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RESEARCH ARTICLE

Selenium nanoparticles increase the testicular antioxidant activity and spermatogenesis in male rats as compared to ordinary selenium

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Abstract

The present study was conducted to investigate the ability of selenium nanoparticles to increase the testicular antioxidant activity and reproductive capacity of male rats and compare the effect of nano-selenium and selenium. The current results showed that nano-selenium has potent effects in increasing the antioxidant capacity by increasing the concentrations of reduced glutathione (GSH) and total antioxidant capacity (TAC), increasing the activities of glutathione reductase (GR) and catalase enzymes, decreasing the level of Lipid peroxidation (MDA). The nano-selenium significantly increases the (metallothionine 1) MT 1 mRNA expression in testes as compared to selenium and control groups. There was a significant increase in the reproductive capacity of rats and this can be seen in the marked increase of the sperm cell count and motility even in the weight and volume of testes and epidiymis in nano selenium treated rats as compared to other groups. Our results were confirmed by histopathological investigation which comes in agreement with our biochemical findings. From the results, it can be concluded that nano selenium improved the spermatogensis and sperm characters as compared to ordinary selenium.

Introduction

Nanotechnology, the design and manipulation of materials at the atomic scale, has the potential to deliver considerable benefits to society. The novel properties that emerge as materials reach the nanoscale open the door to innovations in energy, manufacturing, and medical treatment. At the same time, these novel properties may pose new risks to workers, consumers, the public, and the environment. The limited data now available demonstrate the potential for some nanomaterials to be persistent and mobile in the environment and in living organisms; to cross multiple physiologic barriers (including lung-blood, blood-brain, and placental barriers, and cell membranes) **(Oberdörster et al., 2005; Nel, 2006).**

Oxidative stress is considered to be one of the important mechanisms involved in carcinogenesis and organs damage **(Wang et al., 2006).** ROS is known to play an essential role in the pathogenesis of many reproductive processes. Oxidative stress attacks the sperm membrane lipids and the DNA in the sperm nucleus **(Zalata et al., 2004)**. ROS-induced DNA damage may accelerate the process of germ cell apoptosis, leading to the decline in sperm counts associated with male infertility **(Agarwal et al., 2003)**. Several studies have implicated ROS in male infertility in mammals **(Conte et al., 1999; Agarwal et al., 2005).**

Selenium (Se) is of fundamental importance to human health **(Rayman, 2000)**. In the form of selenocysteine, the 21st amino acid, Se functions as a redox center of an array of selenoproteins **(Driscoll et al.,**

2003), some of which have important enzymatic functions for the redox homoeostasis, such as glutathione peroxidase (GPx) **(Miyamoto et al., 2003)**, phospholipid hydroperoxide glutathione peroxidase (PHGPx) **(Imai et al., 2003)** and thioredoxin reductase (TrxR) **(Arner and Holmgren, 2000; Becker et al., 2000)**. The efficacy of Se in inducing Se-containing enzymes in vivo and in vitro depends on its chemical form **(Ortuno et al., 1996)**. Properties of Se are indeed dependent on its size. Above micrometer size, it is biologically inert **(Zhang et al., 2001)**. In contrast, subnano particles of elemental Se have robust cytotoxicity to leukemia cells **(Sieber et al., 2005)**. It has been reported that Nano-Se has a size effect on redox reactivity **(Mishra et al., 2005)**, and Nano-Se in the range of 5–200 nm has a size dependent effect in directly scavenging various free radicals in vitro, such as 1,1-diphenyl-2-picryhydrazyl (DPPH) and the superoxide anion **(Huang et al., 2003)**.

We undertook the present study to examine the beneficial antioxidant effects of Nano Selenium on spermatogenesis. We evaluated the effect of Nano selenium on semen parameters including sperm concentration, motility and viability in cauda of epididymis.

