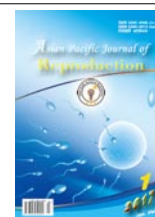


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Plasma antioxidant capacity, sexual and thyroid hormones levels, sperm quantity and quality parameters in stressed male rats received nano-particle of selenium

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ABSTRACT

Objective: To evaluate the effects of nano-particle of selenium (nSe) on plasma antioxidant capacity, sexual and thyroid hormones and spermatogenesis in male rats exposed to oxidative stress. **Methods:** Forty rats were randomly divided into four treatments with ten replicates. Treatment groups were: C, the control group received normal saline as gavage and injection (*i.p.*); OS, received tert-butyl hydroperoxide (0.2 mmol/kg body weight) for inducing oxidative stress; nSe, received nSe (0.3 mg/kg body weight) as gavage, and OS+nSe, received tert-butyl hydroperoxide and nSe. All groups were treated for 28 d and administrations were done each 48 h. **Results:** Oxidative stress decreased and gavage of nSe to stressed rats increased the antioxidant capacity and activities ($P<0.05$). Oxidative stress decreased and nSe increased the levels of thyroid, gonadotropic and testosterone hormones as compared with the control group ($P<0.05$). Gavage of nSe to stressed rat resulted in increase ($P<0.05$) of gonadotropin and testosterone hormones compared to oxidative stress group. There were no significant differences ($P>0.05$) between rats exposed to oxidative stress and those in the control group for sperm quantity and quality. Gavage of nSe to stressed rats had no effect ($P>0.05$) on the sperm parameters, except increased viability and progressive percentages. **Conclusions:** Nano-particle of Selenium administration in stressed rats could ameliorate the negative effects of oxidative stress on the antioxidant capacity and activities, but not on the quantity and quality parameters of sperm.

1. Introduction

Oxidative stress is a condition occurs in the animal body when an imbalance occurs between free radical generation and antioxidants functions[1]. If the generation of free radicals be more than usual, it can damage cells via disruption of macromolecules, such as DNA, proteins and lipids. Adverse effects of oxidative stress on thyroid function[2], testicular tissue[3] and sperm quality[4] is well documented. The imbalance may occur due to a decrease in antioxidant defense system[5], because of selenium deficiency or higher need to selenium in stressful conditions. When an animal

exposed to oxidative stress, selenium is necessary for antioxidant enzymes activity and stability to decline the harmful effects of free radicals on body macromolecules and functions[6–10]. Selenium is the active core of many seleno-proteins implicated in antioxidant defense mechanisms, testicular tissue, sperm capsule, testosterone metabolism and thyroid metabolism[11,12]. During oxidative stress, selenium maybe used for antioxidant activity and selenium deficiency occurs in the animal body for thyroid and testicular

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function. Selenium deficiency causes a decrease in intracellular glutathione peroxidase (GPx) activity[13], sperm parameters[14,15] and decrease in plasma thyroid hormones levels[12]. In the previous studies[16-18], the effects of organic and inorganic sources of selenium on the immunity, growth, health, and reproduction in human and animals were examined; however, effect of nano-particles of selenium on mentioned parameters remained unknown. Nano-selenium (nSe) has recently attracted in animal nutrition attentions, because of high absorbed ability and low toxicity compared to other sources of selenium[8]. It was hypothesized that administration of nSe could satisfy the body needs to selenium during oxidative stress and ameliorate the negative effects of stress on antioxidant capacity and activity, sexual and thyroid hormones and sperm parameters. Information about the effect of nSe on antioxidant capacity, thyroid hormone levels, and reproductive measurements in animals exposed to stress is limited. Therefore, the main purpose of this work was to evaluate the effect of nano-selenium on antioxidant capacity, sexual and thyroid hormones levels, sperm quantity and quality in male rats exposed to oxidative stress induced by tert-butyl hydroperoxide.

