



Selenium nanoparticles are less toxic than inorganic and organic selenium to mice in vivo

Arin Bhattacharjee^{1,2} · Abhishek Basu^{1,3} · Sudin Bhattacharya^{1,4}

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Abstract

Nanoparticles (NPs) provide versatile means to reduce the toxicity, enhance bioactivity and improve targeting of cells. The antioxidant and pro-oxidant effects, or bioavailability and toxicity, of selenium depend on its chemical form. In the present study the effects of nano-selenium (Nano-Se) was compared with inorganic and organic selenium on the basis of their antioxidative activities and hematological parameters in Swiss albino mice. At an oral dose of 2 mg Se/kg b.w. per day administered for consecutive 28 days, both forms of selenium suppressed mice growth rather than Nano-Se. Abnormal liver and kidney function were more pronounced with selenite treatment than Nano-Se as indicated by the increase of hepatotoxic and renal toxic marker in serum and also confirmed by histological examination. After being treated with different forms of selenium it can be seen that the activity of enzymes have increased considerably in case of Nano-Se. Synthesized selenium nanoparticles, caused less bone marrow cell death and prevented DNA damage, compared to other forms of selenium. Our results suggest that Nano-Se as an antioxidant can serve as a potential chemopreventive agent with reduced risk of selenium toxicity.

Keywords Nanoparticle of selenium (Nano-Se) · Oxidative stress · Toxicity · Phase II detoxifying enzymes · Comet assay

Introduction

Oxidative stress is implicated in the development of chronic and degenerative disorders such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. To counteract the oxidative stress human body can produce antioxidants, which are either naturally synthesized endogenously or supplied exogenously through foods and/or supplements. The antioxidants in their turn prevent and neutralize the damages caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS), and

enhance the immune defense and lower the risk of cancer and degenerative diseases [1].

The modern day research in cancer chemotherapy is aiming to find ways to minimize the toxicity of standard cancer chemotherapeutic drugs. The past 20 years have seen significant advances in the treatment of cancer. Despite the improvement in cancer chemotherapy, the outcome is seriously affected due to drug resistance and severe adverse effects and oxidative stress plays a major role in it.

One of the efficient first lines of endogenous defenses against free radicals consists of some selenium containing enzymes. Selenium (Se) is an essential and unique trace element for humans of every age mainly because of the antioxidant effects of the selenoproteins such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR), selenoproteins P etc. and plays a crucial role in health and disease [13]. Selenium as a co-factor of these antioxidants takes part in scavenging free radicals. It protects enzymes and nucleic acids from the harmful effects of ROS, prevents cells, membranes and cell organelles from lipid peroxidation [53]. Being a cofactor of these antioxidant enzymes selenium takes part in scavenging free radicals, thus protecting cells, membranes and cell organelles from lipid peroxidation, enzymes and nucleic acids from the harmful effects of ROS [53]. Thus, Se

Sudin Bhattacharya: Retired.

✉ Sudin Bhattacharya
sudinb19572004@yahoo.co.in

¹ Chittaranjan National Cancer Institute, Kolkata 700026, India

² Present Address: Global College of Pharmaceutical Technology, Palpara More, Krishnagar, Nadia, West Bengal 741102, India

³ Present Address: Bengaluru, India

⁴ Present Address: South 24 Parganas 700148, India

functions in the body as an antioxidant, in thyroid hormone metabolism, redox reactions, reproduction, and immune functions [55].

Diet is the most important Se source, and intake of this essential element depends on its concentration in food and amount of food consumed [45]. A wide variety of selenium compounds exists, both in organic forms as well as in inorganic forms. In daily life, we ingest these selenicals with our ordinary diet. According to the Food and Nutrition Board, Institute of Medicine of the National Academics, US, the recommended dietary allowance (RDA) is 55 µg Se/day and the dose for 19–50 year old man and woman is 45 µg of Se/day as estimated average requirements (EAR). The tolerable upper intake level is 400 µg Se/day [51]. But there are variations in the dietary recommendation between the countries, ranging from 25 to 100 µg Se/day [21]. RDA for children of 1–3 years is 20 µg Se/day and that for breast feeding mothers is 70 µg Se/day [25]. The optimum serum selenium concentration for healthy adults as recommended by the World Health Organization is 39.5–194.5 ng/ml. The maximum allowable concentration (MCL) in drinking water was reported 50 ppb (0.05 mg/l). It was reported that the lowest observed adverse effect level (LOAEL) for selenium is 1540–1600 µg Se/day and above that is toxic. Hair, toe, fingernail loss and garlic like odour in the breath are associated with LOAEL symptoms whereas acute respiratory problem, renal failure and myocardial infarction are the causative effect of selenium induced toxicity [22].

