Naunyn-Schmiedeberg's Archives of Pharmacology (2021) 394:1975–1981
https://doi.org/10.1007/s00210-021-02105-2

ORIGINAL ARTICLE

# Amelioration of autoimmunity and inflammation by zinc oxide nanoparticles in experimental rheumatoid arthritis

Sameh S. Gad[1] · Ahmed M. Fayez[1] · Mahmoud Abdelaziz[1] · Doaa Abou El-ezz[1]

Received: 31 January 2021 / Accepted: 20 May 2021 / Published online: 8 July 2021


## Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the lining of the synovial joints and approximately affects 0.5 – 1% of the total population imposing a socioeconomic burden. The current study aimed at investigating the novel possible beneficial effects of using zinc oxide nanoparticles (ZnO NPs) on such devastating disease. The complete Freund's adjuvant (CFA) model was used to mimic RA in rats where ZnO NPs were given orally (2 mg/kg/day) daily for 14 days; and diclofenac Na, the standard drug, was given intraperitoneally (1 mg/kg/day) the day after CFA, daily for 14 days. Our results displayed that ZnO NPs attenuated adjuvant-induced increased production of inflammatory mediators interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10), and total leukocyte count. Besides, they ameliorated autoimmunity through suppression of anti-citrullinated protein auto antibodies (anti-CCP) levels in rats. In conclusion our results highlight the benefits which could be obtained of nanoparticles either alone or in combination with the known anti-arthritic and/or anti-inflammatory agents, giving rise to new protocols to maximize the control of RA.

**Keywords** Rheumatoid arthritis · Zinc oxide nanoparticles · Chronic disease · Rheumatoid factor · Anti-CCP levels

## Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that approximately affects 0.5 – 1% of the total population (Silman and Pearson 2002; Moreland 2005; Alivernini et al. 2019). Clinically RA is characterized by the presence of persistent symmetrical joint affection including arthralgia, redness, swelling, stiffness, and decreased range of motion (Guo et al. 2018). It is not only characterized by its disabling progressive articular damage but also by the presence of extra-articular manifestations in some cases including hematological, skin, cardiac, ocular, and pulmonary manifestations (Dai et al. 2018; Muravyev 2018). Currently, there is no cure for RA, but receiving proper medical care at early stages of the disease are of high importance and can greatly improve the patient's life by either stopping or retarding the progression of the joint's erosion, and therefore reducing the severity of the disease, or even reaching a state of remission (Heidari 2011).

Pathogenesis of RA is very complicated. Immune system activation and the presence of some pathological manifestations depend on both the adaptive and innate immune pathways, besides the presence of cytokines, growth factors, and intracellular signaling molecules. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are two of the most important pro-inflammatory cytokines that have several roles to play in the progression of RA (Kay and Calabrese 2004). Currently RA management strategies and options are expanding starting from the classical methods of treatment, passing by the conventional synthetic disease modifying anti-rheumatic drugs (DMARDs), and ending up to the biological response modifiers (BRMs) (National Institute for Health and Clinical Excellence 2018). Many novel molecules are now designed to target specific cytokines and/or their receptors especially TNF- α, IL-1β based on their pivotal role in the inflammatory response and consequently on the disease progression (Cascão et al. 2012).

Nanoparticles (NPs) have been extensively studied for their beneficial effects in several fields, for example, food, cosmetics, and agriculture industries. Concerning the medical research field, NPs have been used based on

✉ Doaa Abou El-ezz
dabulez@msa.eun.eg; doaa.ez86@gmail.com

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA University), Giza, Egypt

its astonishing properties, as they have a high penetration power, superb ligand-binding properties, and high safety margins, and some of them can also have distinctive powerful antimicrobial, anti-inflammatory, anti-oxidant, and anti-angiogenic properties (Agarwal et al. 2019). Zinc (Zn) is considered as one of the most important supplements for the human health, based on its ability to modulate several physiological processes (Kim et al. 2014).

Zinc oxide nanoparticles (ZnO NPs) are one of the most important metal oxide nanoparticles due to their exclusive properties, and they are currently merged in several biological applications due to their biocompatibility, low cost, and high safety profile (Jiang et al. 2018). The aim of the current study was to evaluate the antiarthritic effects of ZnO NPs using CFA-induced arthritis rat model.