2. Materials and methods

2.1. Chemicals

Nano-selenium was purchased from American Elements Company (Los Angeles, USA). The average particle size was 42 nm with a purity of 99.95%. Before applying, an aliquot of nano-selenium was poured in the tube containing normal saline with 1% sodium carboxymethyl cellulose (as stabilizer). To disperse the particles, tubes were stirred for 15 min and then put in ultrasonic bath for 25 min. To avoid the aggregation of the particles, fresh suspension was prepared before every use. tert-butyl hydroperoxide (2-Methylpropane-2-peroxol) was purchased from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA).

2.2. Animals and treatments

Forty male Wistar albino rats (120 g body weight) were obtained from the Pasteur Institute (Tehran, Iran). The rats were placed in polycarbonate plastic cages, fed a standard laboratory pelleted diet for rat and fresh water ad libitum. Rats were kept in a clean animal room with air-conditioner under ambient temperature of 23 °C, relative humidity of 55%, and 12 h light/dark period. Rats were handled and cared in accordance with the guidelines on the

laboratory animals approved by the Pasteur Institute. The rats were quarantined for 7 d before starting the experiment. Thereafter, rats were fed a semi-pure pelleted diet. After acclimatization to cages environment (a week), rats were randomly assigned to four treatments with ten replicates. Treatment groups were as follows: C, the control group received normal saline as gavage and the injection (*i.p.*); OS, injected (*i.p.*) tert butyl hydroperoxide 0.2 mmol/kg body weight to induce the oxidative stress; nSe, gavaged nSe (0.3 mg/kg body weight), and OS+nSe injected (*i.p.*) tert butyl hydroperoxide (0.2 mmol/kg body weight) following gavaged nSe (0.3 mg/kg body weight). The same condition of gavage and injection was done for all rats. All experimental groups were treated for 28 d and administrations were done each 48 h.

2.3. Sample collection

Two days after final gavage and injection, feed was removed and rats were fasted overnight with free access to water. Animals were anesthetized with the injection (*i.p.*) of ketamine-xylazine. Blood samples were taken into heparinized tubes from the heart. Then tubes were centrifuged (2 500 g for 15 min) and the plasma as supernatant was collected in the clean tubes and kept at -80 °C for further analysis. Epididymis was cut and removed from the adhering tissues. After that, quantitative and qualitative measurements were made.

2.4. Blood measurements

The plasma activity of GPx was determined according to the method described by Paglia *et al.*[19]. Briefly 10 µL of plasma, standard (0.1-0.3 U/mL purified glutathione peroxidase) or water (as blank) was added to a 96-well plate. Then, to each well a quantity of 290 µL of 0.1 M phosphate buffer solution containing EDTA (5 mmol/L), NADPH (0.88 mmol/L), sodium azide (200 µmol/L), reduced glutathione (2.1 mmol/L), tert-butyl hydro-peroxide (7.9 µmol/L) and glutathione reductase (1 U/mL) were added. The reagents were mixed and the absorbance at 340 nm measured using plate reader. The activity of superoxidase dismutase (SOD) was determined according to the method described by Beauchamp *et al.*[20] using commercial kit of Pars Azmoon (Tehran, Iran). The plasma level of reduced glutathione (GSH) was determined according to the method of Ellman using spectrophotometer at 412 nm[21]. The plasma level of malondialdehyde (MDA) was assayed by the thiobarbituric acid assay using spectrophotometer at 523 nm based on the method described by Ohkawa *et al.*[22]. The plasma

level of gonadotropins hormone (FSH and LH), and testosterone were measured by using enzyme-linked immunosorbent assay using commercial kits (Shibayagi Co., Gunma, Japan), as described in the instructions provided with the kits. For spike recovery test, 10 μ L of analyte is spiked to the sample matrix and standard diluent, and the two sets of responses are compared based on values calculated from a standard curve. The plasma levels of triiodothyronine (T_3) and thyroxine (T_4) were determined using commercially enzyme-linked immunosorbent assay kits (Biocheck Inc., Foster City, CA, USA).