Besides that disruption of endocrine function, synthesis of thyroid hormones and growth hormones and insulin-like growth factor metabolism occurs [36]. These data support the importance of proper selenium intake but Se has a narrow window between beneficial and toxic dose limiting its use in therapy and prevention [42]. Apart from that, depending on chemical form, dose and duration of intake, Se can be toxic to the subjects such as invertebrates [54], fishes [3], amphibians and reptiles [8], birds [16], mammals [2, 15, 33] and humans [2, 34, 59]. Tolerance for Se toxicity depends on, among other factors, the rate of excretion, and Se excretion depends on the rate of methylation of Se [19]. Inorganic forms of Se reacts with glutathione [13] to form seleno-trisulphides and those reacting with other thiols generate oxygen free radicals, such as superoxide anion (O_2^-) by redox catalysis [48]. Inorganic selenium compounds impart genotoxicity resulting systemic toxicity where as organoselenium compounds though not devoid of toxicities are much less toxic. Organic diselenides (e.g. selenocystine and selenocystamine are converted into selenols (RSeH) in presence of thiols which also results in ROS generation. Further reductions that lead to the formation of superoxide under aerobic conditions in the presence of thiol could play a role in the toxicity of diselenide and alkyl selenide [11]. Free radical hypothesis of selenium toxicosis is based

on the methyl-selenide formation, which also results into superoxide radicals and subsequent oxidative stress. Excess selenium in the form of selenocystine inhibits the methylation of selenium and increases the amount of intermediary metabolite, hydrogen-selenide, which can also be toxic [14].

In order to reduce the toxicity of Se, nanotechnology emerged as a promising field with great potential to give breakthroughs that can be applied in real life. Therefore, synthesis and characterization of the nanoparticles gained importance on account of their therapeutic applications [24]. Because of reduced size, large surface to volume ratio and the difference in their properties of surface atom [35] the nanoparticles and have incited more interest and are being exploited by drug developers to gain enhanced efficiency over conventional systems in therapeutics. In this regard, nanoparticles of selenium (Nano Se), as a new form of selenium, emerge as a promising candidate due to their unique biological activities [20].

In our earlier study we have synthesized Nano-Se and evaluated its chemoprotective activity against cyclophosphamide (CP)-induced hepatotoxicity, pulmonary toxicity and genotoxicity in vivo in Swiss albino mice [6, 7]. In this study, we have compared the sub-acute toxicity of three Se (organic, inorganic and nano) forms in Swiss albino mice. We also evaluated antioxidative properties of Nano-Se on murine bone marrow cells for the first time. The results obtained from the above study will help select suitable and the safe form of Se to be further incorporated in the formulation of Se-containing pharmaceuticals available in the market.

Materials and methods

Chemicals

Major chemicals were procured from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. They included Sodium selenite, 1-chloro-2,4-dinitrobenzene (CDNB), ethylene diamine tetraacetic acid (EDTA), reduced glutathione (GSH), pyrogallol, 5,5'-dithio-bis(2-nitro benzoic acid) (DTNB), sodium dodecyl sulphate (SDS), bovine serum albumin (BSA), β -nicotinamide adenine di nucleotide phosphate (reduced), glutathione reductase, normal melting agarose, low melting point agarose, ethidium bromide, sodium azide (NaN_3), dimethyl sulphoxide (DMSO) and Triton-X 100. Giemsa stain and the other chemicals were purchased from Merck (India) Limited, Mumbai, India and were of analytical grade.

Experimental animals

Swiss albino female mice weighing approximately 23–25 g and of 5–6 weeks old were requisitioned from the animal house of Chittaranjan National Cancer Institute (CNCI), Kolkata. The animals were maintained at controlled conditions (20–25 °C and 18/6 h light cycle). They had free access to standard food pellets and drinking water. All experiments were carried out following the Institutional Guidelines for the Care and Use of Laboratory Animals (CPCSEA Registration. No. 1774/GO/RBi/S/14/CPCSEA).

Preparation and characterization of nano selenium

Synthesis of Nano-Se was carried out following the method of Zhang et al. [60] with slight modifications and characterized for physic-chemical properties as reported in our earlier study [7].

The synthesized Nano-Se was dissolved in saline (0.9% NaCl) and administered orally. 1,4-Phenylene-bis(methylene)selenocyanate and sodium selenite (Na_2SeO_3) was used as a suspension and solution in saline (0.9% NaCl), respectively. The Se-solutions/suspensions were prepared on each day just before treatment and were administered by oral gavages for 28 days. Each animal of the normal control group received saline (0.9% NaCl) during the entire experimental period.

Selection of the dose of Nano-Se was based on our preliminary toxicity studies carried for 28 days. Different parameters of toxicity were carried out with 2, 3 and 4 mg Se/kg body weight and 2 mg Se/kg body weight was the non toxic dose preferred (unpublished data).

Experimental design

The animals were divided into four groups containing six ($n=6$) animals in each.

Group I-(Vehicle control): Each animal received saline (0.9% NaCl).

Group II-(Sodium selenite): Animals were gavaged 2 mg Se/kg b.w.