Materials and Methods

Preparation of selenium nanoparticles

One mL of 25 mM sodium selenite (Sigma-aldrich, Egypt) was mixed with 4 mL 25 mM of glutathione containing either 2 mg or 20 mg bovine serum albumin. The mixture pH was adjusted to 7.2 with 1.0 M sodium hydroxide to generate red elemental selenium and oxidized glutathione. The red solution was dialyzed for 96 hours at 4°C against double distilled water which was replaced every 24 hours to separate oxidized glutathione from the selenium nanoparticles. The final solution containing selenium nanoparticles and bovine serum albumin was stored in a 4°C refrigerator, in which the selenium nanoparticle solution Prepared using the low concentration of bovine serum albumin was stable for several months, and the selenium nanoparticle solution prepared by using the high concentration of bovine serum albumin was Stable for several years **(Zhang et al., 2001).**

Experimental design

This study was approved by the Committee of Scientific Ethics at Beni-Suef University, Egypt, and was carried out in accordance with its guidelines for animal use. Thirty male albino Wistar rats weighing on average 150–200 g were used in this study. They were obtained from the animal house of the Research Institute of Ophthalmology, Giza, Egypt. They were kept under suitable conditions for 1 week for adaptation. They were maintained in stainless steel cages in a well-ventilated animal house at normal temperature (22°C ± 5°C) under a 12:12-hour light–dark cycle. They were fed with standard diet and given water ad-libitum. The rats were randomly divided into three equal groups (10 rats each) as follow **Group 1 (control)** rats were given a single dose of sterile PBS (0.5 mL intraperitoneally [IP]) as a single dose. **Group 2** (selenium-treated group) rats were given 0.5 mg/kg body weight dispersed in 0.5 mL PBS IP for seven successive day 24 hours interval. **Group 3** (nano-selenium-treated group) rats were given nano-selenium (0.5 mg/kg body weight dispersed in 0.5 mL PBS IP for seven successive days 24 hours interval. Twenty-four hours after administration of the last dose. Rats were dissected under anesthesia by using 1.37:1 mixture of ketamine:xylazine (1 mL/kg body weight, IP).

Testes tissue preparation

After dissection testes were quickly collected and then washed by physiological saline to remove any clotted blood or tissue debris. The testes were weighted and the caudal epididymis was removed the testes were divided into three parts. The first part (0.5 gm) was suspended in 5 ml physiological saline (0.9 % NaCl) for homogenization (Teflon Homogenizer, India). The tissue homogenates were centrifuged 1500 X g for 20 minutes at 4 ºC. The supernatants were kept at – 20 ºC till the time of determination of oxidative/ antioxidant parameters **(Lin et al 2010)**. The second part was kept for MT-1 mRNA expression. The third part was placed in 10 % formalin solution for histopathological investigations.

Biochemical parameters

All kits of the measured parameters were purchased from Biodiagnostic company (Cairo, Egypt). The following parameters are measured using T80 UV/VIS spectrometer (China).

Determination of antioxidant parameters

Nitric oxide (NO) was measured according to **Montgomery and Dymock (1961**). Total antioxidant capacity (TAC) determination was based on **Koracevic et al. (2001)**. Malondialdehyde (MDA) was measured according to **Satoh (1978)**. Superoxide dismutase (SOD) activity measurement was based on **Nishikimi et al. (1972)**. Reduced glutathione (GSH) content was measured according to **Beutler et al. (1963)**. Glutathione Reductase (GR) activity was determined according to **Goldberg and spooner (1983)**.

Real-time RT-PCR analyses

Total RNA was extracted with Trizol agent, and purified with RNeasy column (Qiangen, CA) according to the manufacturer's instructions. Total RNA was reverse transcribed with MuLV reverse transcriptase and oligo-dT primers.

	Forward primer	Reverse primer
B-actin	GGCCAACCGTGAAAAGATGA	CAGCCTGGATGGCTA CGTACA
MT -1	CTCCGTAGCTCCAGCTTCAC	AGGAGCAGCAGCTCTTCTTG

The SYBR green Power PCR master Mix (Applied Biosystems, Foster City, CA) was used for real-time PCR analysis. The relative differences in expression between groups were expressed using cycle time (Ct) values, and Ct values for interested genes were first normalized with that of b-actin in the same sample, and then relative differences between groups were expressed as relative increases, setting control as 100%.

