Induction of Selenium Nanoparticles Disturbs Behavior, Blood and Serum Biomarkers and Oxidative Stress Markers from Vital Organs of Male and Female Albino Mice

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Summary: Current investigation was focused to determine the biological effects of Selenium nanoparticles (Se NPs) in mice. Se NPs (50mg/ml saline/Kg body weight) were intraperitoneally injected to 5 week old albino mice (N = 22) for 14 days. Control group was intraperitoneally injected with saline water (N = 22). In all subjects, a series of neurological tests, hematological parameters and markers of oxidative stress in vital organs were determined. We are reporting that rota rod and open field test performance remained unaffected in Se NPs injected mice when compared with saline treated controls. Male mice injected with Se NPs had significantly less line crossing (P = 0.02) while performing light dark box. They approached object A less frequently (P = 0.02) and spent lesser time with it (P = 0.001) during novel object recognition test (trial 1). % lymphocytes were significantly reduced (P = 0.03) in these mice while % monocytes were higher than control (P = 0.03). Concentration of cholesterol (P = 0.02) and LDL (P = 0.003) was significantly decreased in male mice. Female Se NPs treated mice spent less time (P = 0.05) with B object in trial 1 and 2 (P = 0.04) of novel object recognition test. They had significantly reduced cholesterol level (P = 0.02) and significantly increased catalase activity in the liver (P = 0.01) than control. Remaining parameters of behavior, blood chemistry and markers of oxidative stress from vital organs were non-significantly different upon their comparison between Se NPs and saline injected mice.

Keywords: Selenium nanoparticles; Field-emission Scanning Electron Microscopy; X Ray diffraction; Mice; Biocompatibility.

Introduction

Selenium and Selenium nanoparticles (Se NPs) has been extensively used in various industries such as medicine, chemical, ceramic, metallurgy and glass making. Due to their enhanced semiconducting, photo conducting, photo electrical and catalytic properties, Se NPs are extensively used in electronics and optics these days [1]. Se NPs are used in medicine as anticancer, anti diabetic, antioxidant, anti inflammatory and antimicrobial agents [2]. Biogenic amorphous Selenium nanoparticles are used to remove mercury from contaminated water [3]. Selenium intake in adequate amounts is required for optimal activity of glutathione peroxidase, thioredoxin reductase and selenoprotein that have antioxidant properties [4, 5]. It has been documented that selenium deficiency increases the risk for infections and cancers, decreases immune response and thyroid functioning. This deficiency has also been correlated with male infertility and with variety of neurological disorders [6]. There are a number of routes through which NPs may get an entry in the living system and may disturb its normal physiology either deliberately or accidently [7]. Selenium (Se) is known to be present in water and soil and hence it has potential to be part of food chain via aquatic animals [8]. Selenite and selenate are considered as toxic forms of Se while their NPs are considered as relatively biocompatible. Se NPs are known to be safe at some doses but they are reported to be toxic at others. Extent of Se NPs toxicity varies from species to species [9, 10]. Current investigation was aimed to document the effects of Se NPs (50 mg/ Kg body weight) intraperitoneal injections for 14 days on behavior, hematology and markers of oxidative stress in albino mice's vital organs.

Experimental

Selenium nanoparticles Synthesis

Procedures of Dwivedi *et al.* [11] was replicated for chemical synthesis of Se NPs. Field-emission Scanning Electron Microscopy (SEM) was carried out to document the morphological appearance of Se NPs through Nova Nano SEM 450 having lens detector. X ray diffraction (XRD) was performed on Bruker D8 advance to confirm the purity and crystallinity of the synthesized NPs.

Experimental design and animals

Albino mice, five week old, (N = 44; 22 male and 22 female) were individually kept in rodent cages. Animals had rodent diet and water available all the time. 22 ± 1^0C was the average temperature of room. A

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rhythm of 14:10 hours was maintained for light and dark respectively. Subjects were distributed into two groups based on specific intraperitoneal injections: group one (N = 22; 11 each male and female) intraperitoneally injected with 50 mg/ml saline/ body weight of Se NPs for 14 consecutive days (single injection in a day). Second group was intraperitoneally injected with saline solution for 14 days and used as control for group one (N = 22; 11 each male and female). Before injecting the dose, body weight of each animal was documented in order to report the effect **of** Se NPs on body weight, if any.

Behavioral testing

From 10th day of specific intraperitoneal injection, rota rod, light dark, open field and novel object recognition tests were performed in both treatments on successive days. Data was recorded and compared based on dose supplementation as well as sex of subjects.

Rota rod Test

This test was performed as reported in Noureen *et al.* [12].