Group III-(1,4-phenylene-bis(methylene)selenocyanate): Animals were gavaged 2 mg Se/kg b.w.

Group IV-(Nano selenium): Animals were gavaged 2 mg Se/kg b.w.

All animals were sacrificed on day 29, and the following parameters were studied.

Hematopathological studies

Isolation of blood and hematopathological studies

Before sacrifice the animals were fasted for 4 h and blood samples were collected under anesthesia from the retro-orbital venous plexus. Red blood cell (RBC) and white blood cell (WBC) counts were made done following standard procedure [12, 57]. Blood hemoglobin was measured following the method of Sahil [46].

Preparation of bone marrow cells and splenocytes

Bone marrow cells were collected from femur bones [7]. The splenocyte cells were processed from spleen and was counted in hemocytometer [6].

Genotoxicity studies

For genotoxicity studies bone marrow cells were prepared for detection of DNA damage (comet assay) and chromosome aberrations following standard procedures as reported [7, 9, 49].

Analysis of chromosomal aberrations included stretching, terminal association, break, fragment, ring formation in metaphase cells. A total of 300 bone marrow cells were observed, 60 from each of 5 mice per treatment set.

Estimation of oxidative and antioxidant stress

The level of lipid peroxidation (LPO) was estimated in liver, lung and kidney microsomal fraction. Thiobarbituric acid (TBA) was used to measure the level of lipid peroxides and expressed as nmol of thiobarbituric acid reactive substances (TBARS) formed per mg of protein using the extinction co-efficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ [39]. Other enzymatic activities were measured in liver cytosols prepared as described earlier [6].

The activity of SOD was determined by inhibition of pyrogallol auto-oxidation and expressed as unit/mg of protein measured as absorbance at 420 nm. One unit of enzyme activity is the amount of enzyme necessary for inhibiting the reaction by 50% auto-oxidation of pyrogallol in Tris-HCL buffer (50 mM, pH 7.50 [30, 32]. Catalase (CAT) activity was measured as the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 per second at 25 °C and expressed as unit/mg of protein at 250 nm [29]. Estimation of reduced glutathione (GSH) level by determination of DTNB reduced by—SH groups in a spectrophotometer at 412 nm. The level of GSH was expressed as nmol/mg of protein [47]. Glutathione-S-transferase (GST) activity was expressed as formation of CDNB-GSH conjugate/min/mg of protein (with CDNB as the substrate) and determined from the increase in absorbance

at 340 nm [17]. Estimation of glutathione peroxidase (GPx) activity was measured by NADPH oxidation using a coupled reaction system consisting of GSH, glutathione reductase and H₂O₂ [40] and expressed as micromole NADPH utilized/min/mg of protein, using extinction co-efficient of NADPH at 340 nm [6]. The activity of thioredoxin reductase activity (TrxR) was measured by a spectrophotometer at 412 nm [37]. The reaction is based on the reduction of DTNB with NADPH to TNB.

Total protein content in tissue homogenate was measured by the method of Lowry using Folin-phenol reagent [27]. The absorbance was measured at 660 nm using the TECAN Infinite® 200 PRO Multimode Reader.

Determination of serum enzymes (ALT, AST and ALP) for liver function test

Blood samples collected from the retro-orbital venous plexus of mice were centrifuged for 10 min at 2000 g. Following standard methods serum alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activity was measured using spectrophotometer [23, 43].

Determination of blood urea nitrogen (BUN) and creatinine levels for kidney function test

Under anesthesia mice blood samples were collected by retro-orbital puncture and were centrifuged at 2000g for 5 min. Serum BUN and creatinine levels were measured by spectrophotometer following standard methods [10, 31].

Processing of tissue samples and histopathological evaluation

For histopathological evaluation, liver lungs and kidney tissues were excised immediately after dissection and processed for histopathology [7]. The stained sections were evaluated by light microscopy (Leica DM 1000) and photomicrographs were taken.

Statistical analysis

For statistical analysis the data were analyzed by One way ANOVA followed by Tukey's Multiple Comparison Test using Graph Pad Prism (Version 5.00). All values are mean \pm SD of six animals. Values were considered significant at $P < 0.05$.

Results

Characterization of Nano-selenium was reported in our earlier study [7]. The particle size ranged between 40 and 55 nm with the average size of 35 nm and a majority of

the nano particles were in the size range of 30–40 nm. The purity of the substance was confirmed by EDAX analysis and confirmed the presence of elemental selenium (64%).

Changes in body weight

Body weight of the animals gavaged with inorganic (Gr. II) and organic selenium (Gr. III) at the dose of 2 mg Se/kg b.w. decreased significantly by 22.13 and 6.32%, respectively, in comparison to the vehicle control group (Gr. I). Oral administration of Nano-Se (Gr. IV) at the same dose showed a significant increase of 9.32% in the body weight of the animals (data not shown for the sake of brevity).

