In vitro and in vivo antioxidant, cytotoxic, and anti-chronic inflammatory arthritic effect of selenium nanoparticles

Sonam Malhotra,1 M. N. Welling,2 S. B. Mantri,3 Krutika Desai4
1School of Science, SVKM’s NMIMS, Vile Parle, Mumbai, Maharashtra 400056, India
2SVKM’s NMIMS, Vile Parle, Mumbai, Maharashtra 400056, India
3SVKM’s Narsee Monjee College of Commerce and Economics, Vile Parle, Mumbai, Maharashtra 400056, India
4SVKM’s Mithibai College of Arts, Chauhan Institute of Science, Amrutben Jivanlal College of Commerce and Economics, Vile Parle (W), Mumbai, Maharashtra 400056, India

Received 24 January 2015; revised 13 April 2015; accepted 2 May 2015
Published online 20 May 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.33448

Abstract: The toxicity of selenium (Se) as an antioxidant supplement in the treatment of arthritis is debatable. In this study, Dextrin stabilized Se nanoparticles (SeNP) of size 64 nm ± 0.158 were used to explore its effects as a potent antioxidant with reduced toxicity in both in vitro and in vivo. In vitro toxicity of SeNP was determined using cytotoxicity assay. In vitro interactions of SeNP with DNA and protein was established. Subacute toxicity of SeNP was studied. Wistar rats with complete Freund’s adjuvant induced arthritis were used. Various concentrations of SeNP per kg body weight were fed orally daily up to 21 days. Arthritic profile based on paw swelling, histopathological changes in joints, blood indices, and antioxidant enzymes level in organs such as liver, kidney, and spleen were investigated. Dextrin-SeNP when interacted with NIH-3T3 cells showed 15% cytotoxicity at 100 μg/mL whereas, bulk Se showed 95% at the same concentration. SeNP at 250 μg/mL showed protective effect on DNA. Interaction of SeNP with BSA showed increase in quenching of BSA fluorescence. SeNP did not show any subacute toxicity at concentration as high as 5 mg/kg b.w. in Wistar rats. SeNP at a concentration of 250 μg/kg b.w. acted as potent anti-inflammatory agent and significantly reduced arthritis induced parameters. The enzymatic antioxidant levels in liver, kidney, and spleen were restored significantly (p < 0.05) at 500 μg/kg b.w. while CRP was regained to normal at concentration of 100 μg/kg b.w. concluding SeNP at 500 μg/kg b.w. can be a potential antiarthritic drug supplement. © 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 104B: 993–1003, 2016.

Key Words: Se nanoparticles, rheumatoid arthritis, SOD, CAT, GPx, CRP, PGE2


INTRODUCTION
Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritus of unknown etiology. It affects 1% of the world population with a higher occurrence in women as compared with men. In the last few years, various studies indicate; involvement of reactive oxygen species and other free radicals as mediators of tissue damage and inflammation of joints via numerous pathways.1–3

Common therapeutic agents for the disease management include three major classes: Nonsteroidal anti-inflammatory drugs (NSAIDS), Corticosteroids, and Disease modifying anti-rheumatic drugs. Increasing evidences for the role played by free radicals suggest that antioxidant therapy may represent an alternative or additional approach. Antioxidant supplements such as Vitamin E, folate, zinc, iron, selenium (Se), and so forth have long been advocated for the treatment of RA, osteoarthritis and other inflammatory arthritus.1,4,5 Epidemiological study proposed that low Se status and low a-tocopherol status may be a risk factor for RA independently of rheumatoid factor status,6 marking the importance of antioxidants in the control of progression of this disease. Se is an essential micronutrient normally available from food such as fish and wheat.7 This metalloid is the cofactor of glutathione peroxidase (GPx), its deficiency affects glutathione (GSH) metabolism.6,8 Although, Se possesses potential as a micronutrient supplement for the treatment of RA, the dose and chemical form play an important role in bioavailability, toxicity, and biological property.7 Alternatively, Selenium nanoparticles (SeNP) can be studied for its reduced toxicity and can be a potential antioxidant therapeutic agent in the treatment of RA.

