Original Article

Comparing The Effects of Selenium Nanoparticles and Selenium Nanocomposites on Food Intake and Anxiety-like Behaviors

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Abstract

Background & Objective: Selenium nanoparticles (Se -NPs) and selenium nanocomposites (Se -NCs) have different biological effects. Therefore, the present study aimed to compare the effects of selenium nanoparticles and selenium nanocomposites (Se –NCs) on anxiety, food intake, and brain histology of rats.

Materials & Methods: Thirty-two male adult Wistar rats were randomly and equally divided into four groups. The control group received saline, the Selenium powder group received 1 mg/kg /day selenium powder orally for 21 days, and the selenium nanoparticle group received three weeks of oral gavage of Se –NPs. The selenium nanocomposites group received three weeks of oral gavage of nanocomposites. Finally, cumulative food consumption and anxiety-like behaviors were assessed, and, after that, rats were bled and sacrificed for further biochemical and histopathological investigations.

Results: Oral administration of Se powder at a dose of 1 mg/kg /day for 21 days had no significant effect on the brain superoxide dismutase (SOD), catalase (CAT) activities. There was also no significant change in the levels of brain glutathione concentration (GSH), brain MDA, and behavioral parameters. Selenium nanoparticles also showed no significant alterations in brain biochemical parameters, behavioral effects, and brain histology. The oral administration of Selenium nanocomposites significantly increased brain superoxide dismutase, catalase, and reduced glutathione content and had positive effects on behavioral parameters.

Conclusion: The present study showed that Se -NCs have behavioral effects and could induce significant biochemical changes in brain oxidative status.

Keywords: nanoparticles, Selenium, behavior, rat, Oxidative Stress

Introduction

Selenium - an essential mineral-is only needed in small quantities but plays an important role in metabolism, DNA synthesis, reproduction, brain function, and hormone secretion. Selenium can act as a powerful antioxidant and can reduce breast cancer risk. Previous studies have shown that Selenium can reduce the risk of cardiovascular diseases and can prevent Alzheimer's disease (1). Selenium is crucial for the proper function of the thyroid gland. Dietary levels of Selenium may help boost the thyroid function of people with Hashimoto's disease.Fish, chicken breast, peanut, mushrooms, and eggs are excellent

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sources of Selenium (2). The selenium concentrations in plant foods depend on the selenium content of the soil.

Selenium deficiency is linked to vitamin E deficiency. Selenium-dependent antioxidant enzymes including, Glutathione peroxidase (G-Px), Catalase (CAT), and superoxide dismutase (SOD), could not work correctly in the selenium-deficient patients (3). Selenium deficiency is linked to various biochemical, metabolic, and behavioral disorders. Selenium has a direct role in regulating the function of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which regulates the function of antioxidant enzymes (4). Specific age groups, including infants, elderly peoples, and pregnant women, are more susceptible to selenium deficiency. Selenium deficiency can lead to myocardial necrosis, leading to the weakening of the heart and congestive cardiomyopathy, the syndrome named Keshan disease (5). Food sources of dietary Selenium are the first-line treatment. Clinicians recommend selenium supplements such as sodium selenite or l-selenomethionine for selenium-deficient patients (6). In most cases, eating selenium-enriched foods along with multivitamins can raise serum selenium levels to a healthy state. In recent years, scientists have introduced newly-synthesized selenium nanoparticles as an alternative for selenium-rich compounds (7). The use of these compounds is increasing due to their unique biological and physicochemical properties, including good absorption, anti-lipid per-oxidative effects, antimicrobial, and anti-inflammatory properties as well as, less toxicity compared to the metallic selenium. Nanotechnology is based on the physicochemical properties of nanomaterial (8). The biological and physicochemical properties of nanoparticles are quite different from the conventional bulk forms of materials. In recent decades, Se -NPs as with unique biocompatibility, optical, catalytic, mechanical, electrical, and antimicrobial properties, have been widely used in industry and medicine (9).

