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# Selenium Nanoparticles Dispersed in Phytochemical Exert Anti-Inflammatory Activity by Modulating Catalase, GPx1, and COX-2 Gene Expression in a Rheumatoid Arthritis Rat Model

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
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**Background:** Literature shows that serum selenium concentration is low in rheumatoid arthritis (RA) patients. Biochemical properties of nanoparticles (NPs) are depend in its medium dispersed. Biochemical properties could effectively alter the therapeutic potential of NPs. Phytochemicals could serve as suitable medium for dispersion of NPs. *P-Coumaric acid (CA)* known to have anti-inflammatory activity.

**Material/Methods:** In the present experiment, we investigated the anti-inflammatory effect of SeNPs dispersed in 1% CA against Complete Freund's adjuvant induced RA. Celecoxib was used as a reference drug.

**Results:** Selenium NPs (SeNPs) size is maintained in 1% CA solution. We observed that supplementation with 500 µg/Kg body weight (b.w.) eNPs significantly restored the levels of thiobarbituric acid reactive substances, COX-2 activity, different antioxidant enzyme activities, and inflammatory cytokines (TNF-α, IL-1β, IL-6, and MCP-1) in RA rats. The mRNA expression of antioxidant enzymes such as MnSOD, Cu/ZnSOD, ECSOD, CAT, and GPx1 was found to be downregulated, whereas COX-2 was upregulated in RA rats; however, the mRNA expression of CAT, GPx1, and COX-2 reverted back to near normal levels in SeNPs-treated animals.

**Conclusions:** The therapeutic potential of SeNPs was confirmed through histological observation of angle joints in different experimental animals. Our results collectively suggest that SeNPs dispersed in CA can be an effective therapeutic agent for inflammatory disorders like acute gouty arthritis.

**MeSH Keywords:** **Antioxidants • Arthritis, Juvenile • Nanoparticles • Selenium**

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## Background

Currently used therapies for rheumatoid arthritis (RA) include nonsteroidal anti-inflammatory drugs (NSAID) for symptomatic relief and disease-modifying anti-rheumatic drugs (DMARD) to change the disease process. However, long-term use of these drugs leaves the individual prone to cardiovascular and gastrointestinal lineage disorders [1,2]. Similarly, treatment with biologic agents mediates epiphenomenon or causes drug resistance in patients [3,4]. Nano-emulsion has been shown to have good therapeutic effects [5–7]; therefore, the present study assessed the essential micronutrients at nano-size dispersed in phytochemical medium that could ameliorate a wide range RA symptoms.

Selenium (Se) is an essential dietary micronutrient and is also an impending drug candidate for the prevention of many diseases, including arthritis. In 2016, a meta-analysis reported that serum Se concentration is low in RA patients [8]. Further, it was shown that Se concentration is low in many soil types in China [9]. Thus, an inorganic form of Se is commonly added to animal feeds in China [9]. The therapeutic dose of Se is above the nutritional dosage [10]. Physiological and pharmacological effects of are caused by selenium-associated proteins [11,12]. Further, Se serves as a co-factor that decreases oxidative stress generated during the metabolic processes through antioxidants defense mechanism with other antioxidants [13,14]. Se at nano-size is known to have superior antioxidant effects than that of the parent substance. Furthermore, Se nanoparticles (SeNPs) shows less *in vivo* toxicity when compared with normal-size Se [15,16]. Dispersed elemental nanoparticles aggregate spontaneously in water or saline [17], which are 2 major media used in drug delivery. Therefore, maintaining the nanoparticle size in drug delivery medium is of paramount importance to achieve the maximum therapeutic effect of NPs.

A hydroxyl derivative of cinnamic acid, referred to as *p*-coumaric acid (CA) (trans-4-hydroxycinnamic acid), is known to have strong anti-oxidative properties. It is commonly found in fruits, vegetables, and beverages [18–21]. CA has ameliorative effects such as antimicrobial [21], anti-ulcer [22], anti-platelet [23], anti-inflammatory activities [24], and anti-mutagenic [25] effects. A recent study by Neog et al. [26] showed the effect of CA in adjuvant-induced arthritic animals. These studies show that CA could be an ideal natural compound for use in drug delivery. In the present study, we evaluated the anti-arthritic effect of SeNPs dispersed in 1% CA and explored the different types of antioxidant gene expression changes in an *in vivo* model. COX-2 inhibitors such as celecoxib have been used as a standard drug candidate [27,28]. Further, different herbal preparations containing CA are shown to have anti-arthritic activity [29,30]. These studies suggest that herbal compounds may have therapeutic value in RA.

Levels of reactive oxygen species (ROS) in rheumatoid joints are greatly elevated. Further, the level of ROS at this site is augmented through the action of inducible nitric oxide synthase (iNOS) [31] and COX-2 [32]. On the other hand, increased ROS leads to a “pro-oxidation environment” in rheumatoid joints that can decrease enzymatic and non-enzymatic antioxidant activity [33]. Elevated ROS and lowered antioxidant levels can damage the protein, lipids, and matrix components [34] in rheumatoid joints. This process accelerates the infiltration of leukocytes at sites of injury. Furthermore, the pro-inflammatory cytokines IL-6, TNF- $\alpha$ , IL-17, and IL-1 $\beta$  exert pleiotropic effects by activating inflammatory signaling cascades. Thus, compounds that control these ROS and upregulate the antioxidants potentially decrease the generation pro-inflammatory mediators, which is vital in maintaining physiologic homeostasis in RA patients.