In this study, dextrin stabilized SeNP were used to study in vitro interaction of SeNP with DNA and protein. Further, it was used as an oral supplement in experimentally...
induced chronic inflammatory arthritis in Wistar rat model. To the best of our knowledge and literature, this is the first report determining the antioxidant and anti-inflammatory activity of SeNP in the rat model.

MATERIALS
All the chemicals such as 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), sodium selenite, ascorbic acid, Dulbecco’s Modified Eagle’s Medium (DMEM), dimethyl sulfoxide (DMSO), Dulbecco’s phosphate buffered saline (DPBS), fetal bovine serum (FBS), antibiotics, hydrogen peroxide, copper sulfate, sodium chloride, Ethylenediaminetetraacetic acid (EDTA), and formalin were purchased from Himedia, Sisco Research Laboratory, Cell Clone Genetix, and Qualigens, India. Fat free bovine serum albumin (BSA) and complete freunds adjuvant (CFA) were purchased from Sigma-Aldrich, St. Louis, MO.

METHODS
SeNP were prepared by a wet chemical approach using ascorbic acid as a reducing agent. They were stabilized by coating with 10% dextrin. They were analyzed using various analytical techniques as describes previously. The size of the coated nanoparticles was 64 ± 0.158 nm.

In vitro cytotoxicity of SeNP
Cytotoxicity of uncoated, dextrin coated SeNP (10–100 μg/mL) as well as sodium selenite (bulk form) were studied by MTT cytotoxicity assay using the NIH-3T3 mouse embryonic fibroblast cell line (a gift from Indian Institute of Technology-Bombay). The cell line was maintained in DMEM medium supplemented with 10% FBS and 1% antibiotic (100 U/mL of penicillin, 100 mg/mL of streptomycin) at 37°C in a humidified atmosphere with 5% CO₂; 10,000 cells/well were seeded in a 96-well plate and were grown for 24 h. After 24 h, the medium was replaced with serum free DMEM containing various concentrations of uncoated SeNP, dextrin coated SeNP, and sodium selenite; 5 mg/mL of MTT reagent prepared in DPBS (20 μL/well) was added to each well and incubated at 37°C on shaker for 4 h in dark. 150 μL of DMSO was then added and the solubilised formazan was quantified spectrophotometrically at 570 and 655 nm in an Elisa Reader (Bio-Rad 680).

In vitro interaction of SeNP with DNA
SeNP were tested for their antioxidant effect on DNA following the method of Battin et al. SeNP were also tested for its in vitro toxic effects when interacted with the DNA. pRsetA plasmid DNA (0.1 pmol) was subjected to oxidative damage using H₂O₂. Reaction mixture contained 4 μM CuSO₄, 5 μM ascorbic acid, 130 mM NaCl, and nanoparticles (100, 250, 500, or 1000 μg/mL). After 5 min DNA was added to the reaction mixture. The reaction mixture with the DNA was first treated with 50 μM H₂O₂ for 30 min on rocker and then with 50 mM EDTA. The samples were then electrophoresed in 1% agarose gel to check for the fragmentation and repair.

In vitro interaction of SeNP with protein
Fluorescence spectra analysis. Fluorescence of BSA in the presence and absence of SeNP was studied on spectrophotometer (Jasco 6500, Japan). Different concentrations of SeNP (10–100 μg/mL) were interacted with 1 mg/mL of BSA in phosphate buffer at pH 7.4 with agitation by keeping it on rotary shaker at 200 rpm at 22–25°C. The fluorescence of BSA was observed at different time intervals (30, 120, and 240 min) at the excitation wavelength of 280 nm and emission spectra in the range of 286–450 nm.