## Materials and methods

### Materials and animals

#### Chemicals

ZnO NPs were supplied by the Egyptian Atomic Energy Authority; diclofenac ampoules were obtained from Novartis. Complete Freund's adjuvant (CFA), Cell suspension [F5881] from Sigma-Aldrich, each ml contains 1 mg of heat killed and dried Mycobacterium tuberculosis [H37Ra, ATCC25177], 0.15 ml mannide monooleate and 0.85 ml paraffin oil. Equipment including centrifuge, refrigerator, test tubes, ELISA kit and a caliper were used.

#### Animals

Twenty-four adult male Wistar albino rats were used, weighing from 150 to 200 g. They were housed six rats in each cage, and kept for 1 week before the experiment for acclimatization at the animal house of October University for Modern Sciences and Arts. They had free access to commercial diet and tap water under standard room temperature and exposed to 12-h light and dark cycles. Handling and disposal of the rats was done according to the local ethics committee of October University for Modern Sciences and Arts guidelines (PH1/EC1/2020PD) that comply with the international laws for the care and use of laboratory animals.

### Methodology

#### Zinc oxide nanoparticles preparation

Gallic acid solution of concentration 1 mg/1 ml was prepared. Zinc oxide (24 mg) was dissolved in 15-ml bi distilled water. Gallic acid solution was heated at 40 °C then Zn solution was added with continuous stirring for 2 h. The reaction parameters, such as pH, reaction time, reaction temperature, and molar ratios, were optimized till the pale-yellow color of ZnO NPs solution was observed. ZnO NPs was characterized and confirmed by TEM: ZnO NPs was prepared with average size 22 nm (Figure s1).

#### Rheumatoid arthritis induction

The induction of rheumatoid arthritis in rats was done using complete Freund's adjuvant. Rats were injected with 0.1 ml S.C. CFA in the left hind paw sub-planter region, followed by 0.1 ml in the root of the tail. On the second day, they were injected with another dose of 0.1 ml CFA in the root of the tail (A Ali et al. 2016).

#### Study design

The rats were allowed to acclimate for 1 week. After that, they were divided randomly into 4 groups, each group containing 6 rats. Group 1 was kept as a normal control group and was injected with saline i.p (CONT), while group 2 was injected with CFA only (CFA). Groups 3 and 4 were injected with CFA, and then the treatment started the day after the induction as follows: Group 3 treated by oral dose of Zn oxide nanoparticles 2 mg/kg/day daily for 14 days (Zn NP); and group 4 treated by i.p. dose of diclofenac Na, 1 mg/kg/day daily for 14 days (Diclo).

#### Collection and processing of samples

At the end of the experiment period, blood was collected from the abdominal aorta, and then animals were euthanized under light anesthesia using sodium thiopental (60 mg/kg, i.p.). Blood samples were collected in non-heparinized tubes and then centrifuged for 15 min at 5000 rpm to separate the serum. The obtained serum was then collected in eppendorff tubes and stored at –20 ºC for biochemical estimation (Ahmed 2015).

#### Biochemical parameters

For the assessment of the inflammation that accompanies the disease, we determined the levels of TNF α using ELISA Kit (Cat. No. CSB-E1218r); IL-10 using ELISA kit (Sandwich Cloud clone Corp.; Cat. No. SEA-0169). IL-1β was determined using ELISA kit cohesion biosciences (Cat. No. # CEK1976), rheumatoid factor [RF] using the kit (Sandwich ELISA; Cat. No. MBS720877), total leucocyte count according to the method of Viana et al. (2012), and anti-CCP using ELISA kit (E-EL-R1521) in the serum.

### Statistical analysis

Data are presented as the mean ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer test for multiple comparisons. Significant difference was considered at $P \leq 0.05$. Data analysis and graph presentation were carried out using GraphPad Prism software version 6.