Serum Se concentration is low in RA patients, and the antioxidant effect of normal-size Se and SeNPs is widely reported in the literature. SeNPs have superior biological effects than normal-size Se. Similarly, dispersed medium could influence biological properties of NPs. The present study through the evidence that phytochemical could serve as NPs stabilizing agent. SeNPs dispersed in CA have been shown to have anti-inflammatory effects.

## Material and Methods

### Chemicals

Nano-Se (purity  $\geq 99\%$ , an average size of 40 nm), reduced glutathione (GSH), ethylene diamine tetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), and acetic acid were obtained from Macklin Biochemical Co., Shanghai, China. Complete Freund's adjuvant (CFA), and CA ( $\sim 98\%$  HPLC) were procured from Sigma-Aldrich, St. Louis, MO. All other chemicals utilized in the current study were of extra-pure grade or analytical grade available commercially.

### Stability of SeNPs

Stability of SeNPs in distilled water and 1% CA were analyzed by monitoring hydrodynamic size of the SeNPs using a Malvern-Zetasizer instrument equipped with a 4-mW He-Ne laser ( $k=632$  nm).

### Animals and development of RA

Wistar albino (WA) rats at the age of 10 weeks were maintained in the Central Animal Facility, Capital Medical University, Beijing, 100041, China. The Institutional Animals Ethics Committee on Experimental Animal Care, Capital Medical University, Beijing,

100041, China approved the experimental procedures (approval no. A40131/2016). Animals were housed at 19–23°C, 40–60% humidity, and 12-h light/dark cycle. During the period of acclimatization (7 days), animals were fed a standard laboratory chow diet. Rats were randomly divided into 6 groups (n=8).

RA was initiated in WA rats through the subcutaneous injection of CFA (0.1 ml of CFA) at the rear surface of the right-hind paw on day 0 of the study [35]. The CFA consisted of 10 mg heat-killed *Mycobacterium tuberculosis* suspended in 1 ml paraffin oil. Periodically, paw swelling was measured using vernier callipers, with increased paw swelling denoting the severity of RA diseases. body weight changes were recorded once a weekly in the control and experimental animals.

### Experimental devise

Group 1: Served as healthy controls administered vehicle alone (0.1% DMSO).

Group 2: RA rats.

Group 3: RA rats treated with SeNPs (250 µg/kg b.w.) in 1% CA medium (day 11 to day 26).

Group 4: RA rats treated with SeNPs (500 µg/kg b.w.) in 1% CA medium (day 11 to day 26).

Group 5: RA rats treated with celecoxib (5 mg/kg) (day 11 to day 26) Group 6: RA rats treated with 1% CA (day 11 to day 26).

0.1% DMSO was used to prepare the SeNP in CA and celecoxib. Drugs and test compound were prepared fresh and used within 24 h. To control the amount of CA administered in different experimental animals, SeNPs dispersed in 1% CA was diluted to 1 ml per animal (irrespective of animal weight) in 0.1% DMSO just before intraperitoneal injection. SeNPs and standard compounds were injected from day 11 to day 26 (consecutively for 16 days). The day of CFA administration was considered as day 0. Experimental rats were euthanized on day 27 by exsanguination, and ankle joints were removed and stored at –80°C until further use.

### Ankle joint tissue sample preparation

The ankle joint tissues were removed immediately and divided into 4 parts and preserved without further delay. One portion of ankle joint was preserved in 10% formalin for histopathological observation. The remaining 3 portions of ankle joints were snap-frozen in liquid nitrogen and stored at –80°C until further analysis. For estimation of biochemical changes, whole frozen ankle joints were pulverized in a liquid nitrogen-filled mortar and pestle. Further tissues were homogenised with tissue homogeniser for 15–20 s. Whole ankle joint homogenates were centrifuged for 12 min at 500 g at 4°C and supernatant was used for further analysis.

**Table 1.** Primer sequences used for quantitative real-time PCR analysis.

S. No.	Gene name	Sequence (5'-3')
1	MnSOD	FW, 5'-ACCGAGGAGAAGTACCACGA-3' RV, 5'-TAGGGCTCAGGTTTTGTCCAG-3'
2	Cu/ZnSOD	FW, 5'-GTGCAGGGCGTCATTCACTTC-3' RV, 5'-GCCTCTTTCATCCGCTGGA-3'
3	ECSOD	FW, 5'-GGGAGAGCTTGTGAGGTGTGG-3' RV, 5'-GCCGCTTCTGCGCTCCTTG-3'
4	GPX-1	FW, 5'-TGAGAAGTGCAGGTGAATG-3' RV, 5'-CGGGGACCAAATGATGACT-3'
5	GPX-2	FW, 5'-TGCCCTACCCTTATGACGAC-3' RV, 5'-TCGATGTTGATGGTCTGAA-3'
6	CAT	FW, 5'-CCTCAGAAACCGATGCTCTG-3' RV, 5'-GTCAAAGTGTGCCATCTCGTCG-3'
7	COX-2	FW, 5'-CTCTCCGAGCTGTGCTGC-3' RV, 5'-TGTGTTGGGTGGGCTC-3'
4	β-actin	FW, 5'-TTCCGGGATGGCCTTCTAC-3' RV, 5'-TTTGCGGATGCATCCAATCTG-3'