In vivo subacute toxicity of SeNP
All animal studies were carried out after the animal ethical clearance from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with the protocol number CPCSEA/IAEC/SPTM/P-37/2012. Wistar rats were purchased from Bharat Serum and Vaccines Pvt. Ltd, Mumbai, India. Before initiation of the study animals were acclimatized to the laboratory conditions. These animals were housed for 7 days under standard conditions of temperature (23 ± 1°C) and 12 h of light and dark cycles before the start of the experiment and were given food and water ad libitum till the end of the study.

Subacute toxicity
Five males and five female Wistar rats (150–200 g: 8–10 weeks) were randomly selected to determine subacute toxicity of the SeNP wherein animals were given a daily oral dose of SeNP at 5 mg/kg body weight adhering to The Organization for Economic Cooperation and Development Guidelines (OECD guidelines 407) for 28 days. The animals were monitored for mortality and physiological changes in breathing (palpitation), eye movement, eye color, writhing effect, vomiting, diarrhoea, body movements, excreta, food and water intake, texture, and color of fur for 28 days daily. At the end of 28 days, the animals were ethically sacrificed. The organs such as liver, kidney, and spleen of 3 male and 3 female rats were dissected and stored in 10% formalin at 22–25°C for histopathological testing.

In vivo anti-chronic inflammatory arthritic study
Grouping of animals. Forty-two male Wistar rats (150–180 g: 8–10 weeks) were randomly divided into the following groups with 6 animals/group:

1. Normal without any disease.
2. Arthritic control which was induced with 0.1 mL of CFA.
3. Standard drug Prednisolone (10 mg/kg b.w.).
4. Se nanoparticles (100 μg/kg b.w.).
5. Se nanoparticles (250 μg/kg b.w.).
6. Se nanoparticles (500 μg/kg b.w.).
7. Se nanoparticles (750 μg/kg b.w.).

Anti-chronic Inflammatory Arthritic study
The treatment groups (Gr IV to VII) were compared with the positive control group, which was the standard marketed drug, Prednisolone (10 mg/kg b.w.) obtained from...
Wyeth, OR. Groups II, III, IV, V, VI, and VII were induced with 0.1 mL of CFA each intradermally in the paw region of the animals. The Gr-III animals were orally fed with prednisolone whereas, Gr-IV to Gr-VII were orally fed 100, 250, 500, and 750 µg/kg b.w. SeNP respectively for 21 days; daily once a day after the onset of the inflammation. The percent increase in inflammation of the paw volume of the animals was monitored using Digital Plethysmometer (ITC life science) daily for 21 days. After 21 days the animals were ethically sacrificed and different organs such as spleen, kidney, and liver were dissected. Blood was collected from each animal by heart puncture. Paw region was dissected and stored in 10% formalin at 22–25°C for histopathological analysis.

Inflammatory marker testing. Blood was collected in vials post sacrifice. Serum was immediately separated from the blood by centrifuging at 1000 g for 10 min at 4°C. Serum was then used for the testing of C-Reactive Protein (CRP) using Aspen-Rapid Diagnostic, India and Prostaglandin E2 (PGE2) using RnD biosystems kit.

Assay of antioxidant enzymes. The dissected organs were weighed 1 g each and homogenized in 5 mL of 10 mM cold phosphate buffer at pH 7.0. Further homogenized samples were centrifuged at 8000 g for 10 min at 4°C. Supernatant was aliquoted and tested for the enzyme levels of superoxide dismutase (SOD), Catalase (CAT), and GPx. The SOD level was estimated using Marklund and Marklund’s method with modifications.17 Autoxidation of pyrogallol was determined for 3 min and used as the sample blank. The reaction mixture consisted of 50 mM Tris-EDTA buffer pH 8.2, 4 mM pyrogallol, and 100 µg of liver protein whereas, 200 µg of kidney and spleen protein. Absorbance was measured at 420 nm for 3 min. The enzyme activity was expressed as units/mg protein, where one unit is the amount of enzyme required to bring about 50% inhibition of the autoxidation of pyrogallol.