2.5. Sperm measurements

After removing of epididymis, the caudal epididymis of left testis was cut and the sperms were extracted into 2 mL Ham's F10 culture medium with 10% fetal bovine serum. Then culture medium was kept at 37 °C and 5% CO₂ for 30 min to disperse the sperms into the medium[23]. Sperm parameters were evaluated in Royan Institute (Tehran, Iran) with a Computer Assisted Sperm Analyzer (CASA) according to the method of Krause[23]. For assessment the percent of sperm with a normal morphology, commercial SpermBlue® kit (Microptic Co., Barcelona, Spain) with fixative and staining solutions was used as described by Van der Horst *et al.*[24].

2.6. Statistical analysis

Kolmogorov-Smirnov test was used to evaluate the normality of data using SAS Software. Thereafter, data was submitted to analysis of variance using the general linear model procedures of SAS. The used model was: $Y_{ij} = \mu + T_i + e_{ij}$. Where μ is overall average, T_i is the treatment effect and e_{ij} is the residual error. Tukey test was used for mean comparison at $P < 0.05$.

3. Results

The effect of treatments on activities of SOD and GPx and level of reduced glutathione, MDA and total antioxidant capacity (TAC) are shown in Table 1. Oxidative stress decrease SOD and GPx activities and nSe increased them ($P < 0.05$). There was no significant difference ($P > 0.05$) between nSe and control group for SOD activity, but GPx activity in nSe was higher than control ($P < 0.05$). Administration of nSe in stressed rats increased ($P < 0.05$) SOD activity compared to OS group. The highest MDA level was for OS group and the lowest one was for nSe group ($P < 0.05$). Administration of nSe in stressed rats decreased ($P < 0.05$) MDA level compared to OS group. The highest

level of TAC and reduced glutathione were for nSe and the lowest ones were for OS group ($P < 0.05$). The level of TAC and reduced glutathione in plasma of stressed rats received nSe were higher than those in OS group ($P < 0.05$).

Table 1

Effects of different treatments on antioxidant capacity and activity (mean \pm SD).

Treatments	Superoxide dismutase IU/mL	Glutathione peroxidase IU/mL	Malondialdehyde μ mol/mL	Total antioxidant capacity	Reduced glutathione μ mol/mL
Control	3.69 \pm 0.15 ^a	2.08 \pm 0.26 ^b	4.92 \pm 0.27 ^b	38.5 \pm 2.84 ^b	13.7 \pm 0.67 ^b
OS	2.22 \pm 0.38 ^c	0.88 \pm 0.07 ^d	5.83 \pm 0.27 ^a	26.8 \pm 2.02 ^d	8.2 \pm 0.30 ^f
nSe	3.92 \pm 0.18 ^a	2.77 \pm 0.13 ^a	3.19 \pm 0.18 ^d	44.6 \pm 2.93 ^a	19.1 \pm 1.17 ^a
nSe+OS	2.84 \pm 0.13 ^b	1.48 \pm 0.16 ^c	4.20 \pm 0.24 ^c	34.1 \pm 2.25 ^c	14.1 \pm 1.38 ^b
F value	44.58	86.85	80.72	34.70	83.32
P-value	0.001	0.001	0.001	0.001	0.001

^{a,b,c,d}Means in column that possess different superscripts differ significantly ($P < 0.05$).

Table 2 shows gonadotropins, testosterone and thyroid hormones levels in plasma of rats. Oxidative stress resulted in decrease ($P < 0.05$) and administration of nSe resulted in increase ($P < 0.05$) the levels of hormones as compared with control group. The highest measured hormones levels were for nSe and the lowest levels were for OS group ($P < 0.05$). Administration of nSe in stressed rat resulted in increase ($P < 0.05$) of gonadotropic and testosterone hormones compared to OS group.