Open Field Test

Open field test was performed to compare locomotory and exploratory behavior of Se NPs treated and untreated mice as documented by Iqbal *et al.* [13].

Light Dark Box Test

Exploration of mice was tested in light/dark box test as reported by Aftab *et al.* [14].

Novel-object recognition test

Apparatus consists of a rectangular chamber **(**40 cm length x 30 cm hight x 35 cm width) with two sample objects (A and B). Both objects were placed in two corners of the arena that were facing each other**.** During first trial, subjects explored two sample objects (A and B) for 5 minutes. In the second trial, the location of objects was not changed but a novel object was introduced in exchange with object A. Animals explored this arena again for 5 minutes. Parameters like line cross, stretch attend reflex, sniffing, number of approaches to object A, B or novel object, time with A, B or novel object, urination and defecation were recorded for each animal to compare their novel object recognition capacity as an indicator of memory formation following Akram *et al.* [15].

Determination of blood and serum parameters

After 14 days of intraperitoneal injections, animals were sacrificed under anesthesia and blood was collected from cardiac puncture. A blood aliquot was used for complete blood count analysis in hematological analyzer (CBC Analyzer, Sysmex 21, Japan). Remaining blood was centrifuged at 15000RPM for 10 minutes to separate serum and to report low density lipoprotein, triglycerides, creatinine, high density lipoprotein and cholesterol by using diagnostic kits following manufacturer's instructions.

Determination of biomarkers of oxidative stress in mice organs

Brain, kidney, heart, liver and lungs were surgically isolated from each subject for the determination of superoxide dismutase, lipid peroxidition and catalase activity following Saleem *et al.* [16].

Statistical analysis

Statistical significance was considered at $P \leq 0.05$ during data analysis. Statistical software Minitab (version 16, Pennsylvania) was implied to analyze data that was presented as mean ± standard error of mean Two sample student's t-test was used to compare various parameters of behavioral tests, hematology and oxidative stress biomarkers between Se NPs treated and untreated animals.

Results and Discussion

Analysis of X-ray diffraction and the surface morphology of the Selenium particles

X-ray diffraction (XRD) peaks matched perfectly with those of JCPDS card No. 06-0362 as presented in Fig 1A. The diffraction peaks showed that the synthesized NPs were crystals with hexagonal structure. The absence of any peak other than the standard pattern indicated that the Se NPs synthesized during present investigation were in single phase and were pure. The size of crystals was calculated through Scherrer**'s** equation

$$D = \frac{K\lambda}{\beta cos\theta}$$

where crystallite size and Scherrer constant (0.94) are presented as D and k, β is the full width at half maximum and diffraction angle is presented as θ. The crystallite size calculated from above equation was found to be in the range of 40-60nm. The scanning electron microscopic (SEM) analysis was done to study the surface morphology the selenium particles and its image is shown in Fig 1B. The particles were agglomerated into blocks which have clear boundaries.

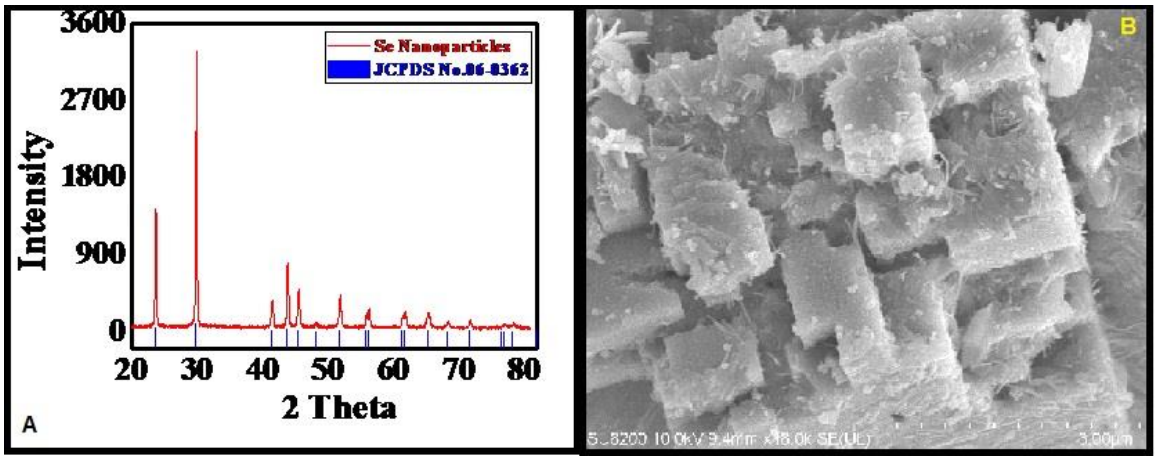

Fig. 1: (A) X ray diffraction (XRD) pattern and (B) Scanning electron micrograph (SEM) of the Selenium nanoparticles synthesized by organic acid induced method during present study.