Previous studies have shown that Se nanoparticles can reduce oxidative damage by preventing free radical formation in different tissues. In the present work, we investigate the behavioral effects of Se -NPs and Se NCs in a Rattus norvegicus model of anxiety and feeding behavior. In the previous studies, Selenium nanoparticles have shown protective effects on animal anxiety models, on the other hand, another experiment showed memory-enhancing effects of Selenium nanoparticles (10). The anxiolytic and memory-enhancing properties of Se could be associated with the role of selenium in the Selene-enzymes. Seleno-enzymes can protect cell organelles and other functional parts of the cells from oxidative destruction (11). However, supra-nutritional levels of Se -NPs could lead to liver damage, neurotoxicity, nephrotoxicity, and reproductive failure (12). Recent studies have shown that nanocomposites have satisfactory biocompatibility and biosafety. Long-term exposure to Selenium could induce behavioral effects such as anorexia, anxiety, and even memory loss effects. However, the behavioral effects of Se nanocomposites have not been thoroughly investigated. Some newly-synthetized nanocomposites have blood-brain-barrier crossing ability and are designed for new diagnostic technics such as highly specific MR Imaging (12). Nanocomposites can carry and tune drug release in the central nervous system which makes them excellent candidates for treating neurodegenerative diseases (13). Nanocomposites and nanoparticles not only could carry the drugs but also could have behavioral effects in humans and laboratory

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rodents (14). Previous studies have shown the anti-anxiety effects of nanocomposites in different animal models, while other experiments showed neurotoxicity of nanocomposites (15, 16).

There is little data regarding the effects of selenium nanocomposites on CNS function and brain histology. Concerning this theme, our objective was to compare the effects of newly synthesized Selenium nanoparticles with Selenium nanocomposites that might provide valuable clues for determining effective supplements for selenium-deficient patients. Nanocomposites, due to their excellent solubility, can easily penetrate different parts of the body. Therefore, we hypothesized that these nanomaterials could induce changes in the central nervous system function, leading to behavioral changes.

Material & Methods

Synthesis of SeNPs by chemical method

Selenium powder were purchased from Sigma-Aldrich (Berlin, Germany). Thiobarbituric acid was purchased from Sigma-Aldrich (Berlin, Germany). In order to synthesize SeNPs, selenium powder (0.25 M) was added to the solution of sodium sulphate (0.50 M) in 100 ml of double distilled water, and mixture was stirred at 70 °C for 9h. A transparent sodium selenosulphate (Na2SeSO3) solution was obtained which was used as a precursor for synthesis of the SeNPs. Sodium selenosulphate solution (0.002 M) was used in a separate Erlenmeyer flask and 0.005 M of acetic acid was added dropwise into sodium selenosulphate solution for the carboxylic group-induced synthesis of the SeNPs.

The appearance of pink color preliminary confirmed the formation of SeNPs. This color indicated the synthesis of Se NPs into the solution. After synthetizing selenium nanoparticles, the supernatant was collected and centrifuged at 11,500 rpm for 20 min at 4 °C. The supernatant was discarded, and pellet was washed with distilled water thrice and the final pellet was suspended into the distilled water and sonicated, and then it was lyophilized. After that, the powder was collected for performing analytical techniques. To stabilize SeNPs, polyvinyl alcohol (PVA) or other biopolymers like starch, chitosan, and cellulose acetate could be considered. The synthesized SeNPs were stabilized by 0.05 ml of 1% aqueous polyvinyl alcohol. To this end, SeNPs were mixed with PVA in water and stirred for 30 minutes. In this step, SeNPs were distributed in the polymer matrix and stabilized and the aggregation of nanoparticles was delayed (17).

Particle characterization

Structural characterization of Se -NPs was performed by using an X-ray diffractometer (XRD). The size of Se -NPs was measured using transmission electron microscopy (TEM) (Philips CM-12 Model operating at an accelerating voltage of 120 kV).

In vivo experiments

Animal grouping

Thirty-two male Wistar rats (205- 220 g) were purchased from animal breeding center of university of Zabol, Zabol, Iran. Rats were kept in standard laboratory conditions in a well-ventilated room, standard temperature (20–23 ˚C), and a complete light/ dark cycle which was as follows: 12 (h) light/ 12 (h) dark cycle. Animals had free access to standard rodent pelleted food (Javaneh-Khorasan, Iran) and sterile tap water. All experimental parts were conducted in accordance with the OECD guidelines and ethical codes of the Animal Ethics Committee of our university

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and with the international ethical codes for performing researches on laboratory rodents. Rats were grouped into four equal groups (eight rats in each group). Control group received physiological saline and three other treatment groups received selenium nanoparticles and selenium powders.