Hematology and clinical chemistry of bone marrow and spleen

The hemoglobin level in blood was low in inorganic (8.58%) and organic (6.50%) selenium treated mice groups and was significantly ($P < 0.05$) high in nano-selenium (7.92%) treated mice in comparison to the control mice group (Table 1). The exposure of inorganic and organic selenium resulted in non-significant ($P < 0.05$) changes in RBC and WBC counts and in bone marrow and splenic cell counts. The cell counts noted in both bone marrow and splenic cells were increased significantly ($P < 0.05$) in Nano-Se gavaged group of mice (Table 1).

Histopathological changes of liver, kidney, lung and spleen

Histology of liver, kidney and lung tissues of Se-treated mice has been shown in Fig. 1. Histopathological examination of Nano-Se treated mice showed normal architecture of liver, kidney and lung tissues similar to that of control animals. Absence of detrimental effects of chronic administration of Nano-Se for a period of 28 days was noted. The cellular structure remained unaltered with the absence of necrosis, inflammation or other lesions. In liver and kidney tissues of mice exposed to inorganic selenium resulted in infiltration of mononuclear cells with pycnosis and renal necrosis. The damages were more in liver and kidney tissues of mice treated with organic selenium exhibiting dilated blood sinusoids in liver and glomerular congestion and renal necrosis in kidney. Lung sections were affected with thickened alveolar septa, congestion and distortion of alveoli with edema in inorganic and organic Se-exposed mice lung tissues.

Alterations in liver and kidney function enzymes

The serum ALT, AST and ALP levels increased significantly ($P < 0.05$) in inorganic and organic selenium treated mice groups than the control mice group. Mice gavaged with of

Table 1 Selenium induced changes in hematological parameters, bone marrow and spleen cells of mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days

Treatment groups	Hb (gm/dl)	RBC ($10^6/\text{mm}^3$)	WBC ($10^6/\text{mm}^3$)	Splenic cell ($\times 10^6$)	Bone marrow cell ($\times 10^6$)
Vehicle control Group I	12.12 \pm 0.60	6.96 \pm 0.40	6.66 \pm 0.37	66.31 \pm 2.83	26.16 \pm 2.25
Inorganic selenium Group II	11.08 \pm 0.72	6.14 \pm 1.09	7.19 \pm 0.41	54.23 \pm 4.30 ^{α}	23.56 \pm 1.92
Organic selenium Group III	11.32 \pm 0.96	6.35 \pm 0.95	7.05 \pm 0.59	63.69 \pm 3.64 ^{β}	26.01 \pm 2.26
Nano-Se Group IV	13.08 \pm 0.38 ^{$\beta\theta$}	7.44 \pm 0.69 ^{β}	7.73 \pm 0.51 ^{α}	84.64 \pm 4.90 ^{$\alpha\beta\theta$}	32.23 \pm 2.43 ^{$\alpha\beta\theta$}

Data are mean \pm SD of six animals; α —significant ($P < 0.05$) as compared with control; β —significant ($P < 0.05$) as compared with inorganic selenium; θ —significant ($P < 0.05$) as compared with organic selenium