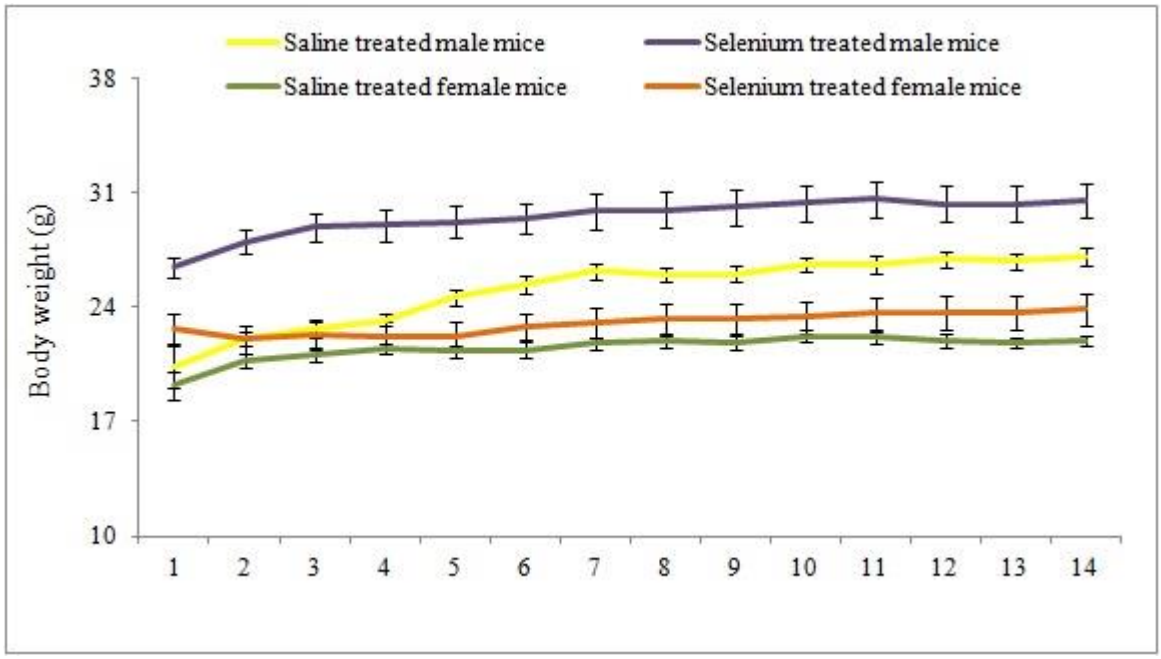

Fig. 2: Comparison of daily change in body weight between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) treated and untreated albino mice. Data is presented as mean ± standard error of mean. P-value indicates the results of two sample t-test.

Body weight analysis

Body weight remained unaffected at all studied time points when it was compared between saline and Se NPs treated mice of both sexes (Fig. 2).

Behavioral test analysis

Rota rod test

Rota rod test data analysis indicated that intraperitoneally injected Se NPs for 14 days did not affect neuromuscular coordination ($P > 0.05$) upon comparison between Se NPs and saline treated mice of both sex (Fig. 3).

Light dark box test

Male mice injected with Se NPs had less line crossing ($P = 0.02$) than their control during light dark transition test indicating decreased exploratory behavior. All other parameters remained unaffected for both treatments (Table-1).