Rats in the treatment group received Se nanoparticles (1 mg/kg /day) and Se nanocomposites (1mg/kg day) orally for 21 days. The doses were selected based on previous works and preliminary experiments (18, 19). Control rats received 1ml of distilled water orally for 21 days. After three weeks of oral gavage treatment, animals were anesthetized by thiopental sodium, and the brain hemispheres were isolated. The fresh brain samples were immediately washed with physiological saline and stored at -80 °C for biochemical analysis of brain tissues.

Measurement of food intake

After three weeks of oral intubations, rats were prepared for determining feeding behavior. Four hours before the food intake measurement, rats were deprived of the food but had free access to water. After the starvation period, animals were returned to their cages and received standard pelleted food. The weight of food was recorded before and after the experiment to measure the four hours of food intake of each rat. The weight of the pelleted diet that every rat consumed during the experiment periods (called cumulative feed intake) was recorded at 2 hours and 4 hours after the starvation period.

Brain antioxidant parameters

The catalase activity in homogenate brain tissues was determined according to the protocols previously described by Goth et al (20). The superoxide dismutase activity in brain homogenates was measured using the colorimetric protocol of Sun et al. with minor modifications (21). The brain concentration of reduced glutathione (GSH), an indicator of the total thiol group's contents, was determined using the protocol of Ellman et al. (22) which had been modified later by Jollow et al (23). The brain malondialdehyde level- a marker of lipid peroxidation- was determined according to the Ohkawa method and described elsewhere (24).

Elevated plus maze and open field test

We designed and performed an animal model of anxiety called elevated plus maze (EPM) to determine the effects of Se nanoparticles on behavior. The overall time of this experiment was approximately five minutes. In the first phase, the animals were placed in a plus-like device, consisting of two close arms. The close arms crossed the open arms. When the rats were calmed, the time spent in two compartments and the number of entries were recorded for each rat. The time of entry was started to record when all four paws of each animal entered one of the two compartments of the device. We also investigated the overall anxiety-like behaviors and locomotion. For this purpose, the animals were placed in the open field test apparatus. The open-field device is a cube-shaped chamber (72×72×35 cm) used to determine the will to explore the movement activity in laboratory rodents. The activities of animals were recorded by a closed-circuit digital recorder camera, located on the top of each room.

Statistical analysis

The results were analyzed with a one-way analysis of variance (ANOVA) followed by post-hoc Turkey's test for multiple comparisons. The SPSS software (version 18.0) was used to measure the behavioral tests. The statistical significance level was set at $P<0.05$.

Results

The results of X-Ray Diffraction (XRD) pattern, Scanning Electron Microscopy image

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and Energy Dispersive X-ray Diffraction analysis of Selenium nanoparticles are presented in figures 1-3.

X-Ray Diffraction pattern of SeNPs

XRD pattern of SeNPs suggests that the sample is nano-crystalline and matches very well with that of the standard selenium powder confirming the formation of selenium nanoparticles, which is shown in figure 1. The calculated lattice constants are a = 4.363 A° and c = 4.952 A°, and the diffraction peaks at 2θ=23.7, 29.8, 41.4, 43.9, 45.5, 51.8, 56.3, 61.9, 65.2, and 68.6° are for the (100), (101), (110), (102), (111), (201), (112), (202), (211), and (113) reflections of the pure hexagonal phase of selenium which are line with previous literature values (JCPDS File No. 06-0362). Also, the crystallite size, D, was calculated from the XRD data using Scherrer's formula. The average particle size of SeNPs was nearly 70 nm.