### Biochemical estimations

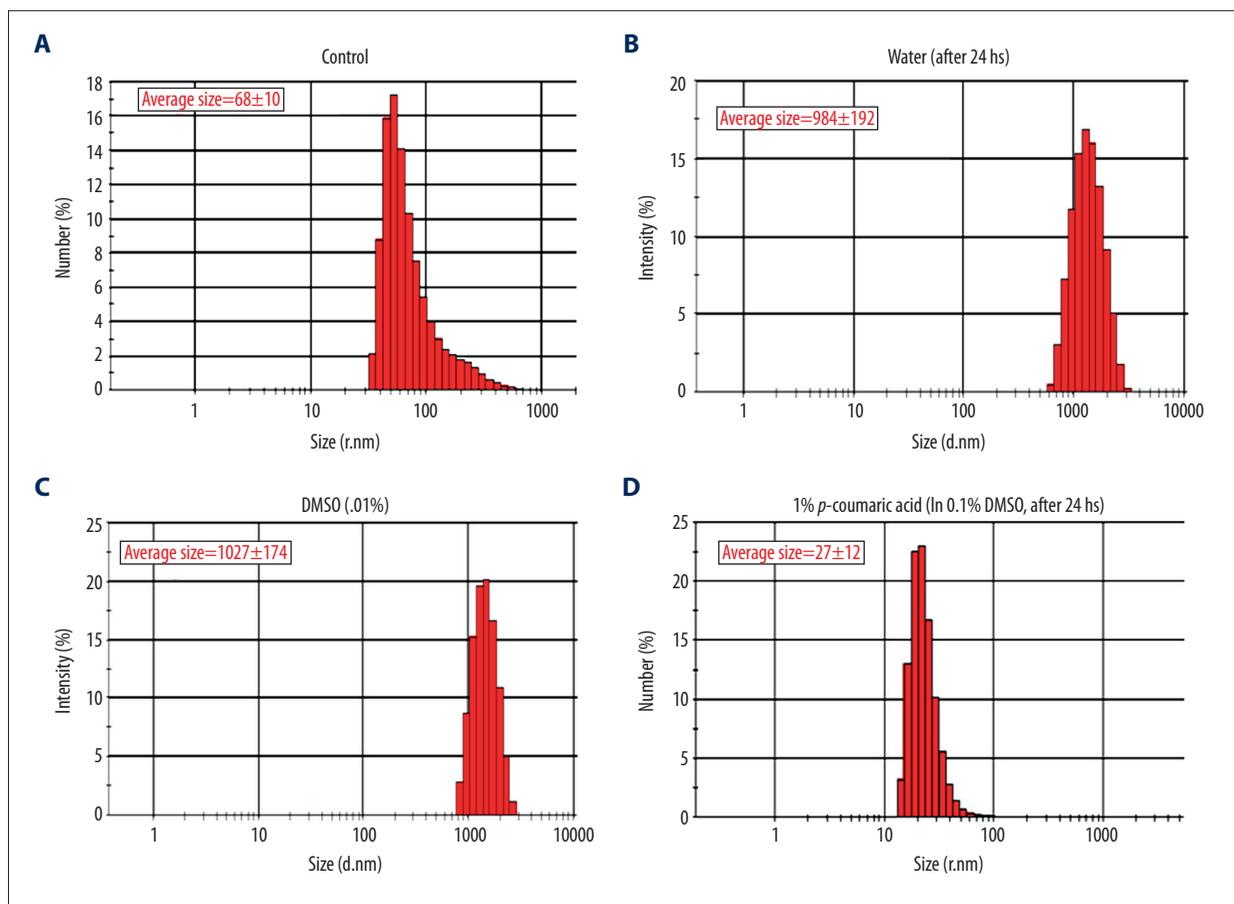
Thiobarbituric acid reactive substances (TBARS) and GSH levels were assayed following the kit manufacturer's procedures (Cayman Chemical Company, USA). Glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and COX-2 activities, and protein contents were estimated using kits obtained from Abcam, USA.

### Isolation of total RNA and Real-time PCR

TRIzol Reagent (Invitrogen, USA) was utilized to isolate the RNA from 100 mg ankle joint tissue of different experimental animals. The RNA was purified through chloroform treatment and precipitation with isopropanol, and were further purified with ethanol wash. cDNA was made from 2 µg total RNA using a high-capacity cDNA reverse transcription kit from Applied Biosystems. A template from freshly made cDNA was used for quantitative real-time polymerase chain reaction (qRT-PCR). qRT-PCR was quantified as described by Alshammari et al. [31]. The primer sequence used to amplify the different genes of interest are shown in Table 1.

### TNF-α, IL-1β, IL-6, and MCP-1 contents

Levels of different pro-inflammatory cytokines in ankle joint tissues were estimated using cytokine kits from Abcam, closely following the kit manufacturer's procedures to quantify the cytokine levels. Contents of different pro-inflammatory cytokines are expressed as pg/mg protein.



**Figure 1.** SeNPs status in different media. (A) SeNPs dispersed in water (immediately after dispersion=0 h). (B) SeNPs dispersed in water (after=24 h). (C) SeNPs dispersed in 0.1% DMSO (after=24 h). (D) SeNPs dispersed in 15 CA (after=24 h). Size determination by DLS analysis. Values are shown as the mean  $\pm$  standard deviation of 3 independent experiments.

### Histological analysis

We used 10% EDTA to decalcify the ankle joints from different experimental animals preserved in 10% paraformaldehyde [35]. The decalcified ankle joints were embedded and were sectioned at 3 mm in a microtome. The sectioned ankle joints were fixed on the slides. The sections were observed under an inverted microscope equipped with digital cameras to assess histopathological changes in control and experimental animals (Olympus photomicroscope, Tokyo, Japan). The slides were scored by a person who was blind to experimental details. A semi-quantitative 5-point scale was used to score the changes in different experimental animals (0-absent, 1-weak, 2-moderate, 3-high, and 4-very high).