The level of CAT was estimated using Chance and Maehly’s method with modifications. The reaction mixture consisted of 200 µg of the protein obtained from the tissue samples and 10 mM potassium phosphate buffer (pH 7.4). The reaction was initiated by adding 19.6 mM H2O2 and the decrease in absorbance at 240 nm was observed for 2 min. The enzyme activity was expressed as units/mg protein, where one unit is the amount of enzyme required to decompose 1.0 µmole of H2O2 per minute.

GPx level was estimated using Rotruck’s method with modifications. The reaction mixture consisted of 0.2 mL of 0.2M phosphate buffer pH 7.6 + 0.1 mL of 10 mM sodium azide + 100 µg of protein from the tissue homogenate + 0.2 mL of 1 mM reduced GSH + 0.1 mL 2 mM H2O2 and the volume was made up to 2 mL with deionized water and incubated at 37°C for 10 min post incubation. 0.4 mL 5% TCA was added and the reaction mixture was centrifuged at 3200g for 20 min; 1 mL of Ellman reagent was added to 0.2 mL supernatant and was incubated at 20–25°C for 5 min. Absorbance was measured at 412 nm. The enzyme activity was expressed as units/mg protein where, one unit of enzyme activity for GPx = 1 µmol of GSH consumed/min.

Statistical Analysis. All the values were expressed as mean (±) standard deviation.

In vitro cell cytotoxicity

Significant difference incell cytotoxicity among the dextrin coated and uncoated nanoparticles as well as sodium selenite was evaluated using Two way ANOVA followed by Bonferroni’s post hoc test considering p < 0.05.

Anti-chronic inflammatory arthritic study in Wistar rat models

Significance in homogeneity among variances (p < 0.05) was tested by Levene’s test. One way ANOVA test was used for
equality of means when there was no significant difference in homogeneity among the variances \( p < 0.05 \) and in case of significant difference \( p > 0.05 \) in homogeneity among the variances Welch and Brown-Forsythe's test was used. This was followed by Games-Howell's post hoc test \( p < 0.05 \). All the analyses of the data were carried out using SPSS version 19.

### RESULTS

**In vitro cell cytotoxicity**

As per the cell cytotoxicity assay (Figure 1) sodium selenite was observed to be highly toxic to the cell retaining 5% viability of cells at the dose of 100 \( \mu \text{g/mL} \), whereas dextrin coated and uncoated SeNP prepared from the same source of Se retained 75% and 65% viability of cells respectively at 100 \( \mu \text{g/mL} \).

---

**FIGURE 2.** Agarose gel electrophoresis (1%) demonstrating the interaction of dextrin coated SeNP with DNA (1) EcoR1/Hind III marker, (2) DNA, (3) DNA + \( \text{H}_2\text{O}_2 \), (4) DNA + \( \text{H}_2\text{O}_2 \) + Se (100 \( \mu \text{g/mL} \)), (5) DNA + \( \text{H}_2\text{O}_2 \) + Se (1000 \( \mu \text{g/mL} \)), (6) DNA + \( \text{H}_2\text{O}_2 \) + Cu/ascorbic acid, (7-10) DNA + \( \text{H}_2\text{O}_2 \) + Cu/ascorbic acid + Se in increasing concentration (100, 250, 500 and 1000 \( \mu \text{g/mL} \)).

**FIGURE 3.** Interaction of dextrin coated SeNP with BSA. Effect of different concentrations of Se nanoparticles (10–100 \( \mu \text{g/mL} \)) on BSA at (A) 30 min, (B) 120 min, and (C) 4 h.
Interaction of SeNP with DNA
SeNP at concentrations of 100 and 1000 μg/mL when interacted with DNA for 30 min, did not show any toxic effect on the DNA (Figure 2). It is interesting to note that SeNP at a low concentration of 250 μg/mL could protect the DNA from the action of H$_2$O$_2$, thus preventing the oxidative damage of the DNA.