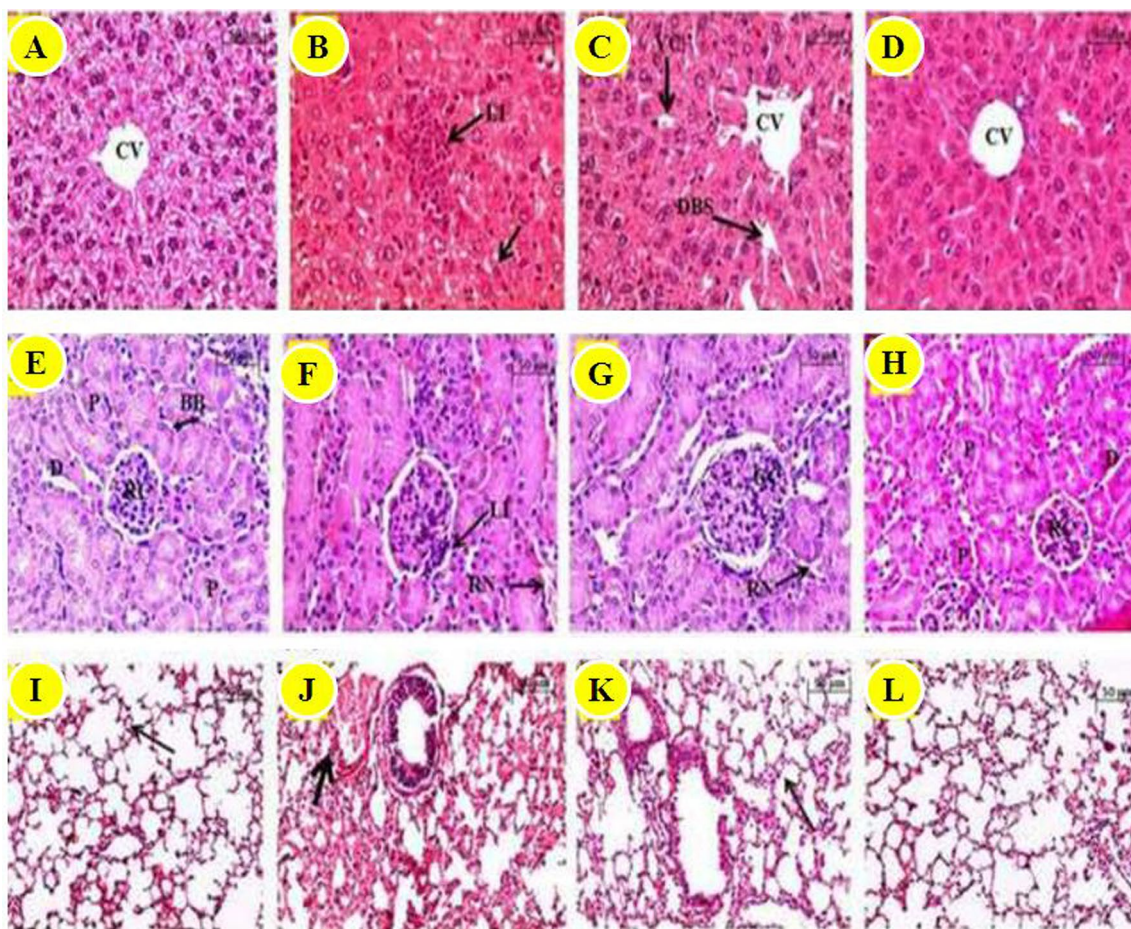


Fig. 1 Histopathological observations of liver, kidney and lung tissue sections of mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days. Magnification $\times 200$. **a** Liver section of the vehicle control group; **b** liver section of inorganic selenium group showing leukocyte infiltration (LI), pyknotic nuclei (PK); **c** liver section of organic selenium showing cytoplasmic vacuole (CV), dilated blood sinusoids (DBS); **d** liver section of nano selenium showing normal liver architecture same as vehicle control group; **e** kidney section of vehicle control group showing proximal (P), distal (D) tubule; **f** kidney section of inorganic selenium group showing leukocyte infiltration

(LI), renal necrosis (RN); **g** kidney section of organic selenium group showing renal necrosis (RN), glomerular congestion (GC); **h** kidney section of nano selenium showing normal kidney architecture same as vehicle control group; **i** lung section of the vehicle control group (arrow) showing normal alveolar architecture without any epithelial damage; **j** lungs section of inorganic selenium group (thick arrow) showing thickened alveolar septa; **k** lungs section of organic selenium (arrow) showing congestion and distortion of alveoli with edema formation; **l** lungs section of Nano selenium showing normal lung architecture same as vehicle control group

2 mg Nano-Se/kg b.w. did not affect serum ALT, AST and ALP profiles (Table 2). Impairment of renal functions was manifested in Se-treated mice. Compared to the control mice group (Gr. I), inorganic(Gr. II) and organic(Gr. III) selenium treated mice showed a significant increase in BUN (~ 1.6- and ~ 1.2-fold, respectively) and creatinine levels (~ 4.8- and ~ 4.3-fold, respectively,) whereas in the Nano-Se treated mice (Gr. IV) levels of BUN and creatinine was not significant ($P > 0.05$) than vehicle control treated mice (Table 2).

Changes in biochemical parameters and antioxidant status

Figure 2 depicts that both inorganic (Gr. II) and organic (Gr. III) selenium compound caused significant ($P < 0.05$) increase in LPO levels in liver, kidney and lung tissues when compared to the vehicle control group (Gr. I). Chronic administration of Nano-Se at the dose of 2 mg Se/kg b.w. showed negligible increase of LPO level ($P < 0.05$) compared to the vehicle control group (Gr. I).

The SOD activity in mice gavaged with inorganic selenium was higher than the control mice but was not statistically significant ($P > 0.05$). The SOD activity increased significantly ($P < 0.05$) by 16.04% following administration of

organic selenium compound compared to the vehicle treated group (Gr. I). At the dose of 2 mg Se/kg b.w. oral administration of Nano-Se resulted in a significant ($P < 0.05$) elevation of SOD activity by 46.5%, in comparison to the vehicle control group (Fig. 3a). The hepatic CAT activity though increased by 12.5% and 16.6% in liver after treating with inorganic and organic selenium compounds, respectively was however not significant ($P > 0.05$). Oral administration of Nano-Se resulted in a significant ($P < 0.05$) elevation of CAT content by 33.87% as compared to the vehicle control group (Gr. I) (Fig. 3b). The GSH content in liver increased by 4.17 and 8.57%, respectively following treatment with inorganic and organic selenium compound but was not significant when compared to the vehicle control group (Gr. I) (Fig. 3c). Administration of Nano-Se showed a 65.24% significant ($P < 0.05$) increase in GSH over the control group (Gr. I). The activity of tissue GST was enhanced in livers of animals treated with inorganic (2.4%) and organic (5.78%) selenium (Fig. 3d). The GST activity increased by 23.49% in mice treated with the same dose of Nano-Se and for the same duration which was significant ($P < 0.05$) as compared to the vehicle control group (Gr. I) (Fig. 3d). The activity of hepatic GPx increased following oral administration of inorganic and organic selenium and was maximum (22.29%) and significant (Fig. 3e) in mice treated with Nano-Se. The