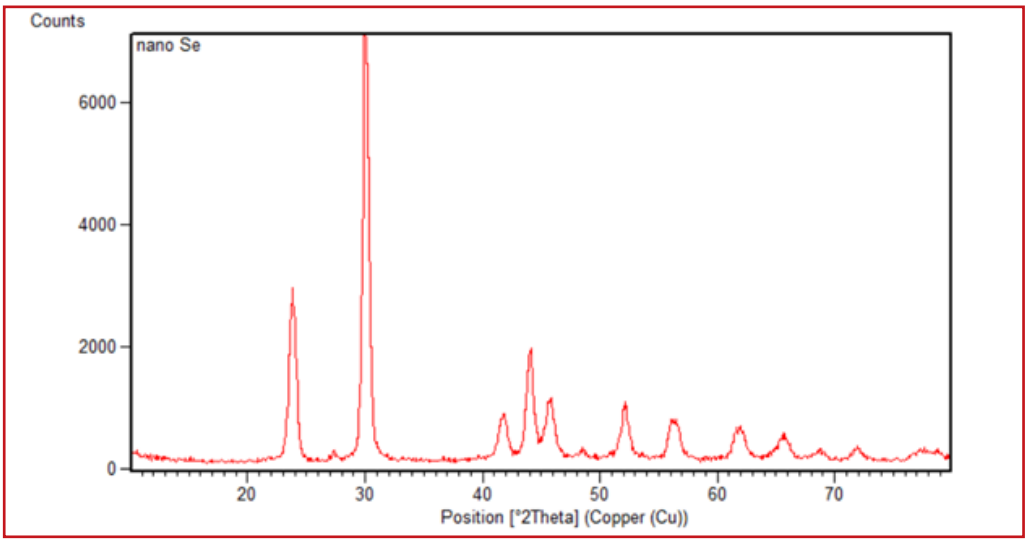


Figure 1. X-ray Diffraction (XRD) pattern of Selenium NPs

SEM was applied to observe the surface morphology of Selenium nanoparticles by using a Hitachi S4160 apparatus. The uniform surface structure was observed when SeNPs were further analyzed by SEM. The topographic structure of SeNPs surface was found to be spherically agglomerated and uniform structure everywhere in figure 2.

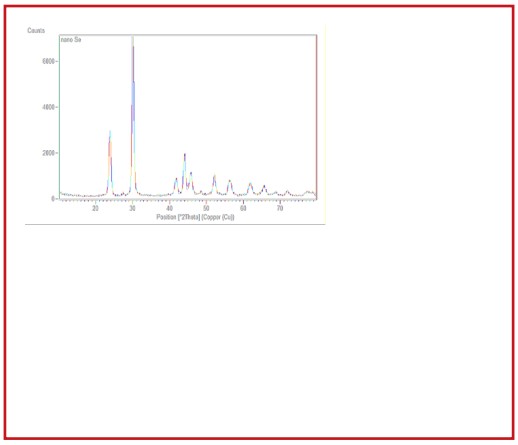


Figure 2. Scanning Electron Microscopy image of SeleniumNCs

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Energy Dispersive X-ray (EDS) Spectrum of Se nanoparticles

Chemical composition of prepared selenium nanoparticles (SeNPs) was confirmed using elemental analysis of EDSspectrum. The spectrum, shown in Figure 3, indicates that nanoparticles of selenium were only synthesized. In this spectrum Se signal is observed.

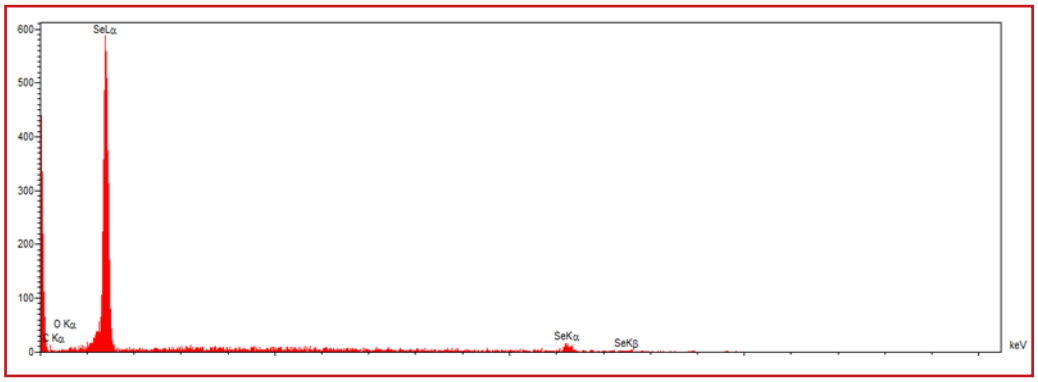


Figure 3. Energy Dispersive X ray Spectrum of SeleniumNCs

Behavioral parameters

The results of food consumption measurements of experimental groups are shown in Chart 1. The food consumption significantly decreased in rats treated with Se nanoparticles, Se powder, and Se NCs at two first hours of experiments (P<0.05). This anorectic effect was more severe in the group treated with the selenium powder. However, no significant changes were observed between groups treated with different formulations of selenium. After four hours, there was no significant difference in the overall food consumption data of experimental groups. The results showed a non-significant decrease in appetite of rats treated with selenium nanoparticles compared to the normal control group. There was also a non-significant decrease in food intake of rats treated with selenium powder compared to the normal control rats (P>0.05). The effects of Se nanoparticles and selenium nanocomposites on food intake began from the start of food intake recording and lasted after two hours. This effect lasted up to two hours.