## Results

### Biochemical Parameters

#### Effect of ZnO NPs on TNF-α, IL-1β, and IL-10 in CFA-induced rheumatoid arthritis in rats

The levels of the inflammatory mediators (TNF-α, IL-1β, and IL-10) in the CFA group showed a significant elevation (150.4 ± 15.92 pg/ml, 374.1 ± 79.60 pg/ml, 234.5 ± 42.66 IU/L), respectively, in comparison to the control group (85.13 ± 10.34 pg/ml, 111.1 ± 12.09 pg/ml, 40.91 ± 5.470 IU/L), respectively, at $P < 0.05$, whereas their levels in the treated groups with ZnO NPs (129.8 ± 13.26 pg/ml, 118.6 ± 15.23 pg/ml, 69.76 ± 9.756 IU/L), respectively, and the standard treatment group with diclofenac (106.3 ± 9.32 pg/ml, 118.9 ± 26.63 pg/ml, 39.78 ± 5.814 IU/L), respectively, were significantly lowered at $P < 0.05$ as shown in Fig. 1.

#### Effect of ZnO NPs on Anti CCP, RF and total leucocytes in CFA-induced rheumatoid arthritis in rats

Evaluation of Anti-CCP in the CFA group (50.55 ± 6.819 IU/ml) showed a significant elevation in comparison to the control group (17.77 ± 2.237 IU/ml) at $P < 0.05$. On the other hand, the level of Anti-CCP in groups treated with ZnO NPs (35.71 ± 3.785 IU/ml) and diclofenac (28.11 ± 3.991 IU/ml) was significantly lowered at $P < 0.05$ as shown in Fig. 2.

Rheumatoid factor levels in the CFA group showed a significant elevation (33.30 ± 4.309 U/ml) in comparison to the control group (18.72 ± 2.632 U/ml). Treatment with ZnO NPs (30.17 ± 0.6524 U/ml) failed to significantly reduce RF level, while diclofenac (22.96 ± 1.842 U/ml) significantly lowered it at $P < 0.05$ as shown in Fig. 3.

Total leucocytes counts in the CFA group (9628 ± 864.2 cells/mm$^3$) showed a significant elevation in comparison to the control group (3154 ± 438.1 cells/mm$^3$) at $P < 0.05$, while their count in groups treated with ZnO NPs (4714 ± 382.0