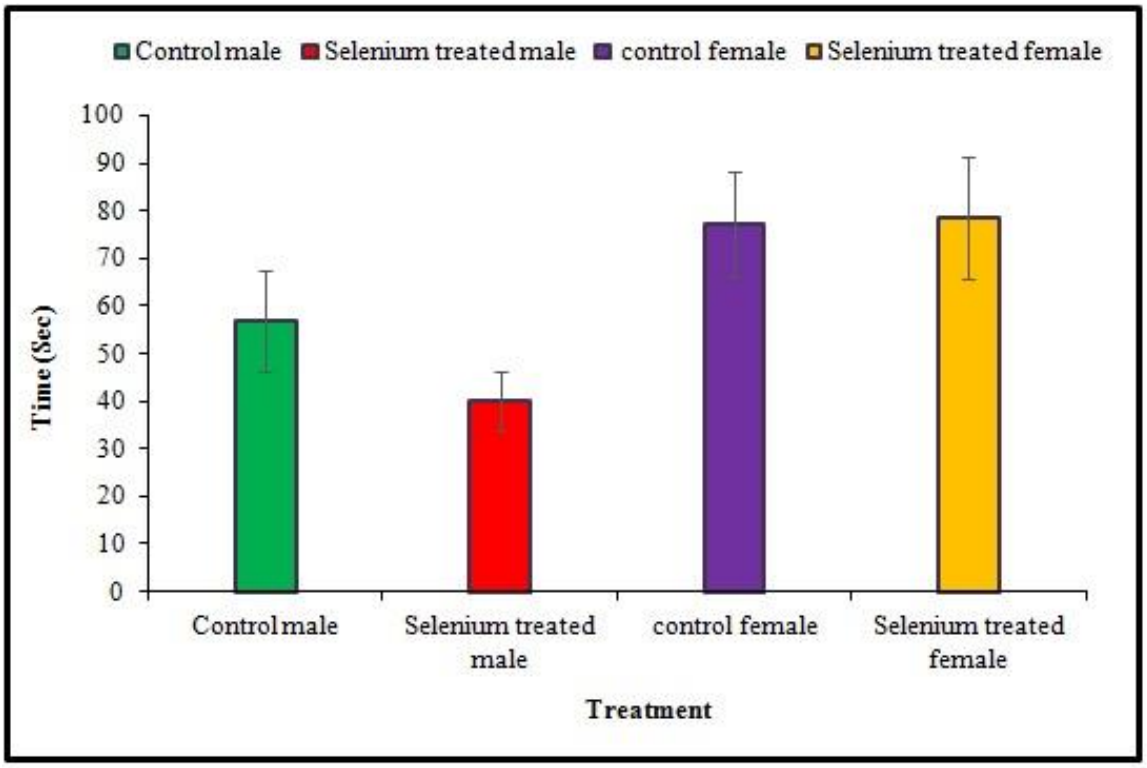

Fig. 3: Comparison of rota rod test performance between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) treated and untreated albino mice. Data is presented as mean ± standard error of mean. P-value indicates the results of two sample t-test calculated for rota rod test performance.

Table-1: Comparison of various studied parameters of light and dark transition test between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) and saline treated albino mice. N = 11 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of two sample t-test calculated for each studied parameter.

Parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Transition frequency	**8.36 ± 1.4**	**5.91 ± 1.1**	**4.0 ± 0.8**	**5.73 ± 0.89**
Line cross	**18.45 ± 1.6**	**12.55 ± 1.7 ***	**13.6 ± 2.1**	**15.45 ± 2.2**
Rearing frequency	**16.36 ± 1.2**	**14.55 ± 2.3**	**12.1 ± 1.1**	**15 ± 1.6**
Stretching frequency	**28.45 ± 2.3**	**20.3 ± 4.4**	**23 ± 3.4**	**25.27 ± 2.8**
Time in light (Sec)	**140.7 ± 17**	**183.1 ± 16**	**178.2 ± 26**	**150.1 ± 18**
Time in dark (Sec)	**159.3 ± 17**	**116.9 ± 16**	**121.8 ± 26**	**149.9 ± 18**
Defecation	**2.18 ± 0.42**	**2.36 ± 0.69**	**2.8 ± 0.39**	**2.64 ± 0.49**

$P > 0.05$ = Non-significant; $P \leq 0.05$ = least significant (*)

Open Field Test

Studied parameters of this test remained unaffected upon comparison between Se NPs and saline treated mice (Table-2).

Novel-object recognition test

Se NPs treated male mice visited object A less frequently (P = 0.02) and spent less time with object A (P = 0.001) than saline injected control during trial 1 (Table-3A). Time spent with object "B" was the only parameter that decreased significantly in Se NPs treated female mice as compared to control during trial 1 (P = 0.05) as well as during trial two (P = 0.05) of novel object test. All other of trial 1 and 2 remained unaffected (P > 0.05) upon comparison between two treatments (Table-3A, 3B).

Blood and serum analysis

Se NPs treated male mice showed lower percentage of lymphocytes (P = 0.03) while increased monocytes percentage (P = 0.03) than saline treated controls. Remaining all other parameters remained unaffected (P > 0.05) (Table-4).

Se NPs male mice had decreased cholesterol (P = 0.02) and low density lipoprotein (P = 0.003) concentration than control group while female mice treated with Se NPs had lower serum cholesterol levels (P = 0.02) than saline treated control (Table-5).

Table-2: Comparison of various studied parameters of open field test between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) and saline treated albino mice. N = 11 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of two sample t-test calculated for each studied parameter.

Parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Time in centre (Sec)	**42.8 ± 10**	**25.4 ± 4.7**	**38.3 ± 8.6**	**29.6 ± 5.6**
Time in corners (Sec)	**557.2 ± 10**	**574.6 ± 4.7**	**561.7 ± 8.6**	**570.4 ± 5.6**
Mobile episodes	**48.6 ± 8**	**54.8 ± 6.7**	**58.8 ± 8.2**	**67.0 ± 11**
Immobile episodes	**48.5 ± 7.9**	**54.5 ± 6.6**	**58.3 ± 08**	**66.3 ± 11**
Rotations	**42.2 ± 4.1**	**54.3 ± 7.3**	**57.5 ± 7.3**	**63.2 ± 09**
Clockwise rotations	**20.1 ± 2.1**	**24.6 ± 3.4**	**24.7 ± 3.3**	**26.7 ± 3.4**
Anti-clockwise rotations	**22.1 ± 2.5**	**29.6 ± 4.3**	**32.9 ± 4.3**	**37.4 ± 6.7**
Defecation	**4.55 ± 0.5**	**4.45 ± 0.7**	**4.60 ± 0.73**	**3.0 ± 0.54**

P > 0.05 = Non-significant

Table-3: (A) Comparison of various studied parameters of novel object recognition test (trial 1) test between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) and saline treated albino mice. N = 11 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of two sample t-test calculated for each studied parameter.

Parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Line cross	**14.55 ± 6.22**	**12.36 ± 7.85**	**11.9 ± 1.4**	**11.27 ± 2.0**
Stretch attend reflex	**19.09 ± 7.76**	**14.3 ± 10.7**	**26.2 ± 3.7**	**19.6 ± 3.3**
Rearing reflex	**11.36 ± 3.70**	**10.91 ± 5.63**	**12.2 ± 1.3**	**9.64 ± 1.2**
Approaches object A	**6.91 ± 2.43**	**4.27 ± 2.57***	**6.3 ± 0.83**	**6.73 ± 1.3**
Approaches object B	**6.45 ± 3.70**	**6.09 ± 3.21**	**5.5 ± 1.1**	**5.64 ± 0.92**
Time with object A (Sec)	**102.2 ± 40.2**	**42.8 ± 34.0*****	**93.3 ± 17**	**127.2 ± 18**
Time with object B (Sec)	**64.3 ± 40.9**	**69.2 ± 53.2**	**74.2 ± 17**	**35.6 ± 4.1***
Defecation	**2.18 ± 1.99**	**2.73 ± 2.37**	**1.9 ± 0.69**	**1.27 ± 0.41**

P > 0.05 = Non-significant; P ≤ 0.05 = least significant (*); P ≤ 0.001 = Highly significant (***)

Table-3 (B). Comparison of various studied parameters of novel object recognition test (trial 2) test between Selenium nanoparticles (50mg/ ml of saline/ Kg body weight) and saline treated albino mice. All values are expressed as mean ± standard error of Mean. P-value represents the results of two sample t-test calculated for each studied parameter.

Parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Line cross	**14.82 ± 8.18**	**17.64 ± 6.85**	**12.7 ± 2.7**	**12.64 ± 2.2**
Stretch attend reflex	**16.8 ± 11.6**	**20.3 ± 15.0**	**18.0 ± 4.6**	**17.18 ± 2.4**
Rearing reflex	**11.36 ± 4.50**	**13.36 ± 4.25**	**11.30 ± 1.2**	**9.55 ± 1.1**
Approaches novel object	**5.55 ± 3.45**	**4.82 ± 1.99**	**6.30 ± 0.96**	**6.55 ± 0.62**
Approaches object B	**6.18 ± 4.33**	**4.45 ± 1.57**	**5.7 ± 1.3**	**4.73 ± 0.47**
Time with novel object (Sec)	**74.3 ± 50.4**	**68.3 ± 52.7**	**76.9 ± 19**	**88.8 ± 18**
Time with object B (Sec)	**51.6 ± 45.9**	**42.5 ± 34.4**	**53.8 ± 8.6**	**32 ± 4.8***
Defecation	**2.18 ± 1.89**	**2.45 ± 1.92**	**1.6 ± 0.50**	**1.09 ± 0.34**

P > 0.05 = Non-significant; P ≤ 0.05 = least significant (*)

Markers of oxidative stress analysis

Analysis of markers of oxidative stress revealed that Se NPs injected male mice had elevated levels of Superoxide dismutase (SOD) in liver (P = 0.003) and kidney (P = 0.01), elevated level of catalase in lungs (P = 0.03) and kidney (P = 0.04) and they had higher Malondialdehyde (MDA) concentration in kidney (P = 0.003) than saline treated control (Table-5). While for Se NPs treated female mice, catalase concentration was significantly increased in the liver (P = 0.01) while MDA levels were found reduced in liver (P = 0.02) upon comparison to saline treated mice. Remaining analyzed parameters from collected organs of both sex remained unaffected upon comparison between the saline and NPs treated animals (Table-6).