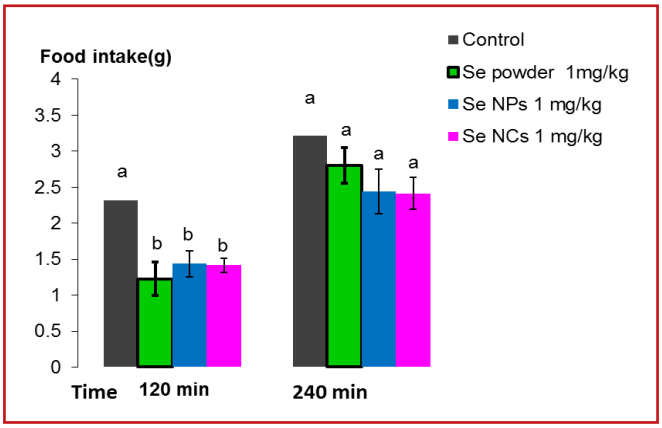


Chart 1. Four hours food consumption of each animal after intraperitoneal administration of Se -NPs. Different letters (a, b) show significant difference compared to the control group (P<0.001)

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The results of the elevated plus-maze test revealed a statistically significant decrease in open arm entries in rats receiving three-week oral gavage treatment of Se nanocomposites. These alterations were not observed in two other experimental groups.

Open field test

As shown in chart 2, the Se -NPs treated rats and Se-NCs treated rats had higher open time entries compared to the healthy normal rats. However, these elevations were not statistically significant (Figure 3) (P>0.05). The results of the overall movement measurements of Se –NPs, Se NCs, and selenium powder were investigated by an open field test, which is an animal model of anxiety and movement. The results of open arm entries showed the prominent behavioral effects of Se nanocomposites.

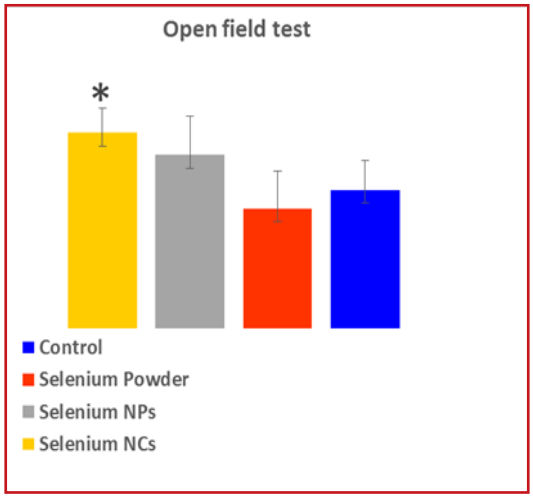


Chart 2. Open field test. Bars shows central area crossing.* shows statisticaly significant difference compared to the normal controls P<0.05

There was a significant increase in latency to start feed intake in rats treated with selenium powder. The latency to start feeding was also increased in rats treated with selenium nanoparticles and selenium nanocomposites (P<0.001) (Table 1). The latency to start feeding was significantly higher in rats receiving selenium powder. The range of latency to start feeding was also longer in the group treated with selenium powder.

Table 1. Latency to start feeding after intraperitoneal administration of Se NPs in rats

Latency of feeding in rats (min)	Mean±SD	Range (s)
Saline (0.5 mL)	5.3± 1.8	3.5-8.5
Se powder 1mg/kg	17.1*±2.8	12.5-20.5
Se NParticless 1mg/kg	16*±2.4	13-21
Se NCoomposites 1mg/kg	14.3*±3.1	12.5-19.5

**significant with respect to control group (P<0.001)

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Biochemical parameters

As shown in Table 2, there was no significant difference in brain superoxide dismutase activities of rats treated with selenium powder or selenium nanoparticles when compared to the normal control groups. The enzymatic activity of brain superoxide dismutase also showed no significant changes in rats treated with Se -NPs compared to the normal rats. On the other hand, the brain SOD activity of Se –NCs treated rats showed a significant increase compared to the healthy control rats (P<0.05). Results of brain catalase activity revealed a similar pattern in which both selenium- powder treated rats and selenium-NPs treated rats showed no changes in brain catalase activity compared to the health controls (P>0.05). The reduced glutathione content significantly increased in rats following oral administration of Se -NPs (P<0.05). Treatment with the Se nanoparticles, or with selenium powder, caused no significant changes in the brain lipid peroxidation levels.