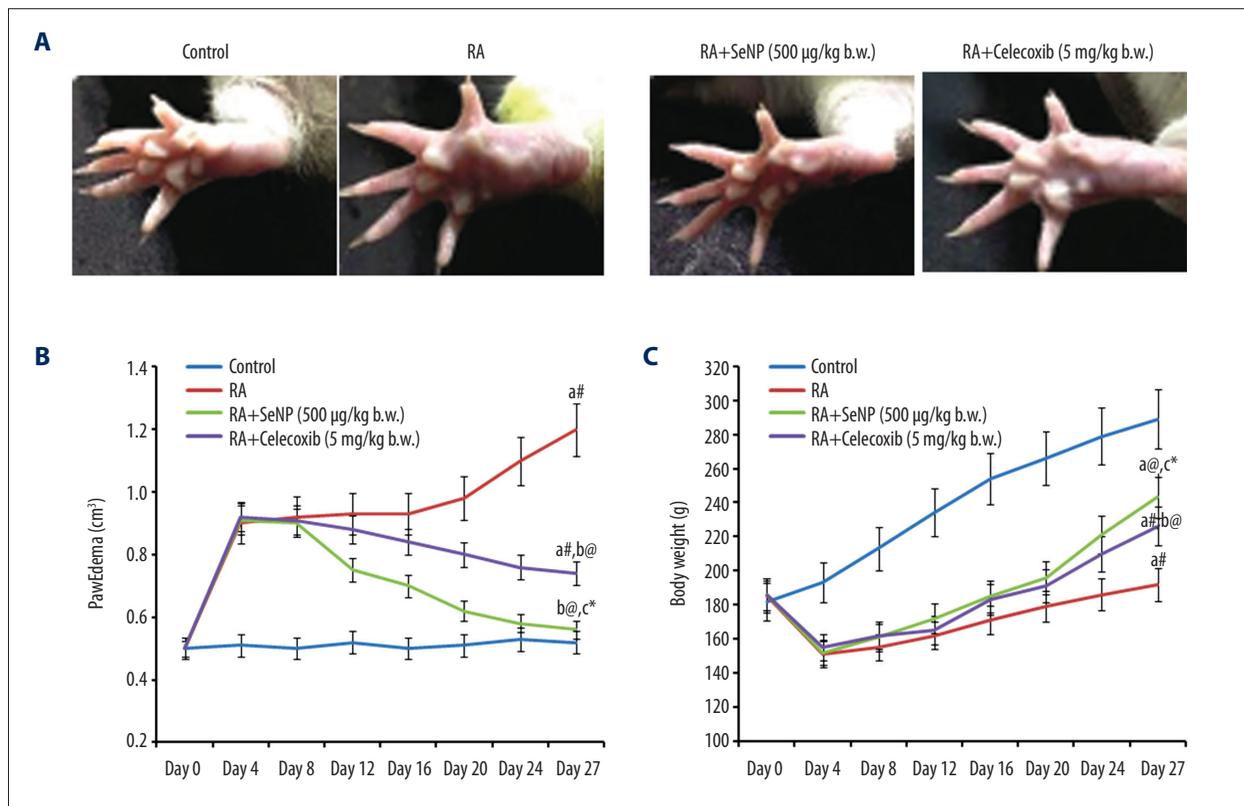
### Statistical analysis

Statistical significance was analyzed by using SigmaPlot 12.0, Systat Software (USA). The comparison of SeNPs supplementation effects were assayed through one-way analysis of variance (ANOVA) followed by appropriate usage of post hoc

Bonferroni *t* tests versus “Control group” or “RA group” or “RA + SeNPs supplementation group” or “RA + Standard drug group” as needed.

### Results

We used commercially available SeNPs. Immediately after the dispersion of SeNPs in double-distilled water, the average size of the SeNPs was  $68 \pm 10$  nm. After 24 h, the average size of the SeNPs was  $984 \pm 192$  nm. The size of the SeNPs was almost 11-fold larger when it is dispersed in water, which could be due to aggregation of NPs. Since phyto-compounds serve as stabilizing agents, SeNPs are dispersed in 1% CA, dissolved in 0.1% DMSO. We found that 1% CA serves as an anti-aggregating substance and as a stabilizing agent. The size of the SeNPs did not increase, even after 24 h (Figure 1).