Despite lot of reported benefits, biocompatibility of Se NPs nanomaterials is needed to be explored further. Selenium (Se) is generally considered as a biocompatible material and it is considered safe over a range of doses but at high doses, Se becomes toxic [17, 18]. The toxicity of Se varies from species to species and it also depends upon that how and how much Se and in which form it is entering in a living system [19]. Keeping these factors in mind, this project was conceived to document the biological effects of Se NPs in albino mice under sub acute exposure conditions.

Table-4: Comparison of studied complete blood count parameters between Selenium nanoparticles (50 mg/ml of saline/Kg body weight) and saline treated albino mice. N = 11 for each treatment. All values are expressed as mean ± standard error of mean. P value indicates the results of 2 sample t-tests calculated for each parameter.

Studied parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
White Blood Cells $\times 10^3$ /µl	11 ± 2.8	11 ± 1.1	15.7 ± 4.8	9.5 ±1.3
Lymphocytes $\times 10^3$ /µl	8.2 ± 1.8	7.8 ± 0.8	12.3 ± 4	6.2 ± 0.8
Monocytes $\times 10^3$ /µl	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
Granulocytes $\times 10^3$ /µl	2.3 ± 0.9	2.5 ± 0.4	3.6 ± 0.7	2.6 ± 0.4
Lymphocytes (%)	78.7 ± 2.6	70.4 ± 2.4*	72.2 ± 2.7	67.5 ± 2.9
Monocytes (%)	4.5 ± 0.9	7.4 ± 0.9*	4.4 ± 0.8	5.8 ± 0.6
Granulocytes (%)	16.8 ± 2.2	22.2 ± 1.9	23.3 ± 2.2	26.6 ± 2.5
Red Blood Cells $\times 10^6$ /µl	6 ± 0.4	6.5 ± 0.4	6.5 ± 0.8	6.5 ± 0.2
Hemoglobin (g/dl)	10.4 ± 0.8	11.6 ± 0.7	11 ± 1.3	10.9 ± 0.5
Hematocrit %	29.6 ± 2	32.1 ± 1.8	30.1 ± 4.1	31.3 ± 1.4
Mean corpuscular volume (μm^3)	49.1 ± 0.7	49.1 ± 1.1	45.3 ± 1.7	47.8 ± 1.3
Mean cell hemoglobin (pg)	17.1 ± 0.4	17.7 ± 0.3	18.3 ± 2.3	16.6 ± 0.3
Mean corpuscular hemoglobin concentration (g/dl)	34.8 ± 0.6	36.1 ± 0.6	41.9 ± 6.3	35.0 ± 0.8
Red blood cells distribution width (%)	23.4 ± 0.8	24.4 ± 2	24.5 ± 1.2	24.2 ± 1.1
Red blood cells distribution width -SD (μm^3)	37.2 ± 1.3	38.4 ± 2.6	36.7 ± 1.5	37.6 ± 1.2
Platelets $\times 10^3$ /µl	395 ± 118	584 ± 211	706 ± 1	448 ± 61
Mean platelet volume (μm^3)	7.7 ± 0.3	7.1 ± 0.1	7.5 ± 0.24	7.7 ± 0.4
Platelet hematocrit (%)	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.04
Platelet distribution width (%)	19.2 ± 2	16.5 ± 0.9	16.9 ± 0.95	17.8 ± 1

P > 0.05 = Non significant; P < 0.05 = Least Significant (*)

Table-5: Comparison of studied serum parameters between selenium nanoparticles (50 mg/ml of saline/Kg body weight) and saline treated albino mice following 14 days of intraperitoneal exposure. N = 11 for each treatment. All values are expressed as mean ± standard error of mean. P-value indicates the results of 2 sample t-tests calculated for each parameter.

Parameters	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Triglyceride (mg/dL)	196.1 ± 13	206.5 ± 56.3	222.8 ± 21	235.8 ± 17
Cholesterol (mg/dL)	143 ± 10	112 ± 5.7*	162.4 ± 9.9	126.4 ± 9.9*
High density lipoprotein (mg/dL)	64.5 ± 5	75.5 ± 4.2	52.9 ± 6.8	61.1 ± 5.9
low density lipoprotein (mg/dL)	70.6 ± 10	23.7 ± 5**	65 ± 14	37.9 ± 27.6
Creatinine (mg/dL)	2.14 ± 0.38	1.58 ± 1.62	0.69 ± 0.44	0.254 ± 0.06

P > 0.05 = Non significant, P < 0.05 = Least Significant (*), P < 0.01 = Significant (**)

Table-6: Comparison of studied antioxidant metabolites of brain, heart, lungs, liver and kidney in albino mice treated with Selenium nanoparticles (50 mg/ml solvent/Kg body weight) with their saline treated control mice of both gender. N = 11 for each treatment. All values are expressed as mean ± standard error of mean. P value indicates the results of 2-sample t-tests calculated for each parameter.