Semen collection and Sperm characteristics analysis

The testes were removed along with its epididymis and weighted separately. Also, The Testes and epididymis were separately immersed in 5 ml normal saline in a measuring cylinder and recording its volumes. The cauda epididymis were minced in normal saline and a drop of this epididymal suspension was picked up for seminal analysis and recording the epididymal spermatozoal characters **(Hafez et al., 1970)**, sperm motility **(Slott et al., 1991)**, sperm cell concentration per ml of semen **(Robb et al., 1987)**, sperm abnormalities and live % of spermatozoa **(Filler et al., 1993)**.

Histopathological examination

Testes were fixed in 10% formalin solution for 48hrs. Then they were processed (washed by water, dehydrated in graduated ethyl alcohol, cleared in xyline and embedded in paraffin wax at 70 ^{0}C) according to **Bancroft and Gamble (2008**). Five microns tissue thickness were mounted on clean glass slides and stained by Heamatoxylin and Eosin.

Statistical analysis

All data were expressed as means± SEM. Differences between the groups were determined by one-way ANOVA followed by least square difference (LSD) post hoc, using SPSS software version 15.0 and results were considered significant when $P < 0.05$ **(Wei et al., 2009)**.

Results

The weight and volume of both testes and tail of epididymis showed a significant increase in Nano – selenium group as compared to both control and selenium groups (Table 1).

Table 1: Effect of Nanoparticle of Selenium on the weight and volume of sexual organs of Albino Rat:

	Testes		**Tail of Epididymis**	
Groups	**Weight (g)**	**Volume (cm^3)**	**Weight (g)**	**Volume (cm^3)**
Control	2.5±0.4 ^{a}	3.30 ± 0.10 ^{a}	0.4±0.03 ^{a}	1.1±0.24 ^{a}
Selenium	2.9±0.8 ^{a}	3.70±0.22 ^{a}	0.5±0.08 ^{a}	1.3±0.10 ^{a}
Nano-Selenium	4.1±0.1^b	5.20±0.20 ^{b}	0.9±0.02 ^{b}	2.1±0.10 ^{b}

Values are expressed as mean ± SE.

Values with different letters in a column are significantly different at level $p<0.05$.

Our results showed a significant increase in the sperm motility, concentration and viability in Nano- Selenium group as compared to control and Selenium groups. The sperm abnormalities % was significantly reduced in Nano – Selenium group as compared to control and Selenium groups (Table 2).

Table 2. Semen parameters of experimental rats treated with Selenium Nanoparticles:

	Sperm parameters (Mean ± SE)			
Groups	**Motility %**	**Sperm cell conc. (X10^6)**	**Viability (%)**	**Abnormalities (%)**
Control	82.66±1.66 ^{a}	22.63±0.81 ^{a}	86.75±0.39 ^{a}	15.81±0.6 0 ^{a}
Selenium	85.50±1.33 ^{a}	25.22±0.60 ^{a}	88.82±0.44 ^{a}	11.24±0.42 ^{a}
Nano-Selenium	94.00 ± 2.00 ^{b}	35.33±0.80 ^{b}	96.22±0.94 ^{b}	6.43±0.61 ^{b}

Values are expressed as mean ± SE.
Values with different letters in a column are significantly different at level p<0.05.

Both Nano-Selenium and selenium significantly increase the GSH concentration, significantly decrease SOD, catalase activities and MDA concentration as compared to control group. Nano- selenium group showed a significant increase of GSH concentration, MDA and catalase activity as compared to selenium group but SOD activity showed a non significant difference between Nano- selenium and selenium groups (Table 3).