Table 2 Effect of selenium on enzymes for liver (ALT, AST, ALP) and kidney (BUN, creatinine) functions of mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days

Treatment groups	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	BUN (mg/dl)	Creatinine (mg/dl)
Vehicle control Group I	31.28 ± 3.28	94.4 ± 6.38	56.2 ± 3.15	10.45 ± 0.21	0.40 ± 0.03
Inorganic selenium Group II	56.00 ± 3.74 ^α	201.2 ± 7.69 ^α	88.61 ± 3.27 ^α	16.63 ± 0.41 ^α	1.95 ± 0.07 ^α
Organic selenium Group III	49.8 ± 3.63 ^{αβ}	140.4 ± 6.54 ^{αβ}	76.32 ± 3.14 ^{αβ}	12.52 ± 0.40 ^{αβ}	1.74 ± 0.04 ^{αβ}
Nano-Se Group IV	34.8 ± 2.28 ^{βθ}	103.6 ± 7.12 ^{βθ}	59.17 ± 4.59 ^{βθ}	8.47 ± 0.24 ^β	0.45 ± 0.02 ^β

Data are mean ± SD of six animals; α—significant ($P < 0.05$) as compared with control; β—significant ($P < 0.05$) as compared with inorganic selenium; θ—significant ($P < 0.05$) as compared with organic selenium

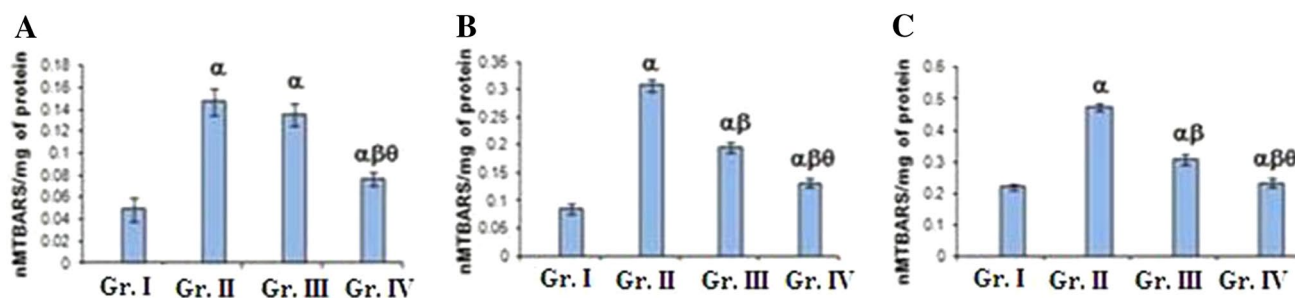


Fig. 2 Selenium induced changes on hepatic (a), renal (b) and pulmonary (c) LPO levels of mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days. Data are mean ± SD of six animals; α—sig-

nificant ($P < 0.05$) as compared with control (Gr. I); β—significant ($P < 0.05$) as compared with inorganic selenium (Gr. II); θ—significant ($P < 0.05$) as compared with organic selenium (Gr. III)