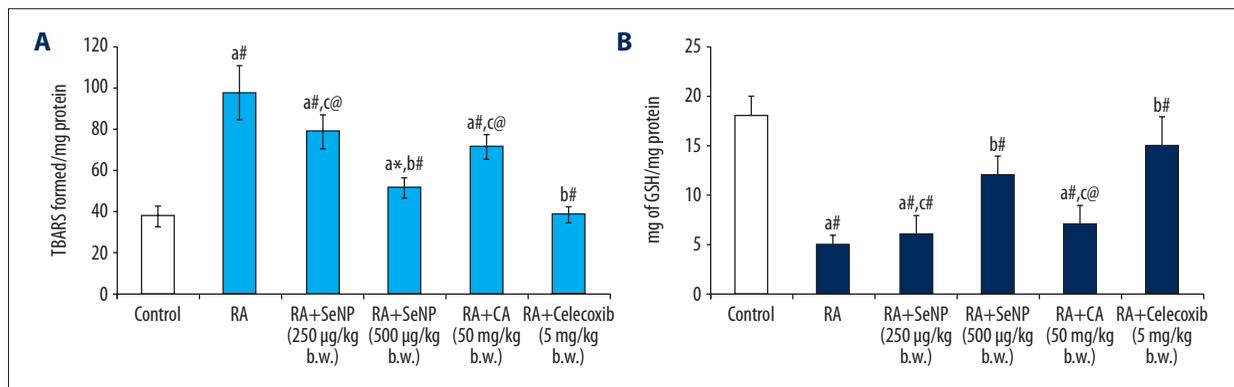
**Figure 2.** Effect of SeNPs on paw edema and body weight changes in different experimental animals. **(A)** Representative image of knee joints in different experimental animals. **(B)** Paw edema (cm<sup>3</sup>) on different days of various experimental groups. **(C)** Average body weight changes in different experimental animals. Values are shown as mean ±SEM (n=6). Differences were analyzed by one-way ANOVA and Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats; 'c' vs. standard drug treated animals. \* *p*<0.05; © *p*<0.01 and # *p*<0.001.

### Effect of SeNPs in physical changes in RA rats

RA animals were treated with 2 different concentrations of SeNPs (250 µg/kg b.w. – Low dose and 500 µg/kg b.w. – medium dose). The SeNPs concentration at 250 µg/kg b.w. did not show statistically significant reduction when compared with standard drug [Celecoxib (5 mg/kg b.w.)] – treated animals. In our experimental conditions, we did not observe a statistically significant effect at 250 µg/kg b.w. of SeNPs. On the other hand, the anti-RA effect of SeNPs was not completely ruled out. RA rats treated with 1% CA alone also did not show statistically significant reduction when compared with standard drug [Celecoxib (5 mg/kg b.w.)] – treated animals. As shown in Figure 2, the paw edema was found to be significantly (*P*<0.001) decreased in SeNPs (500 µg/kg b.w.)-treated animals when compared with RA rats. Interestingly, the reduction (*P*<0.05) in paw edema was significantly greater in SeNPs-treated rats when compared to standard drug [Celecoxib (5 mg/kg b.w.)]-treated animals.

### SeNPs administration attenuated the oxidative stress changes in RA rats

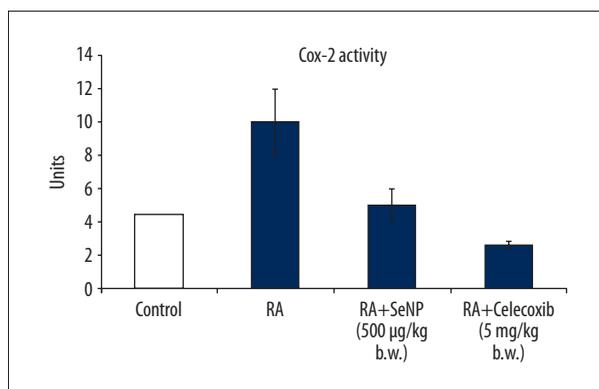
We observed a significant increase (*P*<0.001) in TBARS level in RA rats compared to control animals (Figure 3A), but GSH (*P*<0.001) (Figure 3B) and antioxidant enzymes such as SOD, CAT, and GPx activities (Table 2) declined (*P*<0.001). Upon treatment with SeNPs (500 µg/kg b.w.) the above changes were reverted back to normal. However, we did not observe a statically significant level of protection at 250 µg/kg b.w. of SeNPs. The protective effect of SeNPs (500 µg/kg b.w.) is similar to that of the standard drug used in this study. Further, COX-2 activity (Figure 4) was increased in RA rats (*P*<0.001). Upon treatment with SeNPs (500 µg/kg b.w.) and standard drug, the COX-2 activity was brought back to normal level (*P*<0.01) when compared with normal rats. Further, we carefully assayed the effect of SeNPs (500 µg/kg b.w.) on gene expression of different antioxidant enzymes. We also analyzed mRNA expression of MnSOD, Cu/ZnSOD, ECSOD, CAT, GPx1, GPx2, and COX-2 (Figure 5) in control, RA, SeNPs (500 µg/kg b.w.), and celecoxib-treated animals. MnSOD, Cu/ZnSOD, CAT, and GPx1 levels were found to be lowered (*P*<0.001) in RA rats, but the COX-2 mRNA level was



**Figure 3.** Levels of TBARS and GSH in different experimental groups. (A) TBARS and (B) GSH. Values are shown as mean  $\pm$  SEM (n=6). Differences were analyzed by one-way ANOVA and Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats; 'c' vs. standard drug treated animals. \*  $p < 0.05$ ; #  $p < 0.01$  and @  $p < 0.001$ .

**Table 2.** Effect of SeNP in 1%CA on MSU crystal-induced anti-oxidant status in rats.

Parameter	Control	RA	RA+SeNP (500 µg/kg b.w.)	RA+Celecoxib (5 mg/kg b.w.)
SOD	3.6 $\pm$ 0.08	1.56 $\pm$ 0.02 <sup>a*</sup>	2.89 $\pm$ 0.05 <sup>a*b*</sup>	2.21 $\pm$ 0.08 <sup>b*</sup>
CAT	7.93 $\pm$ 0.36	3.72 $\pm$ 0.04 <sup>a*</sup>	6.42 $\pm$ 0.08 <sup>a*b*c*</sup>	5.86 $\pm$ 0.76 <sup>b*</sup>
GPx	25.9 $\pm$ 2.9	12.2 $\pm$ 1.7 <sup>a*</sup>	21.6 $\pm$ 2.8 <sup>b*c*</sup>	18.9 $\pm$ 2.2 <sup>b*</sup>



**Figure 4.** COX-2 activity in different experimental animals. Values are shown as mean  $\pm$  SEM (n=6). Differences were analyzed by one-way ANOVA and Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats; 'c' vs. standard drug treated animals. \*  $p < 0.05$ ; #  $p < 0.01$  and @  $p < 0.001$ .