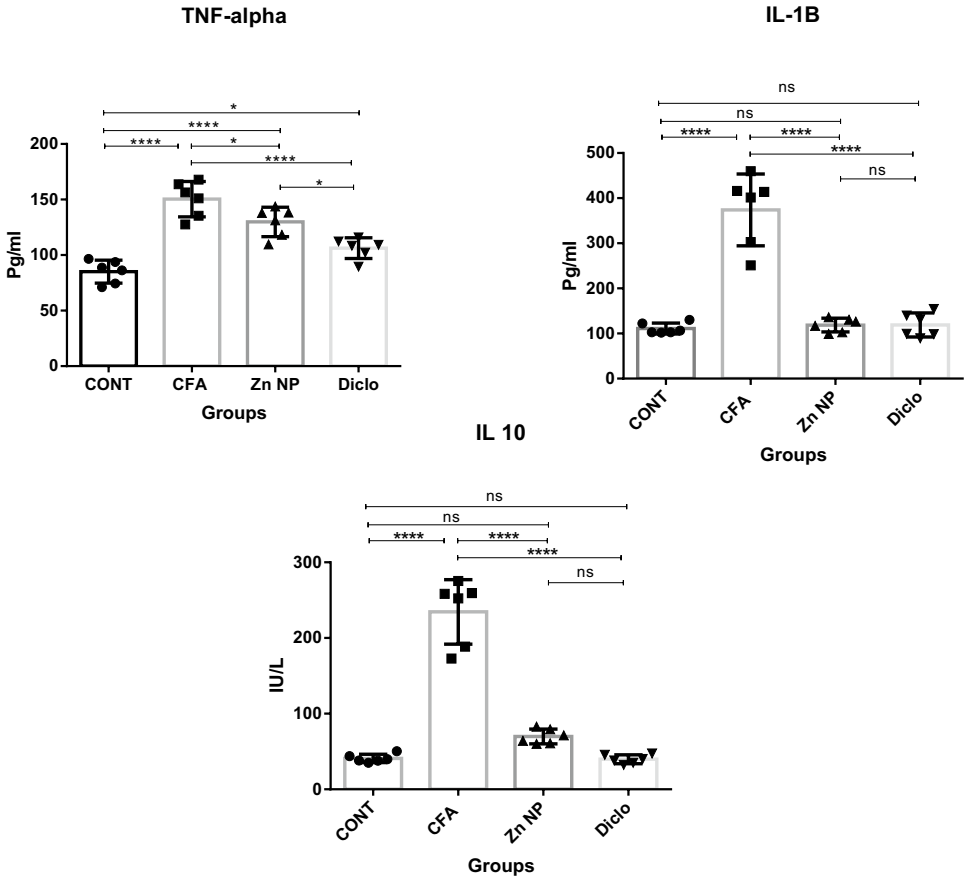


**Fig. 1** Effect of ZnO NPs on TNF-α, IL-1β and IL-10 in CFA-induced rheumatoid arthritis in rats: The control group was injected with saline i.p., while the CFA group was injected by CFA only. ZnO NP group was treated by oral dose of ZnO NPs 2 mg/kg/day daily for 14 days the day after RA induction by CFA; diclofenac group was treated by i.p. dose of diclofenac Na, 1 mg/kg/day the day after RA induction by CFA and continued daily for 14 days. Data are presented as the mean ± SD ($n = 6$). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Results were considered significant at $P < 0.05$

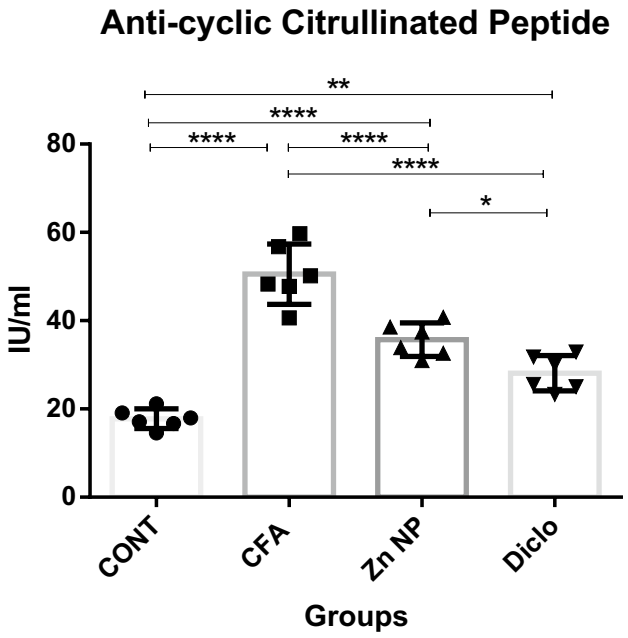


**Fig. 2** Effect of ZnO NPs on Anti CCP in CFA-induced rheumatoid arthritis in rats: The control group was injected with saline i.p., while the CFA group was injected by CFA only. ZnO NP group was treated by oral dose of ZnO NPs 2 mg/kg/day daily for 14 days the day after RA induction by CFA; diclofenac group was treated by i.p. dose of diclofenac Na, 1 mg/kg/day the day after RA induction by CFA and continued daily for 14 days. Data are presented as the mean ± SD ($n = 6$). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Results were considered significant at $P < 0.05$