Table 2

Effects of treatments on gonadotropin, testosterone and thyroid hormones levels (mean \pm SD).

Treatments	FSH ng/mL	LH ng/mL	Testosterone ng/mL	T_3 nmol/L	T_4 nmol/L
Control	379 \pm 27.36 ^b	25.6 \pm 2.35 ^b	1.79 \pm 0.21 ^{bc}	2.11 \pm 0.13 ^b	46.9 \pm 5.39 ^b
OS	307 \pm 34.52 ^c	19.0 \pm 1.43 ^c	1.58 \pm 0.15 ^c	1.76 \pm 0.12 ^c	36.3 \pm 2.13 ^c
nSe	439 \pm 25.35 ^a	31.4 \pm 2.63 ^a	2.19 \pm 0.16 ^a	2.60 \pm 0.17 ^a	53.0 \pm 4.44 ^a
nSe+OS	351 \pm 38.45 ^{bc}	23.3 \pm 2.24 ^b	1.88 \pm 0.14 ^b	1.81 \pm 0.09 ^c	34.7 \pm 2.25 ^c
F value	12.15	21.83	8.82	1.23	20.92
P-value	0.006	0.001	0.002	0.340	0.001

^{a,b,c}Means in column that possess different superscripts differ significantly ($P < 0.05$).

The effect of different treatments on concentration, viability, amplitude lateral head (ALH), beat cross frequency (BCF), and morphology of sperms are presented in Table 3. Higher concentration of sperm was seen in nSe received groups ($P < 0.05$). Oxidative stress had no effect ($P > 0.05$) on sperm concentration as there is no difference ($P > 0.05$) between OS and control group. In stressed rats, administration of selenium has no effect ($P > 0.05$) on sperm concentration as compared with OS group. Higher sperm viability was seen in groups received nSe ($P < 0.05$). There was no difference ($P > 0.05$) for sperm viability between OS group and control. nSe increased viability percentage in stressed rats. There were no differences ($P > 0.05$) among treatments for ALH and BCF.

Table 3

Effects of treatments on quantity, viability and morphology of sperms (mean±SD).

Treatments	Count 10 ⁶ /mL	Viability %	Amplitude lateral head (ALH) μm	Beat frequency (BCF) Hertz	Normal sperm Morphology %
Control	44.6±5.70 ^b	82.5±3.68 ^b	2.07±0.26	6.71±0.75	85.5±3.31 ^b
OS	45.6±6.43 ^b	85.5±2.38 ^{ab}	2.47±0.44	7.35±0.82	86.5±1.70 ^{ab}
nSe	54.2±5.35 ^a	90.5±4.43 ^a	2.37±0.47	7.94±0.93	90.2±2.64 ^a
nSe+OS	51.7±4.92 ^{ab}	90.3±1.70 ^a	2.35±0.27	6.96±0.99	89.2±2.06 ^{ab}
F value	3.92	5.47	0.83	1.46	3.19
P-value	0.036	0.013	0.501	0.273	0.062

^{a,b}Means in column that possess different superscripts differ significantly ($P<0.05$).

Table 4 shows curved line velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR) and oscillation index (WOB) of sperms. Oxidative stress increased ($P>0.05$) VCL. Administration of selenium in non-stressed or stressed groups had no effect ($P<0.05$) on VCL. Treatments had no effect ($P>0.05$) on VSL and VAP, but had significant effect ($P<0.05$) on LIN and STR. The highest LIN was seen in nSe group and the lowest one for stressed rats received nSe ($P<0.05$). WOB was not affected ($P>0.05$) by oxidative stress and/or selenium administration.

Table 4

Effect of different treatments on sperm speed parameters (mean±SD).