Table 3: Changes of GSH level, SOD activity, MDA concentration and catalase activity in testes of different groups:

Groups	GSH (mg /gm tissue)	SOD (U / gm tissue)	MDA (nmol/gm tissue)	Catalase (U /gm tissue)
Control	30.50± 1.50[a]	04.97 ± 0.16[a]	19.09 ± 1.02[a]	6.87± 0.58[a]
Selenium	64.20± 4.10[b]	12.40 ± 0.68[b]	9.90 ± 0.99[b]	18.1 ± 1.60[b]
Nano-Selenium	82.11 ± 1.30[C]	13.54 ±1.01[b]	3.01 ± 2.03[c]	29.8± 1.21[c]

Values are expressed as mean ± SE.
Values with different letters in a column are significantly different at level p<0.05.

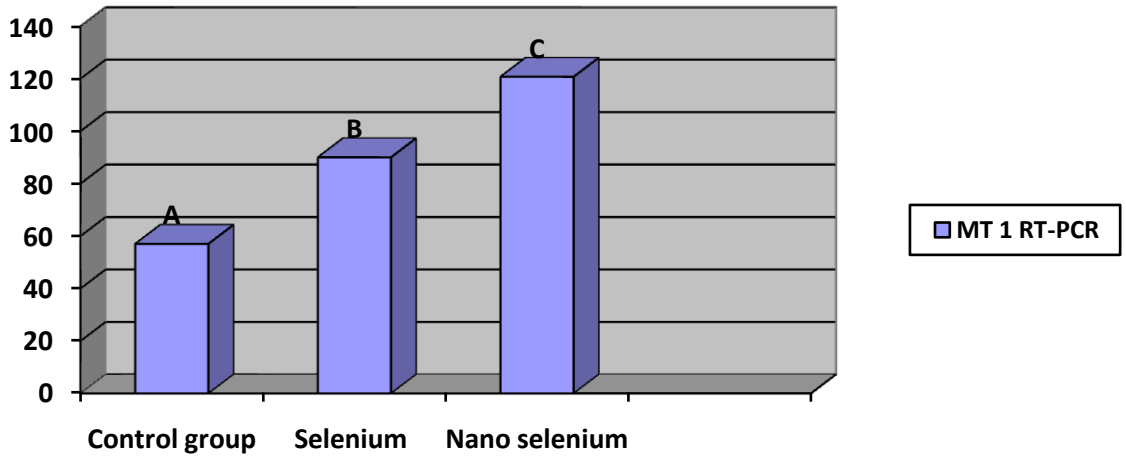
The results of the table indicated a non significant difference of NO in all groups, a significant increase of TAC and glutathione reductase (GR) activity in Nano –selenium group as compared to control and selenium groups (Table 4).

Table 4. changes in niterous oxide (NO) concentration, total antioxidant capacity (TAC) and glutathione reductase (GR) activity in testes of different groups:

Groups	NO (µmol/ gm)	TAC (mM / gm)	GR (U/ gm tissue)
Control	23.43± 1.20[a]	30.21± 1.74[a]	135.90± 5.98[a]
Selenium	22.12±2.03[a]	45.98±0.99[b]	245.80±2.11[b]
Nano-Selenium	19.20±1.40[a]	62.10±2.87[C]	351.06±1.95[C]

Values are expressed as mean ± SE.
Values with different letters in a column are significantly different at level p<0.05.

Figure (1): changes in MT-1 mRNA RT-PCR expression against B- actin: The figure showed a significant increase of MT-1 mRNA expression in Nano- selenium group as compared to selenium and control groups.



Different superscript letters indicate significant differences at P < 0.05.

Figure 2: Gross picture of testes and Couda Epididymis of male albino rat showing; **(A)**Normal sizes (Control group), Enlarged size **(B)** (Selemium group) and hypertrophy Over-enlarged Sizes testes and Epididymis **(C)** (Nano-selenium group).