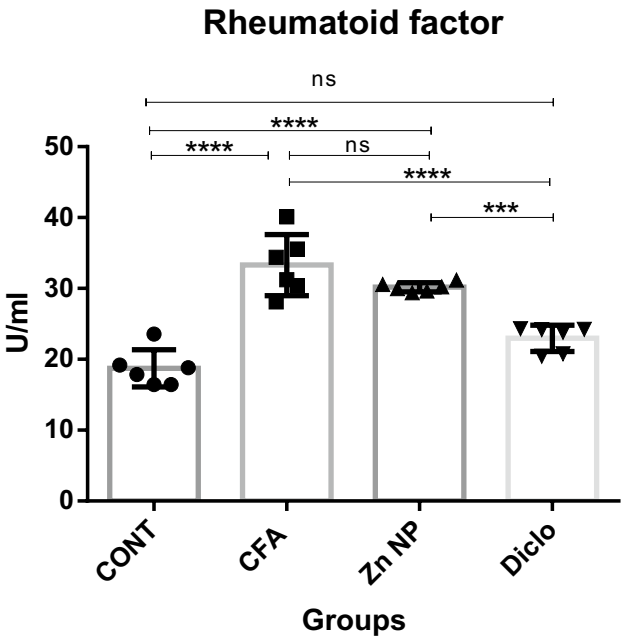


**Fig. 3** Effect of ZnO NPs on RF in CFA-induced rheumatoid arthritis in rats: The control group was injected with saline i.p., while the CFA group was injected by CFA only. ZnO NP group was treated by oral dose of ZnO NPs 2 mg/kg/day daily for 14 days the day after RA induction by CFA; diclofenac group was treated by i.p. dose of diclofenac Na, 1 mg/kg/day the day after RA induction by CFA and continued daily for 14 days. Data are presented as the mean ± SD ($n = 6$). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Results were considered significant at $P < 0.05$

cells/mm$^3$) and diclofenac (3920 ± 785.1 cells/mm$^3$) were significantly lowered at $P < 0.05$ as shown in Fig. 4.

## Discussion

In this study, we tried to shed the light on the possible beneficial effects of using zinc oxide nanoparticles on the rate of RA progression, and the possible mechanisms behind these effects, and based on our results, ZnO NPs significantly reduced inflammation and abrogated autoimmunity after CFA injection in rats.

Adjuvant-induced arthritis in rats has been approved as an experimental model to study the pathogenesis of human rheumatoid arthritis (RA) (Halloran et al. 1996; Pan et al. 2017). In the present study, complete Freud's adjuvant (CFA) induced arthritis in male Wistar albino rats since elevated levels of total leukocyte count, rheumatoid factor, anti-CCP antibody, and inflammatory cytokines were observed in sera of studied rats injected with CFA. Rheumatoid arthritis is characterized by three stages, autoimmunity, chronic inflammation, and joint destruction. The autoimmunity phase involves autoantibodies (rheumatoid factor and anti-CCP) that are produced specific for IgG and is the earliest phase which is often referred to as the pre-articular phase because it precedes the inflammatory and articular destruction phase (McInnes and Schett 2007).

Zinc supplements have been proven to produce beneficial effects in elder population due to reduction of inflammation and oxidative stress (Prasad 2014). Similarly zinc oxide nanoparticles have been recently reported to exhibit various anti-inflammatory effects like reversing LPS-induced liver cell injury and human mononuclear cells inflammation (Prasad 2014; Kim and Jeong 2015).In the present study, zinc oxide nanoparticles (ZnO NPs) reduced the adjuvant-induced increased productions of IL-1β, TNF-α, IL-10, total leukocyte count, rheumatoid factor, anti-CCP levels in rats. Therefore, we hypothesize that ZnO NPs could be useful in treatment of autoimmune RA.

Tumor necrosis factor alpha (TNF-α) and IL-1β are central inflammatory cytokines involved in the pathogenesis of rheumatoid arthritis (RA), since they are involved in bone resorption and cartilage remodeling through maturation

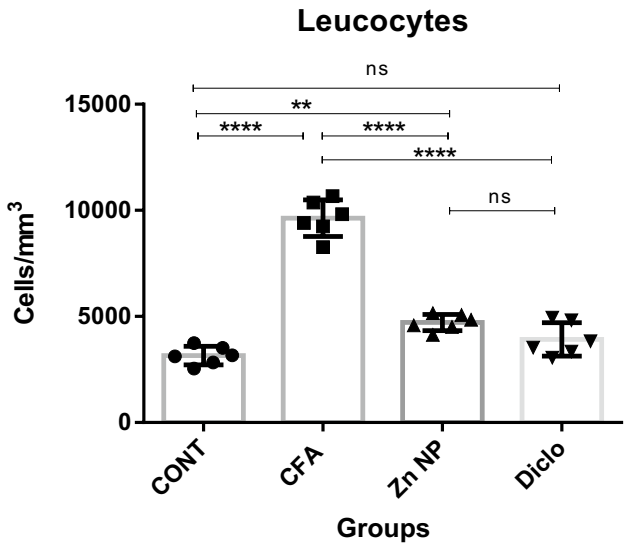


**Fig. 4** Effect of ZnO NPs on total leucocytes in CFA-induced rheumatoid arthritis in rats: The control group was injected with saline i.p., while the CFA group was injected by CFA only. ZnO NP group was treated by oral dose of ZnO NPs 2 mg/kg/day daily for 14 days the day after RA induction by CFA; diclofenac group was treated by i.p. dose of diclofenac Na, 1 mg/kg/day the day after RA induction by CFA and continued daily for 14 days. Data are presented as the mean ± SD ($n=6$). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Results were considered significant at $P<0.05$