Interaction of SeNP with protein
The SeNP when interacted with protein (BSA) at different concentrations [Figure 3(a–c)] showed a fluorescence quenching mechanism measured using fluorescence spectrophotometer. BSA having an excitation wavelength at 278 nm and emission spectra in the range of 286–450 nm showed that the increase in time of interaction from 30 to 240 min with increasing concentrations of SeNP from 10–100 μg/mL showed quenching of the fluorescence in BSA.

In vivo subacute toxicity
The repeat dose toxicity was monitored daily for 28 days. Mortality was not observed. Physiological changes such as abnormal breathing (palpitation), eye movement, eye color, writhing effect, vomiting, diarrhoea, body movements, excreta, food and water intake, texture, and color of fur were not observed in the animals under study. No histopathological changes in organs such as liver, spleen, and kidney were observed [Figure 4(a–c)]. Subacute toxicity study did not show any signs of abnormality as no physiological or histopathological changes were observed in the rats and its organs.

Anti-chronic inflammatory arthritic study
Chronic inflammatory arthritis was experimentally induced in Wistar rats using CFA. All animals showed inflammation in the paw region, which was observed for 21 days using Digital Plethysmometer. Percent increase in the paw size...
(Figure 5) was calculated on day 1 and day 21 in the diseased group of rats. Levene’s test confirmed that there was no significant difference in homogeneity among the variances of arthritic group and the treated groups. Hence, one way ANOVA was considered for the equality of means and it was found that, at \( p < 0.05 \) there was a significant difference between the arthritic group and the treated groups. Games-Howell’s test showed a significant difference \( (p < 0.05) \) in percent paw volume when treated with SeNP at 250 \( \mu \)g/kg b.w. concentration and prednisolone as compared with the arthritic group. From the descriptive statistics it is evident that SeNP at 250 \( \mu \)g/kg b.w. concentration and prednisolone at 10 mg/kg b.w. decreased the paw volume when compared with arthritic group.

**Antioxidant enzyme levels**

Antioxidant enzyme, namely, SOD, GPx, and CAT levels in liver, spleen, and kidney in normal control, arthritic control, and treated groups were checked at the end of 21 days. Levene’s test confirmed that there was no significant difference in homogeneity among the variances of normal control group, arthritic group and the treated groups from each of the organs, liver, kidney, and spleen. Hence, one way ANOVA was considered for the equality of means for these enzymes in each of the organs. A significant difference \( (p < 0.05) \) was observed amongst the normal control group, arthritic group and the treated groups in the levels of SOD, CAT, and GPx in each of the organs. Games-Howell’s test showed that the antioxidant enzyme status of these animals in their liver, kidney, and spleen had changed significantly \( (p < 0.05) \) in the diseased group of animals with respect to normal control group [Figure 6(a–c)]. From the descriptive statistics, it was observed that antioxidant enzyme status of the diseased group of animals had decreased significantly as compared with that of normal control group in all the organs studied.

According to the Games-Howell’s test it was observed that the SOD and GPx level in liver, restored normal levels in SeNP treated group at 500 \( \mu \)g/kg b.w. concentration \( (p < 0.05) \), whereas CAT level was restored in SeNP treated group at 750 \( \mu \)g/kg b.w. In kidney and spleen SOD level, restored normal levels in SeNP treated group at 500 \( \mu \)g/kg b.w; GPx and CAT level were restored in SeNP treated group at 100 and 250 \( \mu \)g/kg b.w.

**Inflammatory biomarkers**

**Level of CRP**

Levene’s test confirmed that there was significant difference \( (p < 0.05) \) in homogeneity among the variances of normal control group, arthritic group and the treated groups. Hence, Welch & Brown-Forsythe’s tests were considered for the equality of means for these groups. In arthritic control group CRP level increased 8.5 fold [Figure 7(a)]. According to Games-Howell’s test SeNP treated group at a concentration of 100 \( \mu \)g/kg b.w could restore the normal CRP level significantly \( (p < 0.05) \).