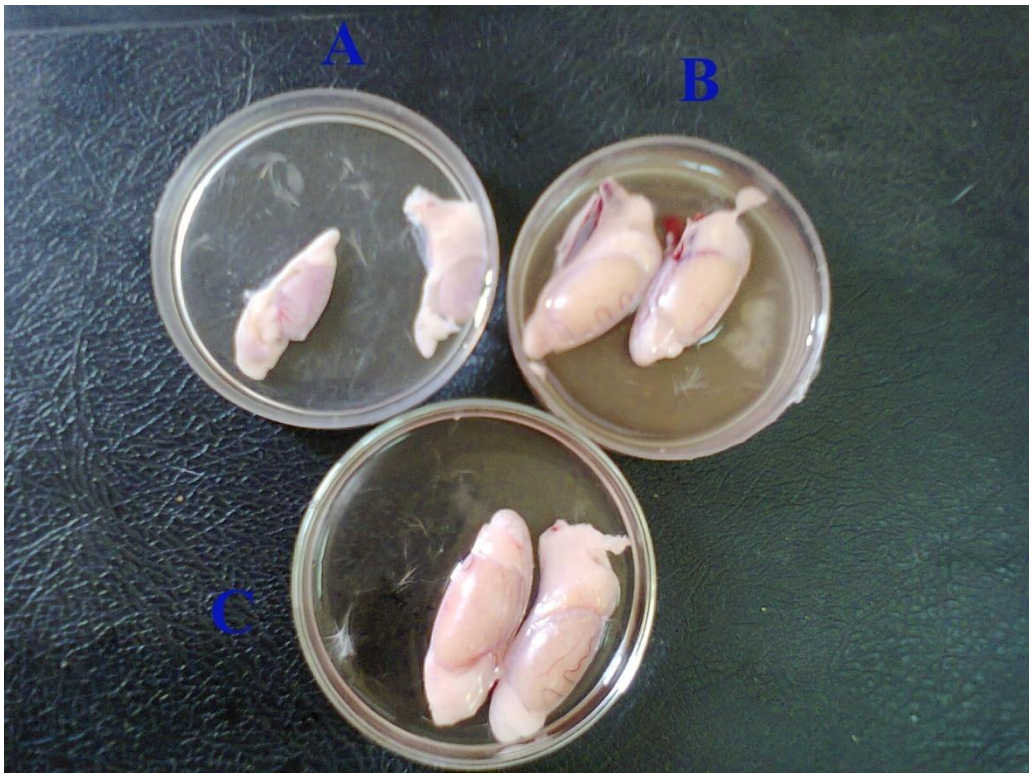
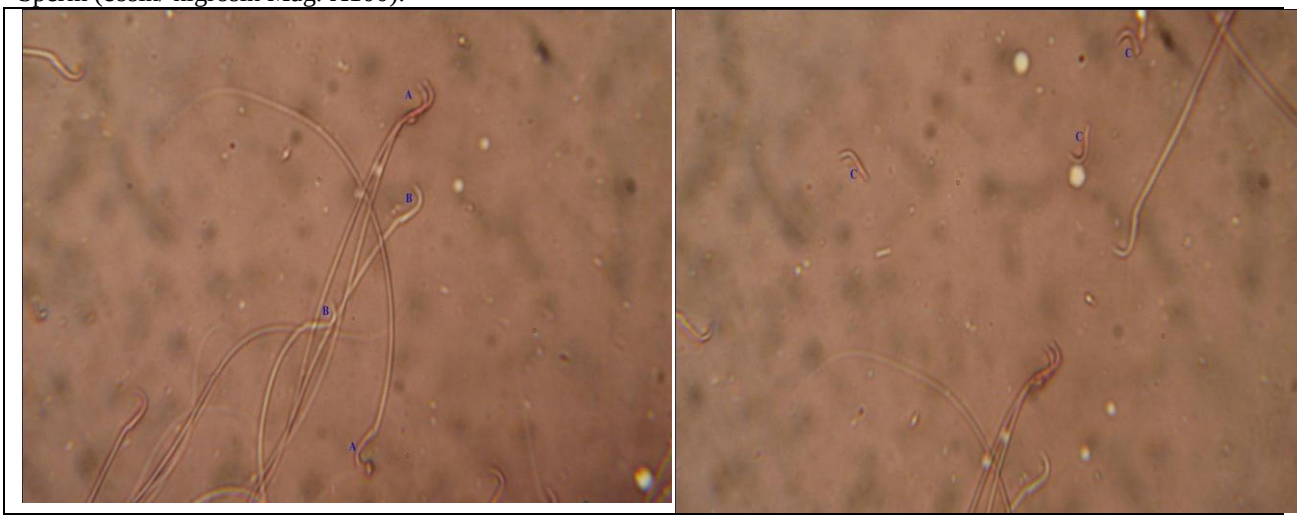