of osteoclast cells and inhibition of collagen synthesis (Goldring 2003). In addition, they augment several inflammatory mechanisms such as angiogenesis, fibrosis, and cell adhesion (Szekanecz et al. 2000). In the current study ZnONPs suppressed the serum levels of these cytokines to a level comparable to that of diclofenac Na, the standard drug. It has been identified that ZnO NPs work against inflammation by several ways. As reported, zinc itself can strengthen the antioxidant defense mechanisms; through the activation of some antioxidant proteins and enzymes, e.g., catalase (Jarosz et al. 2017). Their anti-inflammatory properties have been attributed to their ability to suppress the production of nitric oxide, nuclear factor kappa B (NF-κB), caspase-1 which is responsible for the activation of the pro-IL-1β, and as a consequence, they have the ability to suppress the production of IL-1β and TNF-α (Lawrence 2009; Jiang et al. 2018; Agarwal et al. 2019). In addition, ZnONPs were found to suppress NF-κB nuclear translocation and increase A20 levels which downregulates NF-κB eventually inhibiting TNF-α and IL-1β production (Agarwal et al. 2019).

Interleukin 10 (IL-10) is an immunoregulatory cytokine produced by Treg cells that plays a key role in prevention of tissue damage due to inflammation. Autoimmune diseases show high level of this cytokine during the early stages of the disease; however, immune equilibrium is reached during later stages (Mingomataj and Bakiri 2016). It is mainly produced by monocytes/macrophages, and it possesses a strong anti-inflammatory effect against functions of both T-lymphocytes and monocytes by inhibiting T-lymphocyte proliferation and decreasing antigen presentation by monocytes (Isomäki and Punnonen 1997).

In the present study, IL-10 was elevated after 2 weeks post injection of CFA; however its level might not have been sufficient to counteract the elevated levels of proinflammatory cytokines, namely, TNF-α and IL-1β. On the other hand, ZnO NPs and diclofenac were able to reduce the level of IL-10 which reinforces their suppressor effect on autoimmunity induced by CFA reducing the severity of arthritis in rats. Although our study highlights the positive role of ZnO NPs might have on suppression of inflammation associated with RA, Roy et al. 2014 have reported that nanoparticles (< 50 nm) may produce detrimental effects on macrophages by inducing cytotoxicity and provocation of inflammation through increased reactive oxygen species (ROS) production. This discrepancy could be attributed to the changes in concentration of nanoparticles which affects their aggregation and potential cytotoxicity; in addition, the size and shape of nanoparticles and the experimental conditions used for their preparation play an essential role in cellular signaling pathway (Kim et al. 2014). In our study we used ZnO NPs prepared at 22 nm which is relatively smaller than those used by Roy et al. 2014 in the in vitro macrophages model and that might explain the anti-inflammatory effect seen in our results. However, further studies are needed to elucidate the effect of different concentrations and experimental conditions of nanoparticle preparation on the progression of RA in-vivo model.

T-cells, B-cells, and macrophages are highly involved in the pathogenesis of RA, for example, B-lymphocytes have critical roles to play in this process, as they are the source of the RF and anti-CCP auto-antibodies, which contribute to immune complex formation and complement activation (Silverman and Carson 2003; Kay and Calabrese 2004). Rheumatoid factor (RF) is an autoantibody which targets the Fc region of IgG and is the first antibody discovered in RA. It was reported to contribute to the pathogenicity of the disease by enhancing immune complexes formation therefore increasing the arthritogenicity of itself and other autoantibodies like anti-CCP (Song and Kang 2010). In the current study, treatment with ZnO NPs was able to reduce the level of RF, however unlike the standard drug diclofenac Na, its effect was non-significant.