Table 2. Effects of Se NPs on antioxidant/ pro-oxidant parameters of rats' brain

Item	Treatment			
	Control	Se -Powder 1 mg/kg	Se -NPs 1 mg/kg	Se -Ncs 1 mg/kg
Superoxide dismutase (U/L)	55.2±10.6	57.7±7.8	58.2±9	68.2*±8.4
Catalase (U/L)	32.0±4.5	37±6.5	34.8±6.1	41.3*±4.5
Reduced glutathione (µmol/mL)	45.8±10.4	57.6±7.6	57.0±7.7	71.2***±11.4
Malondialdehyde (nmol/mg tissue)	610.5±59.7	663.3± 56.7	618.7± 26.1	705.8*±81.4

*Statistically significance difference between letters (P<0.05)
**statistically significance difference between letters (P<0.01)
***statistically significance difference between letters (P<0.01)

Histopathological results

The brain morphological and histopathological investigations of different experimental groups showed valuable data, which is shown in figure 4. The brain histopathological analysis of the normal control rats showed the normal morphological appearance of brain tissues, with distinct neurons and neuroglia cells surrounding the neurons (figure 4a). The histopathological difference of the second group -rats treated with selenium powder- was quite different and the normal morphology of the brain was not present. In this group, there was a slight congestion of blood vessels in the brain (figure 4b). In the selenium powder treated group, other brain cells had normal morphology, and large pyramidal cells were present (figure 4b). As shown in figure 4c, histopathological investigation of rats received Selenium nanocomposites revealed normal histopathological pattern with intact neurons and the surrounding neuroglia around them. The histopathological appearance of the Selenium nanocomposites treated rats was quite similar to the histopathological morphology of the saline-treated group. As shown in figure 4d, the cellular arrangement of neurons, neuroglial cells, and the neuronal dendrites and axons was quite normal.

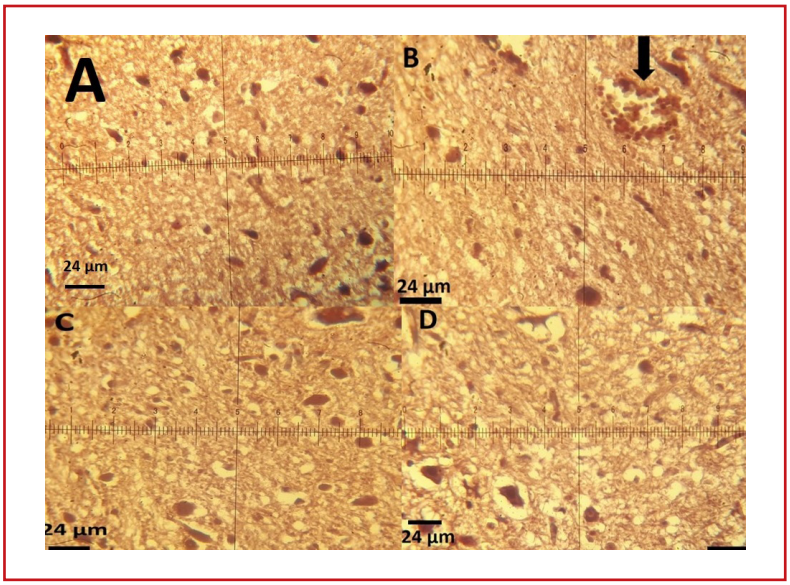


Figure 4. Light micrograph (trichrome mason stain. Mic. Mag. ×40) of brain section of (a) a control rat; showing normal histological appearance; (b) a rat treated with Se -NPs showing normal pattern; (c) a rat treated with Se -NPs Arrow shows slight blood congestion; (d) a rat treated with Se -NCs normal histology of the brain