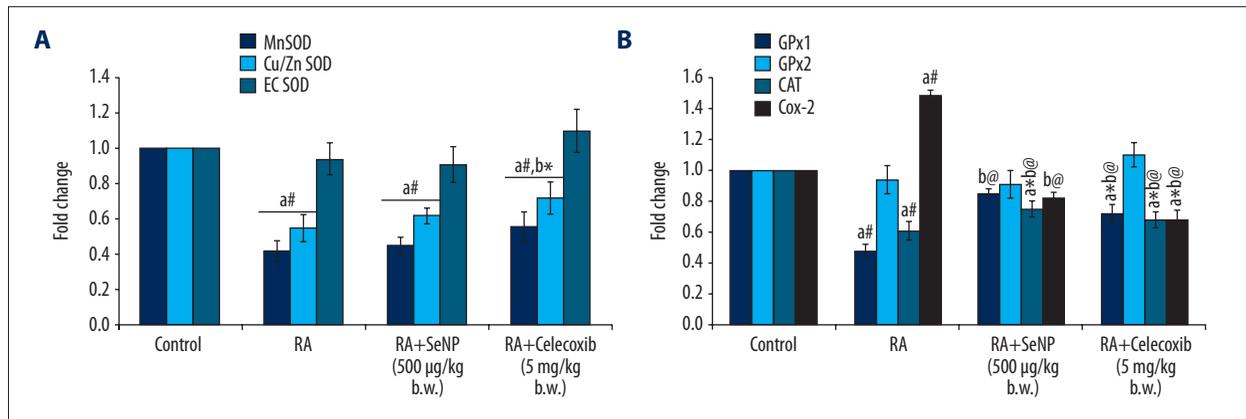
high ( $P < 0.001$ ). CAT and GPx1 mRNA levels were found to be increased ( $P < 0.05$ ), whereas the COX-2 mRNA level was lower in SeNPs-administered rats. We did not observe any statistically significant change in MnSOD, Cu/ZnSOD, ECSOD, or GPx2 of SeNPs-treated animals. To the best of our knowledge there are no reports available on the effect of Se on different types of antioxidant enzyme gene profile in RA rats.

### Inflammatory cytokines

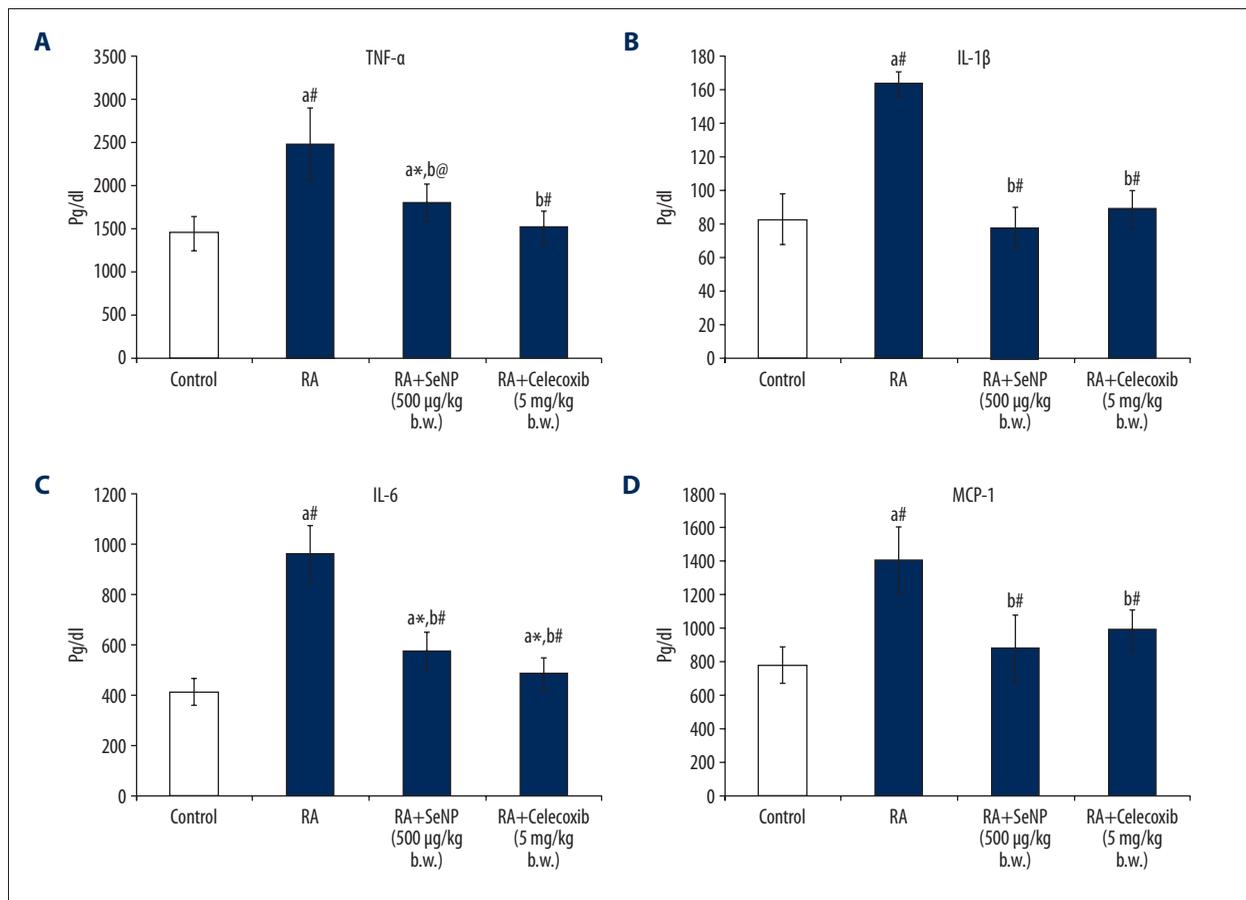
We estimated the TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and MCP-1 levels in angle joint homogenate (Figure 6). These cytokine levels were elevated in ( $P < 0.001$ ) RA rats when compared with control animals. Upon treatment with SeNPs (500 µg/kg b.w.) and standard drug, these inflammatory cytokines were brought back to normal levels (except for TNF- $\alpha$ , at  $P < 0.05$ ) when compared with normal rats. However, when a comparison was made between RA rats and drug-treated animals, TNF- $\alpha$  at  $P < 0.01$  level and IL-1 $\beta$ , IL-6, and MCP-1 were at  $P < 0.001$  level were reduced. When SeNPs-treated animals were compared with standard drug-treated animals, there was no statistically significant difference, indicating that the therapeutic effect of SeNPs is similar to that of standard drugs such as celecoxib. Increased levels of different cytokines in RA rats has been observed by different investigators.