**Level of PGE2**

Levene’s test confirmed that there was no significant difference \( (p < 0.05) \) in homogeneity among the variances of normal control group, arthritic group and the treated groups. Hence, one way ANOVA was considered for the equality of means for these groups. The PGE2 levels [Figure 7(b)] did not show a significant difference \( (p < 0.05) \) in the arthritic group of rats as compared to the normal control. From descriptive statistics it was observed that on an average...
PGE$_2$ levels were not significantly higher in the arthritic group as compared with normal control group. Surprisingly, the animals treated with concentration of 100 $\mu$g/kg b.w.of SeNP showed significant difference ($p < 0.05$) in the PGE$_2$ levels as compared to normal control group and arthritic group. From the descriptive statistics it was observed that the PGE$_2$ values in case of SeNP treated group at 100 $\mu$g/kg b.w. concentration were significantly less than those in case of normal as well as arthritic control groups.

Histopathological results [Figure 8(a–g)] of the paw region supported the data as minimal inflammatory cells were observed in the group treated with SeNP (750 $\mu$g/kg b.w.).

**DISCUSSION**

*In vitro* cytotoxicity of dextrin coated SeNP, uncoated as well as sodium selenite was evaluated using MTT on NIH-3T3 fibroblast cell line. At 100 $\mu$g/mL, coated SeNP showed least toxicity of 15% and uncoated showed 35% toxicity.
whereas, bulk Se showed 95% toxicity indicating changes in the properties of Se, which possibly resulted in decrease of cell toxicity (Figure 1). The IC50 values of coated SeNP, uncoated SeNP and bulk Se estimated were 149 ± 0.5, 87 ± 0.7, and 50 ± 0.36 μg/mL, respectively. In the studies demonstrated by Forootanfar et al., biogenic SeNP showed IC50 at 41.5 ± 0.9 μg/mL; whereas bulk Se (SeO2) showed IC50 at a low concentration of 6.7 ± 0.8 μg/mL, which concluded that the SeNP prepared by different methods as well as from different sources show difference in the toxicity levels. Bulk Se is more toxic as compared with Se nanoparticles irrespective of their method of preparation.

In addition to cytotoxicity, nanoparticles were interacted with macromolecules such as DNA and protein. Interaction of metal nanoparticles with macromolecules is a major concern with respect to its toxicity, bioavailability, and altering these molecules. SeNP not only prevented DNA damage but also protected the DNA from oxidants such as H2O2. DNA was subjected to damage using copper and H2O2, a Fenton-type reaction and the inhibition of this damage was monitored by treating different concentrations of SeNP. The Se compounds are known to inhibit the oxidative damage by the metal coordination mechanism (Battin et al., 2006). Different forms of organic Se compounds such as selenomethionine and selenocystine at 10 μM concentration inhibited 46 and 96% DNA damage respectively whereas, 2-aminophenyldiselenide a seleno-organic compound showed no effect. In this study, SeNP at the concentration of 250 μg/mL inhibited DNA damage. This is the first study reporting interaction of SeNP with DNA.