Treatment	VCL mm/s	VSL mm/s	VAP mm/s	LIN %	STR %	WOB %
Control	66.8±15.72 ^b	18.3±7.26	33.3±6.91	24.5±2.39 ^{ab}	47.3±1.78 ^b	51.6±3.18
OS	98.8±16.41 ^a	26.8±8.36	44.6±10.01	25.1±1.03 ^{ab}	51.6±2.44 ^{ab}	50.8±3.25
nSe	83.1±16.52 ^{ab}	27.1±9.19	39.7±5.43	29.5±5.56 ^a	56.0±8.83 ^a	54.0±4.71
nSe+OS	92.5±12.57 ^a	28.5±4.22	38.7±7.38	22.6±1.93 ^b	47.6±2.15 ^b	48.7±4.29
F value	3.28	1.53	1.48	3.19	2.91	1.25
P-value	0.058	0.257	0.270	0.062	0.078	0.333

^{a,b} Means in column that possess different superscripts differ significantly ($P<0.05$).

The effect of treatments on motile percentage, progressive percentage and different motility types of sperms are presented in Table 5. The highest motile percentage and PR was observed in groups received nSe ($P<0.05$). Oxidative stress had no effect on PR ($P>0.05$). No differences ($P>0.05$) was found for type A among treatments, but differences ($P<0.05$) appeared for types B, C and D. oxidative stress decreased types C and nSe decreased Type D ($P<0.05$) as compared with the control group. nSe administration in stressed groups had no effect ($P<0.05$) on different types compared to OS group, except increased type B and progressive percentage.

Table 5

Effect of different treatments on various motility parameters (values as percent) of sperms (mean±SD).

Treatments	Motile	PR	type A	type B	type C	type D
Control	82.0±3.89 ^b	57.1±6.89 ^b	22.7±5.95	35.7±4.75 ^b	23.7±4.77 ^a	17.9±3.88 ^a
OS	84.2±4.58 ^{ab}	59.9±3.99 ^b	29.7±6.08	30.1±4.72 ^b	24.3±4.32 ^a	15.7±3.36 ^{ab}
nSe	90.1±3.35 ^a	76.3±9.01 ^a	31.5±5.77	44.5±3.26 ^a	13.5±8.24 ^b	9.8±4.58 ^c
nSe+OS	88.7±1.57 ^{ab}	70.9±7.19 ^a	27.1±5.07	43.6±4.37 ^a	18.5±5.09 ^{ab}	11.3±1.55 ^{bc}
F value	4.57	6.70	1.78	10.20	3.01	4.54
P-value	0.0234	0.006	0.204	0.001	0.072	0.024

^{a,b,c}Means in column that possess different superscripts differ significantly ($P<0.05$).

4. Discussion

The aim of the present work was to find the effect of oxidative stress on plasma antioxidant capacity, the concentration of gonadotropic and steroidal hormones, and quality and quantity of sperms and the main purpose was to evaluate the protective effect of nano Selenium on mentioned parameters. In the literature, no report was found on the effects of nano Selenium on plasma antioxidant capacity, the thyroid, gonadotropic and steroidal hormones and sperm parameters in animals under stress.

In our study, the activities of SOD and GPx decreased in plasma of rats exposed to oxidative stress. Under oxidative stress, antioxidant defense systems included of SOD and GPx are essential factors to scavenge the reactive oxygen species[25]. Our finding is in line with the previous reports[8-10], who revealed that oxidative stress resulted in a decrease in the antioxidant enzymes activities of organs in the body of human and animals. There are discrepancies among various studies concerning the effect of oxidative stress on SOD and GPx activities. It seems that the activities of SOD and GPx depend mostly on duration of exposure and intensity of oxidative stress. Rats in this study were exposed to oxidative stress for four weeks, that is very long and the intensity of oxidant agent was very high. When the oxidative stress is chronic and the intensity is very high, the proteins damage became profound and a decrease in SOD and GPx activities may occur either via direct oxidative damage of the SOD and GPx molecules, or via oxidative stress-altered SOD or GPx gene expression, or both[10,13]. On the other hand, when the oxidative stress is for a short period (acute), the defense mechanisms will increase the activities of SOD or GPx to enervate the oxidative stress. During chronic and high intensity of oxidative stress, mitochondrial enzymes also damaged that resulted in a generation of free radicals and a decrease in energy production, thus the cells could produce less enzymatic defenses against the oxidative stress. Moreover, oxidative stress resulted in an increase of DNA damages and also DNA methylation profile[26].