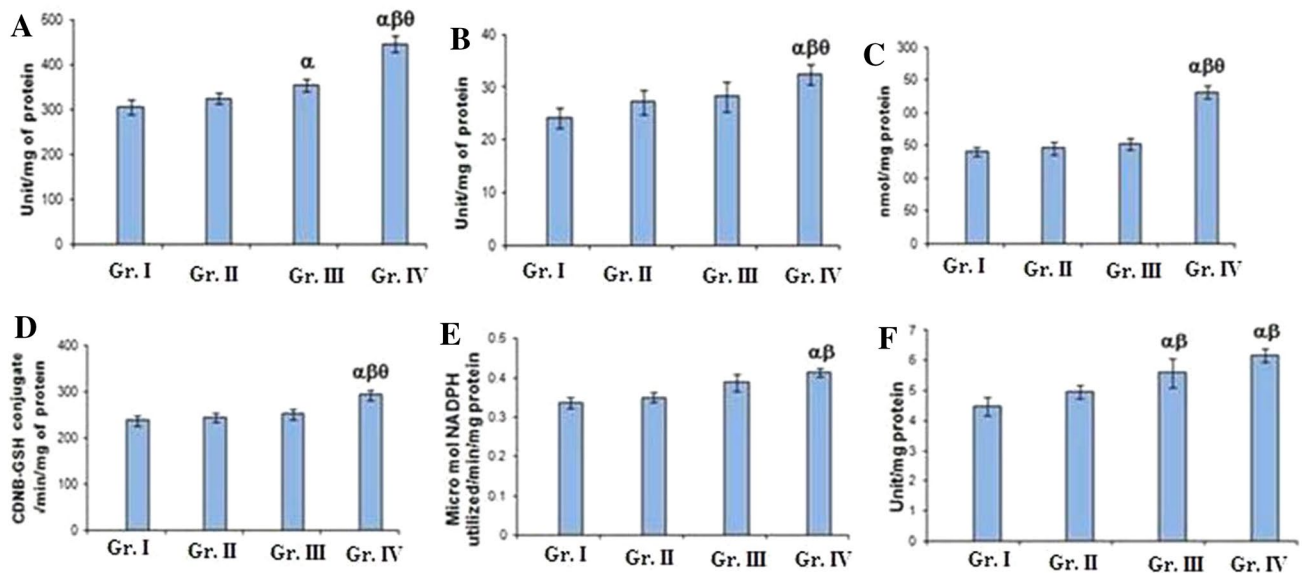


Fig. 3 Selenium induced changes of antioxidant enzymes GSG (a), GST (b), SOD (c), CAT (d), GPx (e) and TrxR (f) in mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days. Data are mean \pm SD of six animals; α —significant ($P < 0.05$) as compared with control

(Gr. I); β —significant ($P < 0.05$) as compared with inorganic selenium (Gr. II); θ —significant ($P < 0.05$) as compared with organic selenium (Gr. III)

TrxR activity increased by 10.65, 24.69 and 37.53% in liver following treatment with inorganic, organic selenium and Nano-Se compounds, respectively, compared to the vehicle treated group (Gr. I) (Fig. 3f).

Genotoxic effects of selenium compounds on bone marrow cells of mice

Comet assay was utilized to evaluate selenium-induced DNA damage in bone marrow cells of mice. The percentage of damaged cells showing comet formations and the average tail length of comet was measured.

Percentage of damaged cells in each group is presented in Table 3. The frequency of cells showing DNA damage was 9.10 in control, 19.41 and 16.52% in inorganic and organic selenium, respectively. They were significant ($P < 0.05$) when compared to control. The percentage of DNA damaged cells in Nano-Se treated group was 10.78%, and was not significant ($P > 0.05$). Tail length of comet due to DNA break in each group ranged from $7.89 \pm 0.86 \mu\text{m}$ in control to $20.23 \pm 1.75 \mu\text{m}$, $18.34 \pm 2.24 \mu\text{m}$ in bone marrow cells of mice treated with inorganic and organic selenium respectively. Administration of Nano-Se resulted in the induction of average tail length of $8.97 \pm 1.51 \mu\text{m}$, that was not significant when compared to control (Table 3). The extent of

Table 3 Effect of selenium on bone marrow cells of mice DNA mice gavaged with 2 mg Se/kg b.wt. for 28 consecutive days by comet assay and Chromosomal aberration assay

Treatment groups	Comet parameters		Total chromosomal aberrations (%)
	% of damage cells showing comet	Average tail length (μm)	
Vehicle control Group I	9.10 ± 0.81	7.89 ± 0.86	14.17 ± 2.24
Inorganic selenium Group II	$19.4 \pm 1.69^\alpha$	$20.23 \pm 1.75^\alpha$	$26.06 \pm 2.24^\alpha$
Organic selenium Group III	$16.5 \pm 1.20^{\alpha\beta}$	$18.34 \pm 2.24^\alpha$	$17.08 \pm 2.40^\beta$
Nano-Se Group IV	$10.7 \pm 1.55^{\alpha\beta\theta}$	$8.97 \pm 1.51^{\beta\theta}$	$13.34 \pm 2.24^\beta$

Data are mean \pm SD of six animals; α —significant ($P < 0.05$) as compared with control; β —significant ($P < 0.05$) as compared with inorganic selenium; θ —significant ($P < 0.05$) as compared with organic selenium

total chromosomal aberrations was estimated to be 14.17% in bone marrow cells of control mice. The proportion of CA was raised significantly ($P < 0.05$) to 26.06% in inorganic selenium-treated mice. Treatment with organic selenium and Nano-Se, showed increase in CAs but was not statistically significant ($P > 0.05$) Table 3. The types of aberrations mainly included chromatid breaks, stretching of chromosomes and ring chromosomes in metaphases.

Discussion

In the present study Nano-Se was synthesized which was less toxic with higher activity of seleno-enzymes than the inorganic and organic Se compounds. As nutritional supplement selenium compounds at high dosage can show cancer preventive properties but have limited applications due to toxicity. Literature survey revealed that the chemo preventive property was mainly carried out with inorganic Se and organic Se compounds [20]. The studies also indicated that inorganic and organic Se compounds were toxic as their pro-oxidant properties that generate superoxide (O_2^-) damage cellular components [56]. Therefore, there has been a growing interest in the synthesis of less toxic form of selenium that could be used as an antioxidant, enzyme inhibitor, neuroprotectant, anti-infectious and as cancer chemo preventive agent and as an immunomodulator.