### SeNPs administration on attenuate cartilage degradation in RA rats

After the experimental period, the histological examination was carried out on ankle joints to understand the features such as joint space narrowing and cartilage degradation (Figure 7). In the current study, RA rats showed tattered cartilage and formation of lacunae (Figure 7A). Upon administration of SeNPs to the RA rat model, curtailed cartilage degradation and joint space



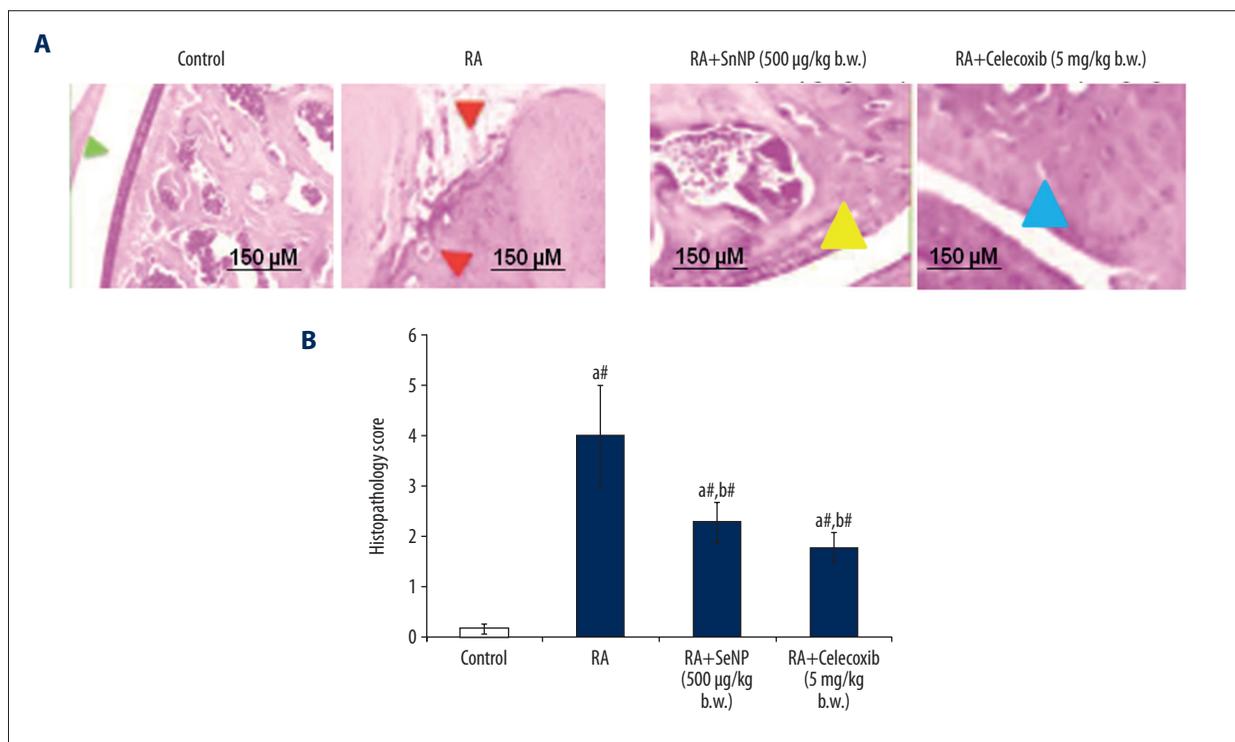
**Figure 5.** Levels of different antioxidant enzyme gene expression levels in control and experimental animals. (A) MnSOD, Cu/ZnSOD, and ECSOD. (B) CAT, GPX1, GPX1-, and COX-2. Values are shown as mean  $\pm$ SEM (n=6). Differences were analyzed by one-way ANOVA and Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats; 'c' vs. standard drug treated animals. \*  $p < 0.05$ ; @  $p < 0.01$  and #  $p < 0.001$ .