As seen in Table 1, exposure of rats to the oxidative stress resulted in an increase of MDA, a decrease of TAC and a decrease of reduced glutathione. During stressful conditions, the generation of free radicals increased that resulted in a suppression of antioxidant system. As a consequence, formation of MDA, depleting antioxidant capacity and consume of GSH and finally damage to cell membrane occur in this conditions. This finding are in agreement with the reports of Zhang *et al.*[8], who speculated that oxidative stress caused a decrease in the antioxidant capacities and an increase in the MDA

level of body organs.

nSe administration increased the activities of GPx and SOD, increased plasma antioxidant capacity and decreased MDA level. Selenium is necessary for the stability and function of GPx and SOD[27]. An interesting study[13] showed that selenium deficiency is associated with significant decrease in the activity of GPx. In line with our results, Shi *et al.*[15] and Hao *et al.*[28] reported that the activities of GPx and SOD significantly increased in the tissues of groups fed selenium, and the lowest MDA level was observed in selenium supplemented group.

Rats in stressed group had lower and those received nSe had higher the level of reduced glutathione. The GPx enzyme consume reduced GSH to reduce the hydrogen peroxide to water and alcohol[29]. Boostani *et al.*[18] reported that selenium supplementation increased the plasma level of GSH in chicks.

In our study, rats exposed to oxidative stress had lower the plasma levels of LH and FSH, which may be related to high plasma corticosterone level[30]. High concentration of corticosterone cause a reduction in the plasma levels of gonadotropin and steroids hormones[31]. Consistent to our finding, in the study of Gökçe *et al.*[32], the oxidative stress reduced sex hormones levels. Previous studies[33-35] revealed that oxidative stress has an important role in the pathogenesis of human and animal infertility.

nSe administration increased plasma gonadotropin and steroids levels in this study. Oishi *et al.*[36] found that feeding low selenium diet to the pullets resulted in a significant decrease in the plasma levels of LH. The findings of Behne *et al.* [37] revealed that morphology and function of testis influenced by selenium deficiency. They also speculated that selenium is necessary for biosynthesis of testosterone and the spermatozoa formation and normal development.

In the present study, oxidative stress resulted in decrease and selenium administration in non-stressed rats increased T₃ and T₄. When the balance between free radicals and antioxidants disrupt, pathological defects of oxidative stress occur. Depending on the nature, amount, and duration of the free radicals generation, these defects cause damages to biomolecules such as enzymes effective in hormones synthesis and organs function, especially to thyroid gland[35]. The thyroid gland has high content of selenium mostly for function of thyroid deiodinases, which catalyse the conversion of T₄ to T₃[6,7].

Induce of oxidative stress in this study decreased the quantity and quality of sperms. Previous study demonstrated that oxidative stress impaired the motility, concentration, and morphology of sperms[38]. The membrane of mammalian spermatozoa made from high levels of polyunsaturated fatty acids which are very sensitive to oxidative

damages via lipid peroxidation[39]. Thus sperms membranes are susceptible to attack of free radicals which results in decreased the sperm motility, presumably by a rapid loss in pool of intracellular ATP. When these events occur, axonemal damage appears which results in a decrease of sperm viability and an increase in the morphological defects of sperm and loss of motility[4].

In the present work, selenium administration resulted in an increase of the sperms parameters in the non-stressed group. It was demonstrated[11,14-16,37] that selenium is an essential factor for normal testis function and spermatogenesis. Selenium can reduce free radicals as a cofactor for antioxidant enzymes such as GPx and through this function can increase the sperm quality and quantity parameters and finally its fertility. Shi *et al.*[15] found that selenium deficiency resulted in abnormal mitochondria of spermatozoa. In their study, nSe supplementation enhanced the selenium content of testis, GPx activity in the testicular tissue and semen, decreased sperm abnormality and protected the integrity of membrane.

