatinine were found to be higher in CFA-induced arthritic rats compared to the control group. On the other hand, treated and protective groups with Cur and Cur-Nps show a reduction in serum Creatinine and Urea in comparison with the RA group.

Our data revealed a significant elevation in Rheumatoid factor (RF) **Mohapatra, T. K.*et al* [52],** anti-cyclic citrullinated peptide (Anti-CCP) **Amer, N. E. S.*et al* [53],** and ESR (Erythrocyte Sedimentation Rate)**Patel, R.*et al* [53]**,in RA group ($P< 0.001$) compared to control group, while there was a highly significant decrease in treated and protective groups with Cur and Cur-Nps in comparison with RA group.

Our data also showed a highly significant increase in Tumor necrosis factor-alpha (TNF-α)**Wahba, M. G. *et al* [54]**and Interleukin-6 (IL-6)**Patel, R.*et al.*,2021 [53]** in the RA group as compared to the control group. There was a highly significant decrease in treated and protective groups with Cur and Cur-Nps in comparison with the RA group.

Additionally, in the present study, protective and treated groups of Cur and Cur-Nps recorded a highly significant decrease in serum MDA in comparison with the RA group, but there was a highly significant increase in the RA group as compared to the control group ($p< 0.001$), These data are supported by the results of**Abdelmawgoud, H., & Saleh, A. [55].**

5. Conclusion

The present study demonstrated that Cur and Cur-Npsdministration provided effective protectionand treatment against RA. The efficacy of Cur and Cur-Nps treatment in RA is possibly attributed to its antioxidant and anti-inflammatory effect.

6. Abbreviation

Curcumin (Cur); Curcumin nanoparticles (Cur-Nps); Complete Freund's Adjuvant (CFA); Rheumatoid arthritis (RA); Body Weight (BW); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Protective (Pr)**;** Treated (Tr); Hemoglobin (Hb); Red Blood Cells (RBCs); White blood cells (WBCs); Hematocrit (HCT); Erythrocyte sedimentation rate (ESR); Rheumatoid factor (RF); Anti–citrullinated protein antibody (Anti-CCP); Tumor necrosis factor alpha (TNF-α); Interleukin-6 (IL-6); Malondialdehyde (MDA); Total Antioxidant Capacity (TAC); Statistical Package of the Social Science (SPSS); Standard Deviation (S.D.).

7. Conflicts of interest

There are no conflicts of interest

8. References

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