Se toxicity has been extensively studied in animals. One of the major characteristic of Se toxicity results in growth retardation through the reduction in growth hormone and somatomedin C production [52] which might be the best indicator of toxic effects from selenium [38]. In the present study we have observed that oral administration of inorganic and organic selenium reduced the body weight of mice as compared with the vehicle treated group and was toxic compared to Nano-Se. The reason behind the increase in body weight of Nano-Se treated mice is not known at this juncture, it may be in part due to nutritional effect.

Hematology and clinical chemistry of bone marrow and spleen confirm that Nano-Se did not affect Hb level, bone marrow and spleen cell counts. Normal architecture of liver, kidney and lung tissues similar to that of control animals were observed in histopathological examination of Nano-Se treated mice. In liver and kidney tissues of mice exposed to organic selenium for 28 consecutive days, the damages were more pronounced than the inorganic Se-treated mice. Lung sections were affected with thickened alveolar septa, congestion and distortion of alveoli with edema in inorganic and organic Se-exposed mice lung tissues.

In the present study, we found both inorganic and organic selenium compound caused alterations in liver and kidney function. Animal studies have demonstrated that the liver is the major target organ of Se toxicity [41]. Generally high

levels of transaminases such as ALT, AST and ALP are good indicators of hepatic damage [4]. Inorganic selenium compound was more potent in inducing high levels of ALT, AST and ALP in serum. The nephrotoxicity markers BUN and creatinine levels were significantly increased in inorganic and organic selenium treated group. The amount of urea nitrogen, a waste product of protein metabolism in the blood is measured by blood urea nitrogen (BUN), and creatinine. The levels of blood BUN and creatinine rise if the function of kidney is impaired and the filtration of waste products is affected. Nano-Se did not show adverse effect on liver and kidney function. Lipid per oxidation is a biomarker of oxidative stress. It causes cell membrane damage leading to gradual loss of membrane fluidity, decrease in membrane potential and increased permeability of ions [5]. Both inorganic and organic selenium forms caused increase in malondialdehyde (MDA) level but its accumulation in liver tissues of inorganic selenium treated mice was higher than that for Nano-Se. Similar observations was also observed in kidney and lung tissues.

Alterations of oxidative stress response induced by inorganic, organic and Nano-Se was evaluated. The antioxidant and detoxifying enzymes manifested that Nano-Se was an efficient enhancer of host antioxidant defense system such as SOD, CAT, GST, GSH, GPx, and TRxR. The activity of the enzymes was higher in Nano-Se than that of inorganic and organic selenium compound.

Reactive oxygen species (ROS) are known to cause damage to cell membranes resulting into break down of key biological components and are involved in the initiation, propagation and maintenance of both acute and chronic inflammatory processes [18]. Therefore organisms have developed a number of defense mechanisms to protect cell membranes and other cellular component from oxidative damage. As a first line of defense the antioxidant and detoxifying enzymes (GST, GPx, SOD, CAT, TrxR) are activated. Chiefly SOD act by quenching superoxide and active oxygen free radicals, produced in different aerobic metabolism [58]. GPx which contains amino acid selenocysteine in its catalytic center [50] reduces hydrogen peroxide via its selenocysteine containing active site-selenols. Two equivalents of glutathione are oxidized to the disulfide and water, through a redox cycle and the hydroperoxide is reduced to the corresponding alcohol [28]. Another selenoenzyme, TRxR, catalyzes the reduction of oxidized thioredoxin by using NADPH as the electron donor. As a key enzyme in Se metabolism, reduce Se compounds and provide selenide to synthesize all selenoproteins. Additionally, other antioxidant functions of TrxR directly scavenge lipid peroxides and hydrogen peroxide [44]. In the present study Nano-Se was highly efficient in reducing oxidative stress producing elevated levels of the antioxidant enzymes. The reason for better seleno enzyme activities upon treatment with nano selenium may be due

to enhanced internalization of nano selenium compared to inorganic and organic selenium. It may be also implicated that the antioxidant enzymes generated to scavenge the ROS were not adequate to counter the oxidative stress produced by inorganic and organic Se. Additional studies with ROS inhibitors are needed to find the actual reasons behind these observations.

Chromosomal aberrations (CA) and DNA damage by comet assay are sensitive biological indicators of genotoxicity of a drug [26]. Absence of significant frequency of chromosomal damage and DNA damage were noted in mice group administered with Nano-Se.

Based on the results of the present study we can say that compared to inorganic and inorganic Se, Nano-Se at the same dose was less genotoxic to the mice and possessed ability to modulate the antioxidative defense system and reduce oxidative stress induced during a chronic treatment of 28 days in vivo. Therefore the use of Nano-Se can be promoted as an effective supplement for chemoprevention and incorporated in the formulation of Se-containing pharmaceuticals available in the market.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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