It was concluded that nano-particle of selenium administration in the stressed rats could ameliorate the negative effects of oxidative stress on antioxidant capacity and activities, but not on quantity and quality parameters of sperm. Further studies are required to research the novel elemental nSe with characterization of bioavailability and fertilization capability of sperm.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

- [1] Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Intern J Biochem Cell Biol* 2010; **42**(10): 163-165.
- [2] Campos C, Casado A. Oxidative stress, thyroid dysfunction & down syndrome. *Indian J Med Res* 2015; **142**(2):113-119.
- [3] Turner TT, Lysiak JJ. Oxidative stress: A common factor in testicular dysfunction. *J Androl* 2008; **29**(5): 488-498.
- [4] Bansal AK, Bilaspuri GS. Impacts of Oxidative Stress and Antioxidants on Semen Functions. *Vet Med Int* 2010; **2010**: 7
- [5] Burton GJ, Jauniaux E. Oxidative stress-best practice and research. *Clin Obstet Gynaecol* 2010; **25**(3): 287-299.
- [6] Beckett GJ, Arthur JR. Selenium and endocrine systems. *J Endocrinol* 2005; **184**(3): 455-465.

- [7] Köhrle J, Jakob F, Contempré B, Dumont JE. Selenium, the thyroid, and the endocrine system. *Endocr Rev* 2007; **26**(7): 944-984.
- [8] Zhang JS, Wang XF, Xu TW. Elemental selenium at nano size (nano-Se. as a potential chemopreventive agent with reduced risk of selenium toxicity: Comparison with Se-methylselenocysteine in mice. *Toxicol Sci* 2008; **101**(1): 22-31.
- [9] Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum MDA in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. *J Cancer Res Ther* 2010; **6**(4): 487-491.
- [10] Miyamoto Y, Koh YH, Park YS, Fujiwara N, Sakiyama H, Misonou Y, et al. Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses. *Biol Chem* 2003; **384**(4): 567-574.
- [11] Ahsan U, Kamran Z, Raza I, Ahmad S, Babar W, Riaz MH, et al. Role of selenium in male reproduction-A review. *Anim Reprod Sci* 2014; **146**(1-2): 55-62.
- [12] Köhrle J, Gärtner R. Selenium and thyroid. *Best Practice Res Clin Endocrinol Met* 2009; **23**(6): 815-827.
- [13] Styblo M, Walton FS, Harmon AW, Sheridan PA, Beck MA. Activation of superoxide dismutase in selenium-deficient mice infected with influenza virus. *J Trace Elem Med Biol* 2007; **21**(1): 52-62.
- [14] Shalini S, Bansal MP. Role of selenium in regulation of spermatogenesis: Involvement of activator protein 1. *Biofactors* 2005; **23**(3): 151-162.
- [15] Shi LG, Yang R, Yue W, Xun W, Zhang C, You-she E, et al. Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. *Anim Reprod Sci* 2010; **118**(2-4): 248-254.
- [16] Ganabadi S Jr, Halimatum Y, Amelia Choong KL, Nor Jawahir A, Mohammed Hilmi A. Effect of selenium supplementation on spermatogenic cells of goats. *Mal J Nutr* 2010; **16**(1): 187-193.
- [17] Boostani A, Sadeghi AA, Mousavi SN, Chamani M, Kashan N. The effects of organic, inorganic, and nano-selenium on blood attributes in broiler chickens exposed to oxidative stress. *Acta Sci Vet* 2015; **43**(1): 1-6.
- [18] Boostani A, Sadeghi AA, Mousavi SN, Chamani M, Kashan N. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. *Livest Sci* 2015; **178**: 330-336.
- [19] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**(1): 158-169.
- [20] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1971; **44**(1): 276-279.