Organ	Metabolite	Saline treated male mice	Selenium treated male mice	Saline treated female mice	Selenium treated female mice
Brain	Superoxide dismutase (unit/gm)	7.09 ± 0.9	5.9 ± 0.7	8.5 ± 1.2	7.8 ± 1.7
	Catalase (mg/dL)	72.5 ± 7.7	61 ± 5.1	147 ± 12	112 ± 13
	Malonaldehyde (picomol/gm)	136.4 ± 11	128.8 ± 8.9	64.7 ± 3	64.8 ± 5
Heart	Superoxide dismutase (unit/gm)	0.32 ± 0.09	0.38 ± 0.13	4.84 ± 2.15	4.78 ± 2.61
	Catalase (mg/dL)	0.39 ± 0.13	0.61 ± 0.18	0.54 ± 0.1	0.64 ± 0.19
	Malonaldehyde (picomol/gm)	0.52 ± 0.15	0.71 ± 0.1	0.61 ± 0.19	0.63 ± 0.14
Lungs	Superoxide dismutase (unit/gm)	0.38 ± 0.13	0.59 ± 0.6	0.38 ± 0.15	0.39 ± 0.2
	Catalase (mg/dL)	0.42 ± 0.07	0.64 ± 0.1*	0.55 ± 0.08	0.63 ± 0.17
	Malonaldehyde (picomol/gm)	0.45 ± 0.27	0.7 ± 0.09	0.45 ± 0.27	0.7 ± 0.09
Liver	Superoxide dismutase (unit/gm)	0.44 ± 0.09	0.79 ± 0.09***	0.43 ± 0.03	0.67 ± 0.16
	Catalase (mg/dL)	0.43 ± 0.03	0.67 ± 0.16	0.38 ± 0.06	0.65 ± 0.11*
	Malonaldehyde (picomol/gm)	0.45 ± 0.27	0.7 ± 0.09	0.74 ± 0.17	0.41 ± 0.07*
Kidney	Superoxide dismutase (unit/gm)	0.39 ± 0.07	0.61 ± 0.09*	0.62 ± 0.14	0.66 ± 0.08
	Catalase (mg/dL)	0.56 ± 0.12	0.77 ± 0.03*	0.56 ± 0.55	0.61 ± 0.6
	Malonaldehyde (picomol/gm)	0.56 ± 0.06	0.87 ± 0.09**	0.48 ± 0.11	0.51 ± 0.09

P > 0.05 = Non significant; P < 0.05 = Least significant (*); P ≤ 0.01 = significant**; P ≤ 0.001 = Highly significant (***)

During present study, body weight remained unaffected on all studied time points upon comparison between Se NPs and saline treated mice of both sex (Fig. 2). Similar to our observations, Hadrup *et al.* [18] had reported that rats treated with Se NPs (0.05 mg) or sodium selenite (0.5 mg/Kg body weight/day) for 14 days exhibited no significant decrease in body weight than control. Our findings are in contrast to Shakibaie *et al.* [10] who had reported that Se NPs at the dose of 20 mg/Kg resulted in significant reduction in mice body weight, while body weight of the animals that received 2.5, 5 and 10 mg/Kg Se NPs increased as compared to their respective controls. Observed differences are probably due to different composition, concentration and size of applied Se NPs and also due to the fact that two different drug delivery methods used in these three studies.