FIGURE 8. Microphotograph of paw region of arthritic group compared to normal rats. (1) Skin layer. (2) Subcutaneous tissue with fluid filled vacuoles and infiltrated mononuclear cells. (3) Muscle layer with inflammatory changes. (4) Necrosis in the bone tissue. Microphotograph of paw region of prednisolone (10 mg/kg b.w.) treated group shows (1) Skin layer. (2) Subcutaneous tissue. (3) Muscle layer around bony tissue. (4) Normal Bone marrow tissue. Microphotograph of paw region of SeNP (100, 250, 500, and 750 μg/kg b.w.) treated group shows (1) Skin layer. (2) Subcutaneous tissue with mild to minimal inflammatory changes from (100 to 750 μg/kg b.w.). (3) Muscle layer surrounding the bone. (4) Normal Bone marrow tissue. H&E stain.
Protein interaction was monitored by the fluorescence quenching mechanism which was reported for the first time with SeNP. As the nanoparticles enter the system they interact with proteins forming a nanoparticles-protein corona and modifying the surface of the nanoparticles in the biological medium influencing cellular uptake, inflammation, accumulation, degradation and clearance of the nanoparticles. Intrinsic fluorescence emission spectra was studied giving a defined view. It was observed that as the interaction increases; the amino acids emitting fluorescence present in the protein sample decreases its fluorescence; showing a fluorescence quenching mechanism. This quenching could be due to protein bound to substrates, protein conformational changes or denaturation. Although, BSA acquires overall negative charge at pH 7.2 but is still capable of binding to SeNP, which are also negatively charged as BSA is covered with 60 surface lysine residues that can be protonated, which might help in binding to the SeNP electrostatically. Additionally hydrophobic interactions may be involved in binding of BSA to SeNP. Spectrofluorometric study showed time dependent decrease in the intrinsic fluorescence of BSA with different concentrations of SeNP as the fluorescence emitting amino acids present on BSA such as tryptophan, tyrosine and phenylalanine get altered if nanoparticles bind to it. BSA contains two tryptophan residues that is, Trp-213 present in the hydrophobic environment and Trp-134, which is situated on the protein surface and is available for interaction with the phenylalanine and tyrosine, which are responsible for intrinsic fluorescence. The fluorescence is widely used to study the changes in the proteins as its excited state dipole moment is relatively large and its emission is sensitive to the polarity of the environment at $\lambda = 350$ nm. As reported by Shi et al. it is suspected that majority of the quenching is from the Trp-134 as it is more readily exposed on the surface of the BSA hence, available for the SeNP to form a conjugate and the conformation of the protein was not distorted as there was no large significant difference in the peak shift of the protein.

Following the in vitro toxicity, SeNP were analysed for their effect in the animals using Wistar rats wherein none of the rats showed any physiological or histopathological signs of toxicity at 5 mg/kg.b.w. of SeNP deciphering the limit of the safety index of drug administration in the body for the treatment of the disease. As studied earlier by Zhang et al. sodium selenite at 6 mg/kg.b.w. induced sub acute toxicity over 10 days disrupting their antioxidant balance and a threefold increase in lipid peroxidation concluding Nano Se is less toxic as compared to selenite in short term toxicity. Toxicity of Se is found not only in inorganic but in organic forms as well. According to Spallholz and Hoffman, nonspecific long term retention in proteins makes the organic forms such as selenomethionine and selenocystiene more toxic in long term consumption.