- [21] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**: 70-77.
- [22] Ohkawa H, Ohish N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid. *Anal Biochem* 1979; **95**(2): 351-358.
- [23] Krause W. Computer-assisted semen analysis systems: Comparison with routine evaluation and prognostic value in male fertility and assisted reproduction. *Hum Reprod* 1995; **10** (Suppl 1): 60-66.
- [24] Van der Horst G, Maree L. Sperm Blue: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. *Biotech Histochem* 2009; **84**(6): 299-308.
- [25] Atencio L, Moreno I, Jos Á, Prieto AI, Moyano R, Blanco A, et al. Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom. *Toxicon* 2009; **53**(2): 269-282.
- [26] Zribi N, Chakroun NF, Elleuch H, Abdallah FB, Ben Hamida AS, Gargouri J. Sperm DNA fragmentation and oxidation are independent of malondialdehyde. *Reprod Biol Endocrinol* 2011; **9**: 47-52.
- [27] Köhrle J, Brigelius-Flohé R, Böck A, Gärtner R, Meyer O, Flohé L. Selenium inbiology: Facts and medical perspectives. *Biol Chem* 2000; **381**(9-10): 849-864.
- [28] Hao X, Ling Q, Hong F. Effects of dietary selenium on the pathological changes and oxidative stress in loach (*Paramisgurnus dabryanus*). *Fish Physiol Biochem* 2014; **40**(5): 1313-1323.
- [29] Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C, Lillig CH. Thiol redox control via thioredoxin and glutaredoxin systems. *Biochem Soc Trans* 2005; **33**(Pt6): 1375-1377.
- [30] Artykova MP, Perfileva IF, Chumburidze ES. Excretion of adrenaline, noradrenaline and luteinizing hormone during treatment with arsenate mineral water of chronic adnexitis. *Vopr Kurortol Fizioter Lech Fiz Kult* 1977; **3**: 72-74.
- [31] Hardy MP, Gao HB, Dong Q, Ge R, Wang Q, Chai Feng X, et al. Stress hormone and male reproductive function. *Cell Tissue Res* 2005; **322**(1): 147-153.
- [32] Gökçe A, Oktar S, Koc A, Yonden Z. Protective effects of thymoquinone against methotrexate-induced testicular injury. *Human Exp Toxicol* 2011; **30**(8): 897-903.
- [33] Muthuvel R, Venkataraman P, Krishnamoorthy G, Gunadharini DN, Kanagaraj P, Jone Stanley A, et al. Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. *Clin Chim Acta* 2006; **365**(1-2): 297-303.
- [34] Yang L, Zhang B, Yuan Y, Li C, Wang Z. Oxidative stress and DNA damage in utero and embryo implantation of mice exposed to carbon disulfide at peri-implantation. *Human Exp Toxicol* 2014; **33**(4): 424-434.
- [35] Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian J Exp Biol* 2005; **43**(11): 963-974.
- [36] Oishi T, Yoshida K, Moriguchi S, Inuzuka S. Effects of low-selenium diet on levels of plasma luteinizing hormone, follicle-stimulating hormone and estradiol in pullets and laying hens. *Jpn Poult Sci* 1988; **25**(5): 337-342.
- [37] Behne D, Weiler H, Kyriakopoulos A. Effects of selenium deficiency on testicular morphology and function in rats. *J Reprod Fertil* 1996; **106**(2): 291-297.
- [38] Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, Wei YH. Increase of oxidative stress in human sperm with lower motility. *Fertil Steril* 2008; **89**(5): 1183-1190.
- [39] Khosrowbeygi A, Zarghami N. Fatty acid composition of human spermatozoa and seminal plasma levels of oxidative stress biomarkers in subfertile males. *Prostaglandins Leukot Essent Fatty Acids* 2007; **77**(2): 117-121.