**Figure 6.** Levels of pro-inflammatory cytokines in control and experimental animals. (A) TNF $\alpha$ , (B) IL1 $\beta$ , (C) IL6, and (D) MCP-1. Values are shown as mean  $\pm$ SEM (n=6). Differences were analyzed by one-way ANOVA and Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats; 'c' vs. standard drug treated animals. \*  $p < 0.05$ ; @  $p < 0.01$  and #  $p < 0.001$ .

was found to be preserved, similar to that of healthy control animals (Figure 7A, control), whereas in celecoxib-treated animal, ankle joint showed reduced joint space and consequently

degraded cartilage. Histological scoring (Figure 7B) shows that SeNPs have significant therapeutic potential in RA rats.



**Figure 7.** Histological observation of knee joints. (A) Representative images of knee joints of different experimental groups. (B) Histopathological score. Values are mentioned as mean  $\pm$  SEM (n=6). Differences were analyzed by one-way ANOVA Tukey's post hoc test. 'a' vs. control; 'b' vs. RA rats. \*  $p < 0.05$ ; @  $p < 0.01$  and #  $p < 0.001$ .

## Discussion

Recent studies show that nano-compounds could have better efficacy than that of the parent compounds [32]. Further, nano-sized compounds have various advantages in therapy, including low adverse effects. In the present study, we planned to test the effect of SeNPs in RA rats. Before administering SeNPs to RA rats, we tested the stability of SeNPs in water and saline, showing that these are not suitable media. A recent study shows that phytochemical compound could serve as anti-aggregation substances [17]. Since our focus is on the effect of SeNPs against RA, we intended to test a phytochemical already proved to have anti-RA effects; therefore, we tested the anti-aggregation property of CA [36]. A review of the literature showed that CA at 100 mg/Kg b.w. is not toxic to rodents and has anti-RA activity (100 mg/Kg b.w.) [26]. The concentration of CA used in our study is 2.5-fold lower than that used in previous studies. In the present study we tested the effect of SeNPs dispersed in 1% CA against adjuvant-induced RA rats.

As observed by other investigators [35–38], CFA administration reduces body weight in the first week, after which the body weight is spontaneously increases, and the body weight gain is more in SeNPs (500 µg/kg b.w.) – treated rats than in RA rats, indicating that SeNPs dispersed in 1% CA has a promising effect in RA rats. The present study was better designed than

the study by Malhotra et al. [37], which used dextran as a stabilizing agent, but in the present study we used a proven phytochemical as the stabilizing agent. Further, CA can reduce the average size of SeNPs, due to the reductant property of CA. Pain and inflammation at joints develop because of the onset of oxidative stress imbalance in RA [39–41]. Further, it was observed that different antioxidant enzyme activities are reduced in RA rats. Inflammatory cytokines are major culprits in RA. COX-2 is a vital enzyme involved in the generation of different inflammatory cytokines through the modulation of different prostaglandins [42,43]. In the present study we observed increased COX-2 activity in RA rats, and similar results were reported by other investigators [35,40,44].

The effect of normal Se and SeNPs on RA is known [34,45], and epidemiological studies show that serum Se concentration is low in RA patients [8]. These studies indicate demand of Se in RA patients. To the best of our knowledge, this the first report showing that SeNPs restore antioxidant level in ankle joints of RA rats. However, the literature shows that supplementation of Se or SeNPs can restore the antioxidant levels in liver, kidney, and spleen [37,46–49]. SOD, CAT, GPx, and COX-2 activities are reverted in SeNPs-supplemented groups. Gene analysis shows that SeNPs alter CAT, GPx1, and COX-2 levels. Interestingly, we found that a single CAT molecule can convert millions of hydrogen peroxide molecules to water and oxygen every second.

GPx1 is universally expressed by most tissues, where it protects cells from oxidative stress [50]. Further, it localizes to the cytoplasm and mitochondria [50]. It detoxifies hydrogen peroxide to water and also reduces other organic hydroperoxides [50,51]. Increased expression of GPx1 is directly correlated with increased levels of GSH observed in SeNPs-supplemented rats because GPx1 uses GSH as the reductant. We also observed that SeNPs restores COX-2 gene expression in RA rats.

Antioxidant molecules were developed as therapeutic agents to treat various human diseases, and studies show that antioxidants with anti-inflammatory effects are promising drug candidates. Various Se compounds are known to have antioxidant and anti-inflammatory activity [52–54]. In the present study we also assessed the anti-inflammatory effect of SeNPs dispersed in CA. Pro-inflammatory cytokines play major roles in joint pain and other pathological disorders in RA patients [51]. Various studies showed that Se can alter the pro-inflammatory cytokines in different oxidative stress conditions [55–57].

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In the present study we also observed similar findings in RA rats. In addition, studies showed that metalloproteinase activities are altered in RA subjects [58]. Targeting metalloproteinase activity is a new approach to treat RA [58]. In the present study we found that SeNPs can restore normal structure of cartilage, which could be due to restoring the metalloproteinase activity.

## Conclusions

SeNPs dispersed in CA treatment restored different biochemical changes observed in the RA rat model. The antioxidant property of SeNPs was found to increase mRNA expression of CAT, GPx1, and COX-2 levels. Furthermore, pro-inflammatory cytokines and histological changes were stored in SeNPs-treated animals. SeNPs dispersed in CA suggest remarkable therapeutic potential against RA hallmarks, which warrants clinical research.

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