RA is a devastating inflammatory disease of unknown etiology, disturbing antioxidant balance of the tissues. Deficiency of certain micronutrients and vitamins accelerate the progression of the disease. Patients with RA have decreased Se level as compared with normal individuals indicating that Se supplements can be used as a complementary medicine in the treatment of RA. Due to the thin line between the essentiality and toxicity of Se the form of Se that is beneficial and least toxic has been under debate. Hawkes et al. reported the tolerable dose of selenomethionine to be 150 $\mu$g Se/kg b.w. Similarly, another study carried out by Institut Rosell for 28-days in the Wistar rats showed that at 1 mg/kg.b.w. of yeast enriched with organic Se reduced the food consumption and body weight of the animals. They also observed that there were hematological, histopathological, and clinical chemistry changes indicative of hepatotoxicity in rats. Various studies carried out using organo Se enriched yeast contained selenomethionine as the source of Se. These results conclusively prove that at higher doses even organic forms of Se or yeasts enriched with Se show toxicity. Hence, SeNP can be used as a better alternative form of Se as micronutrient. In this study, the SeNP a new form of Se with reduced toxicity was administered orally for 21 days at different concentrations in comparison with the standard drug (Prednisolone 10 mg/kg b.w) in rats, which were all experimentally induced chronic inflammatory arthritis using CFA. The prognosis of the disease was studied daily for 21 days which further helped in understanding the day by day swelling in the paw region where CFA was injected and showed that from 1st day till the 21st day there was a reduction in the paw volume of the rats treated with SeNP. Dose dependant effect of SeNP was observed in the reduction of paw volume wherein 100 $\mu$g/kg b.w. did not show satisfactory reduction as compared with 250 $\mu$g/kg b.w. Various enzymatic and non-enzymatic parameters such as SOD, CAT, GPx, and CRP, PGE$_2$, respectively, were analyzed for monitoring the effect of the drug in the treatment of chronic inflammatory arthritis after 21 days. CRP and PGE$_2$ test were carried out to analyse the disease condition in comparison with the normal group of animals. PGE$_2$ did not show a significant increase in the arthritic group which indicates that the inflammation created by CFA did not show its action on the COX 2 enzymes. PGE$_2$ did not show significant decrease in the levels in the treated group except for the 100 $\mu$g/kg b.w. dosage, which showed a significant decrease from the disease group levels. This could be because of the standard drug being a glucocorticoid and the selective activity of Se nanoparticles on the COX 2 enzyme; given that COX 1 and 2 both enzymes are responsible for the increased levels of PGE$_2$ in the system. These results are in line with the previously reported study by Prempeh and Mensah wherein, nimesulide, a glucocorticoid was concluded to possess a selective activity toward COX 2 enzymes and the drugs did not show any activity on decreasing the levels of PGE$_2$, which could also be because the dosage of the drugs used. It might not be sufficient enough to alter the levels of PGE$_2$ or that COX 1 and COX 2 coexist in noninflammatory state with COX 2 occurring in minute quantity.

The antioxidant enzyme status was observed in various organs such as liver, kidney, and spleen after 21 days of the treatment. SOD, CAT, and GPx levels decrease in case of
disease group of animals due to oxidative stress in the body in all the organs as reported by Devi et al.\textsuperscript{34} stating the use of CFA as an inducer of chronic inflammatory arthritis and \textit{Vitex negundo} leaf extract upto 50 mg/kg b.w. as the dietary cure for the disease. Also the traditionally marketed drugs such as prednisolone was given at 10 mg/kg b.w. as reported by Suike et al.\textsuperscript{15} which is a higher concentration with increased toxicity as compared with the nontoxic SeNP administered at a lower concentration. An interesting aspect of the study was that animals treated with SeNP showed a significant restoration of the antioxidant enzyme levels than the prednisolone drug (10 mg/kg b.w.).

Histopathological study was carried out to analyse the tissue level damage in the local area surrounding the bone of the paw region. Microphotographic data also represented the positive effect of treatment of the Se nanoparticle at various concentrations from the severe damages observed in the disease group of animals to the minimal changes observed in the SeNP treated group (750 μg/kg b.w.).

**CONCLUSION**

SeNP prepared using a facile wet chemical approach, coated with 10% dextrin were found to be non-toxic at \textit{in vitro} and \textit{in vivo} conditions unlike the bulk form of Se at the same concentration. Further, their antioxidant properties were explored to be used as a potent therapeutic drug supplement against RA. Considering all the parameters studied it was observed that oral administration of SeNP at a concentration of 500 μg/kg b.w. upto 21 days daily showed a significant effect on experimentally induced chronic inflammatory arthritis in Wistar rats and can be considered as a potential complementary medicine for the treatment of RA. In future, combination studies of the drugs and SeNP at different concentrations can be carried out which will help in reduction of the doses of the steroidal drugs hence reducing their side effects.

**ACKNOWLEDGMENTS**

The authors would like to thank Dr. V. G. Gaikar from Institute of Chemical Technology, Mumbai, India for providing the laboratory facility for fluorescence spectroscopy.

**REFERENCES**


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具