Blood brain barrier (BBB) helps to regulate transport of materials to brain for its normal functioning. Burk *et al.,* [20] had injected Se-labeled Selenoprotein (SelP) to Selenium-deficient rats, it rapidly vanished from the serum and increased uptake was detected in brain. SelP were found in higher concentrations in endothelial cells of brain vasculature but not in brain itself. This raises questions regarding transportation of SelP through BBB and Burk *et al.,* [20] speculated that SeIPs were absorbed by the endothelial cells forming the inner BBB layer. We assume the Se NPs crossed BBB and were unequally distributed in mice brain during present investigation as we observed significant changes in the performance of some tests like light and dark test and novel object recognition test but rota rod and open field test results remained unaffected (Table 1-3; Fig. 3). Our results are in contrast with Boylan *et al.* [21] who had reported that mice fed for 8 weeks with 1 mg Se as selenite per Kg body weight exhibited greater activity and entered significantly more squares in the open field test than mice fed with Se deficient (0.2mg Selenium/Kg) diets. The difference in results is due to use of selenite in the report of study of Boylan *et al.* [21] at a different dose and for long term exposure as compared to the present study. Light and dark box is a characteristic tool used in the assessment of anxiety and exploratory behavior in rodents. We observed significantly reduced line crossing in Se NPs treated male mice than male mice injected with saline (Table-1) indicating decreased exploration. A diminished number of line crossings during light and dark transition test were associated with anxiolytic (anti-anxiety) activity. Our results are in agreement with Aburawi and Baayo [22], as they had reported that intraperitoneal Se administration (200µg/Kg) in three doses 24, 5 and 1 hours before scoring resulted in anxiolytic effects in mice. During novel object recognition test, Se NPs treated female albino mice spent more time with object "B" (that remained unchanged during trial two). Apparently, the applied dose of Se NPs did not influencing object recognition capacity of mice during this study. Our results are in agreement with Bastug *et al.* [23] who had reported no difference in learning and memory formation when compared between rats fed with Se adequate diet (1mg/ Kg bodyweight) and Se deficient (0.2mg/ Kg body weight) diets.

Hematological parameters are considered as good indicators of health as they are affected by nutritional deficiencies and stress as well as with changes in environmental factors [24]. During present study we observed a significant decrease in lymphocyte (%) in Se NPs treated mice than control (Table-4). Similarly, Keyhani *et al.* [25] had reported reduced white blood cell and platelet count in mice administrated with 30 mg/ Kg body weight of Se and they had proposed that Se administration had weakened the immune system and prevented the lymphocytes production. These findings are in contrast with Yazdi *et al.* [26] who had observed no significant changes in the levels of total white blood cells as well as no change in various types of white blood cells in the mice receiving Se NPs (100 µg/day). We assume that they applied a lower dose to affect white blood cells. We also observed a significant increase in monocyte (%) in male mice treated Se NPs than control group (Table-4). Increase in the number of monocyte in experimental group is due to effect of Se it causes impairment in the monocyte's attachment to endothelium and hence number of monocytes increases in blood of Se treated group [27].

Analysis of our results showed that the cholesterol concentration in both male and female mice and low density lipoprotein concentration in male mice decreased significantly in Se NPs treated mice as compared to saline treated controls (Table-5). Similar are the findings of Bunglavan *et al.* [28] as they had reported a significant decrease in total cholesterol in the Se NPs supplemented (150 ppb) Wistar rats as compared to control group. Se supplementation is known to enhance expression of receptors for low-density lipoprotein [29] and it has also been documented that Se administration decrease the 3-OH-methyl-glutaryl CoA reductase activity that results in decreased plasma LDL and total cholesterol concentrations [30].

Lipid peroxidation is known to be important event associated with cell death and Malondialdehyde is major chemical produced during this process. Lipid peroxidation results in impaired membrane functions, cytotoxicity and eventually in cell death [31]. During present study, we observed a significant decrease in Malondialdehyde concentrations in liver ($P = 0.02$) of Se NPs treated female albino mice than control. These observations are in line with Abubakar *et al.* [32] who had observed a significant decrease in Malondialdehyde concentrations in liver ($P < 0.001$) of Se NPs treated rats.

During present investigation, superoxide dismutase concentrations remained unaffected in all organs under investigation when compared between Se NPs and saline treated female mice. Our results are in agreement with Agarwal and Behari [33] and Fawaz and Moustafa [34] who had reported unaffected superoxide dismutase concentrations in kidney and lungs respectively in mice exposed to sodium selenite. We observed a significant increase in catalase concentrations in liver ($P = 0.04$) of Se NPs treated male albino mice. Our results are in parallel with those of Agarwal and Behari [33] who had reported higher catalase activity in liver ($P < 0.05$) of mice exposed to sodium selenite.

In conclusion, intraperitoneal injection of 50mg Se NPs/ Kg body weight for 14 days resulted in decreased line cross during light and dark transition test while rota rod, open field and novel object recognition test performance remained unaffected. Se NPs treatment disturbed the complete blood count, serum cholesterol levels and markers of oxidative stress in lungs, liver and kidney of albino mice. Toxic effects were visible in male mice than in females

Disclosure of Interest

Authors declare no conflict of interest.

Author's Contribution

Study was designed and supervised byFI, nano material was synthesized and characterized by MNA and LN, behavioral testing was done by MS and RT, hematology was done by MNK and MS, GM and GS did markers of oxidative stress analysis. All authors contributed in preparation of manuscript.

Compliance with ethical standards

Procedures and experimental protocols were reviewed and approved by the ethical committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan (Pakistan).

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