Figure (3). Photomicrograph showing sperm from the cauda epididymis of control group: the figure showed many sperm abnormalities; **A:** Dead sperm, **B:** Live Sperm, **C:** tail less sperm, **D:** Curve tail **E:** coiled tail and **F:** Normal Sperm (eosin/ nigrosin Mag. X100).



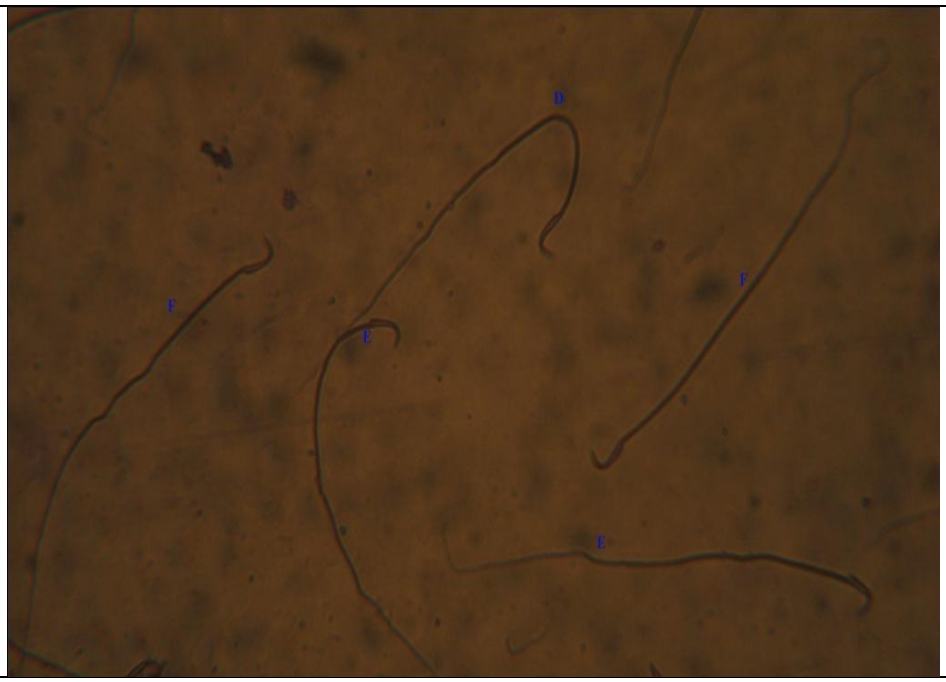
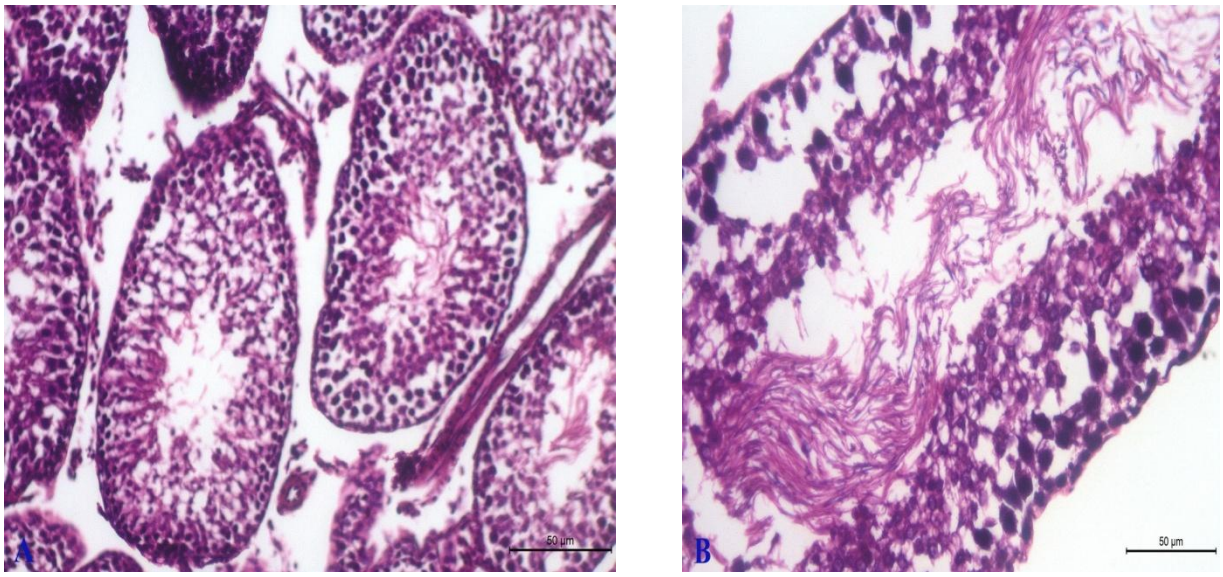
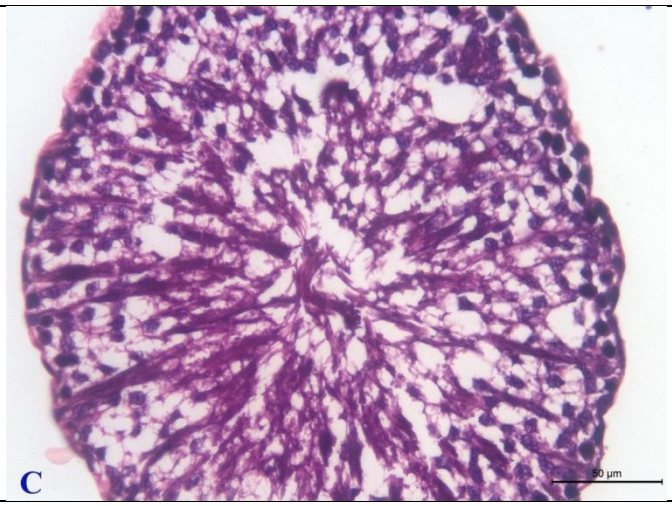