Discussion

Many studies have investigated the effects of Se -NPs on brain function and different behavioral parameters. However, the biological effects of different formulations of selenium nanoparticles are not completely understood. However, it is acceptable that selenium-enriched materials could have unwanted biological effects on the brain and behavior. There are conflicting data about the effects of Selenium nanoparticles on various organs. Previous studies have reported the anxiolytic and anticonvulsant effects of Se -NPs in laboratory rodents, while other experiments showed positive effects of Se -NPs on the rat's brain (25). As seen in this study, intraperitoneal administration of Se -NPs induced significant alterations in brain oxidative status. Based on previous studies, selenium nanoparticles and selenium powder at proper doses had no toxic effects on laboratory animals (26). Our results were in accordance with the results of previous studies that reported the antioxidant effects of Se nanoparticles on brain and behavior (27). It seems that the effects of selenium nanoparticles have dose-dependent effects on behavior and brain antioxidant status (28). In the current work, an increase in the brain catalase, superoxide dismutase, and reduced glutathione content was observed. These biochemical changes showed antioxidant effects of Se nanoparticles. It could be concluded that the increase in the brain catalase and superoxide dismutase activity and an increase in brain GSH content are indicators of the antioxidant effects of Se -NPs. Catalase and superoxide dismutase enzymes are the most important free radical scavengers in the body. Free radicals are highly reactive molecules with unpaired electrons that tend to take electrons from surrounding molecules. The enzymatic and non-enzymatic antioxidants are key factors in preventing reactive oxygen production in the cytoplasm (29). Glutathione peroxidase, catalase, and superoxide dismutase are the most important enzymatic free radicals. The antioxidant enzymes could reduce the

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oxidative damage by reducing reactive oxygen species production. Mammalian cells are endowed with low-molecular-weight free-radical scavengers such as vitamin A, ascorbic acid, alpha-tocopherol, and N-acetyl-5-methoxytryptamine (melatonin) (30). In the current work, the increase in catalase and superoxide dismutase activity could be associated with the decrease in generating reactive oxygen species or increased availability of NADPH - a key molecule in the antioxidant defence system. In this study, a non-significant increase in brain GSH levels was observed in the selenium powder group. Previous studies have shown that Selenium nanoparticles can increase the brain GSH levels (31). In previous studies, Selenium nanocomposites significantly increased brain glutathione content (32).

The current results were in line with other studies showing the positive effects of Se -NPs on brain function. Also, the results of our study were in line with previous studies indicating the neuroprotective effects of the selenium powder. However, the biochemical effects of Selenium powder were quite different from the behavioral and histopathological effects of Selenium powder.

Selenium powder caused a significant alteration in rats' behavior. The most prominent behavioral effects of Se NPs, Se NCs, and Selenium powder was a decrease in the amount of food that every rat consumed (called food intake). However, this anorectic effect did not last more than two hours after injection. We conclude that these anorectic effects might be due to the pain and distress of injection and handling rather than direct behavioral effects. Also, in the histopathological analysis, there was slight congestion in blood vessels. Whether these changes are signs of selenium toxicity is unclear. However, it is demonstrated that selenium has the ability to cross the blood-brain barrier.

In the current experiment, animals showed signs of anxiety-like behaviors and behaviors associated with anxiety. The two behavioral tests used in the current work were the open-field test and the elevated plus-maze. The anxiety-like behaviors that were observed in the current work could have multiple interpretations. One explanation could be the effects of pain and restriction stress following animal handling. Our current findings were in contrast with the previous works that showed a decrease in anxiety following treatment with Se – NPs (33). This result might be due to the short duration of administration. One of the earlier experiments on Selenium-induced behavioral effects proved the ability of selenium powder to induce behavioral and pathological changes in a balb/c mice model (34). Former researches have shown that selenium neurotoxicity is accompanied by locomotion dysfunction, hind limbs paralysis, dysrhythmia, and, muscular paralysis, and respiratory and cardiac arrest (35).

Conclusions

In summary, selenium nanocomposites showed antioxidant potential and had significant effects on behavior. In contrast, Selenium powder showed detrimental effects on behavior parameters, which was confirmed by the histopathological results. Our results also showed that Selenium powder or selenium nanoparticles could cross the blood-brain barrier and could induce prominent histopathological and biochemical changes. More experiments are needed to completely understand the effects

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of selenium nanoparticles on behavioral parameters and brain oxidative status.

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Conflict of Inrests

The Authors declare no conflict.

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