Anti-cyclic citrullinated peptide (anti-CCP) has a higher specificity to RA than RF and is detected before the clinical development of RA. Induction of RA involves the formation of citrullinated proteins within the joint like fibrin, vimentin, and collagen followed by a local chronic

immune response that provokes the erosive disease (Kinloch et al. 2006; Niewold et al. 2007). Our study has shown that CFA significantly increased anti-CCP level in rats, which came in accordance with previous study of Salem et al. (2020). Both ZnO NPs and diclofenac successfully reduced anti-CCP level which shows the potential use of ZnO NPs for suppression of autoimmunity associated with RA. In addition, CFA-induced arthritis in rats showed a higher level of total leucocyte count compared with control rats which mean that leucocytes were recruited to site of inflammation in the arthritic group, contrarily groups treated with Zn NPs and diclofenac showed significantly decreased total leucocyte counts which further proves the antiarthritic potential of ZnO NPs. Taken together these results suggest the anti-arthritic and anti-inflammatory activity of ZnO NPs and their potential usefulness as a promising tool for the treatment of RA.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00210-021-02105-2.

**Authors' contribution** AMF and SS conceived and designed research. AMF and MA conducted the experiment. DA, SS and MA analyzed the data and wrote the manuscript. All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

**Funding** No funding sources are available.

**Data Availability** All data generated or analyzed during this study are included in this published article (and its supplementary information files).

## Declarations

**Ethics approval and consent to participate** All experimental procedures adopted for in vivo studies were in accordance with the local ethics committee guidelines at Modern Sciences and Arts University (PH1/EC1/2020PD) that comply with the international laws for the care and use of laboratory animals.

**Conflict of interest** The authors declare that there is no conflict.

## References

A Ali A, S El-Zaitony A, Al-Haleem ENA (2016) Evaluation of therapeutic efficacy of vinpocetine in adjuvant induced arthritis model in rats. J Pain Manag Med 02: https://doi.org/10.35248/2684-1320.16.2.115

Agarwal H, Nakara A, Shanmugam VK (2019) Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: a review. Biomed Pharmacother 109:2561–2572. https://doi.org/10.1016/j.biopha.2018.11.116

Ahmed WMS HO (2015) Effect of atorvastatin and vitamin D on FreundÂ's adjuvant-induced rheumatoid arthritis in rat. J Bioequiv Availab 07. https://doi.org/10.4172/jbb.1000221

Alivernini S, Tolusso B, Petricca L, et al (2019) Rheumatoid arthritis. In: Mosaic of autoimmunity: the novel factors of autoimmune diseases. Elsevier, pp 501–526

Cascão R, Vidal B, Raquel H et al (2012) Effective treatment of rat adjuvant-induced arthritis by celastrol. Autoimmun Rev 11:856–862. https://doi.org/10.1016/j.autrev.2012.02.022

Dai Q, Zhou D, Xu L, Song X (2018) Curcumin alleviates rheumatoid arthritis-induced inflammation and synovial hyperplasia by targeting mTOR pathway in rats. Drug Des Devel Ther 12:4095–4105. https://doi.org/10.2147/DDDT.S175763

Goldring SR (2003) Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheumatology 42:11–16. https://doi.org/10.1093/rheumatology/keg327

Guo Q, Wang Y, Xu D, et al (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res 6. https://doi.org/10.1038/s41413-018-0016-9

Halloran MM, Szekanecz Z, Barquin N et al (1996) Cellular adhesion molecules in rat adjuvant arthritis. Arthritis Rheum 39:810–819. https://doi.org/10.1002/art.1780390514

Heidari B (2011) Rheumatoid arthritis: early diagnosis and treatment outcomes. Casp J Intern Med 2:161–170

Isomäki P, Punnonen J (1997) Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann Med 29:499–507

Jarosz M, Olbert M, Wyszogrodzka G et al (2017) Antioxidant and anti-inflammatory effects of zinc. Zinc-Dependent NF-Kb Signaling Inflammopharmacology 25:11–24. https://doi.org/10.1007/s10787-017-0309-4

Jiang J, Pi J, Cai J (2018) The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications[1] J. Jiang, J. Pi, and J. Cai, "The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications," vol. 2018, 2018. Bioinorg Chem Appl 2018:18

Kay J, Calabrese L (2004) The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. Rheumatology (oxford) 43(Suppl 3):2–9. https://doi.org/10.1093/rheumatology/keh201

Kim MH, Jeong HJ (2015) Zinc oxide nanoparticles suppress LPS-Induced NF-κB activation by inducing A20, a negative regulator of NF-κB, in RAW 264.7 Macrophages. J Nanosci Nanotechnol 15:6509–6515. https://doi.org/10.1166/jnn.2015.10319

Kim MH, Seo JH, Kim HM, Jeong HJ (2014) Zinc oxide nanoparticles, a novel candidate for the treatment of allergic inflammatory diseases. Eur J Pharmacol 738:31–39. https://doi.org/10.1016/j.ejphar.2014.05.030

Kinloch AJ, Lundberg KE, Moyes D, Venables PJ (2006) Pathogenic role of antibodies to citrullinated proteins in rheumatoid arthritis. Expert Rev Clin Immunol 2:365–375. https://doi.org/10.1586/1744666X.2.3.365

Lawrence T (2009) The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 1:1–11. https://doi.org/10.1101/cshperspect.a001651

McInnes IB, Schett G (2007) Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 7:429–442. https://doi.org/10.1038/nri2094

Mingomataj E, Bakiri AH (2016) Regulator versus effector paradigm: interleukin-10 as indicator of the switching response. Clin Rev Allergy Immunol 50:97–113. https://doi.org/10.1007/s12016-015-8514-7

Moreland L (2005) Unmet needs in rheumatoid arthritis. Arthritis Res Ther 7:2–8. https://doi.org/10.1186/ar1736

Muravyev Y V. (2018) Extra-articular manifestations of rheumatoid arthritis. Nauchno-Prakticheskaya Revmatol 56:356–362. https://doi.org/10.14412/1995-4484-2018-356-362

National Institute for Health and Clinical Excellence (2018) Rheumatoid arthritis in adults: management NICE guideline

Niewold TB, Harrison MJ, Paget SA (2007) Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. QJM 100:193–201. https://doi.org/10.1093/qjmed/hcm015

Pan T, Cheng T, fang, Jia Y ran, et al (2017) Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats. J Ethnopharmacol 205:1–7. https://doi.org/10.1016/j.jep.2017.04.020

Prasad AS (2014) Zinc is an antioxidant and anti-inflammatory agent: its role in human health. Front Nutr 1:1–10. https://doi.org/10.3389/fnut.2014.00014

Roy R, Parashar V, Chauhan LKS et al (2014) Mechanism of uptake of ZnO nanoparticles and inflammatory responses in macrophages require PI3K mediated MAPKs signaling. Toxicol Vitr 28:457–467. https://doi.org/10.1016/j.tiv.2013.12.004

Salem HF, Nafady MM, Kharshoum RM et al (2020) Mitigation of rheumatic arthritis in a rat model via transdermal delivery of dapoxetine HCL amalgamated as a nanoplatform: In vitro and in vivo assessment. Int J Nanomedicine 15:1517–1535. https://doi.org/10.2147/IJN.S238709

Silman AJ, Pearson JE (2002) Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 4:S265–S272. https://doi.org/10.1186/ar578

Silverman GJ, Carson DA (2003) Roles of B cells in rheumatoid arthritis. Arthritis Res Ther 5:1–6. https://doi.org/10.1186/ar1010

Song YW, Kang EH (2010) The pathogenic role of rheumatoid factor in rheumatoid arthritis. Int J Clin Rheumtol 5:651–658. https://doi.org/10.2217/ijr.10.62

Szekanecz Z, Halloran MM, Volin MV et al (2000) Temporal expression of inflammatory cytokines and chemokines in rat adjuvant-induced arthritis. Arthritis Rheum 43:1266–1277. https://doi.org/10.1002/1529-0131(200006)43:6%3c1266::AID-ANR9%3e3.0.CO;2-P

Viana MT, Perez MC, Ribas VR et al (2012) Leukocyte, red blood cell and morphological adaptation to moderate physical training in rats undernourished in the neonatal period. Rev Bras Hematol Hemoter 34:285–291. https://doi.org/10.5581/1516-8484.20120073