Figure (4): Histological architecture of the testis in different treatment groups: (A) Control group and selenium (B) groups showed normal testicular histology with the lumen of seminiferous tubules filled with sperm and showed poor recovery in comparison to complete recovery in Nano-Selenium groups evidenced by densely filled seminiferous tubules and healthy Sertoli cells attached to the basement membrane (C).





Discussion

Oxidative stress is a constant threat to all living organisms and an endogenous antioxidant defense system is employed by the body to eliminate or attenuate it **(Ceconi et al., 2003)**. Oxidative damage occurs when forces that favor oxidation outweighs antioxidant protection within cells **(Marczin et al., 2003)**. The increased oxidative stress in reproductive system that defined as stress induced by increased numbers of molecules containing reactive oxygen species (ROS) **(Yesilbursa et al., 2005)**. Oxidative stress, free radical formation and decrease antioxidant activity are among the causes of testicular degeneration and spermatogensis alteration **(Nabela et al., 2010)**. ROS are produced by numerous metabolic and physiologic processes in the body, but the increased level of ROS is toxic to cells including spermatozoa **(Rodriguez et al., 2002)**. It is reported that ROS in a balanced concentration is necessary for capacitation, acrosome reaction, sperm motility and fertility **(Agarwal et al., 2004)**. The oxidative stress induced by ROS was estimated in rat testes of control group by measuring some oxidative/ antioxidant parameters such as GSH, CAT, SOD, GR, MDA, TAC and NO in testicular tissue.

Selenium is an essential dietary trace element, which has an antioxidant role in the protection of the cell from oxidative damage. Selenium is an integral part of many proteins with catalytic and structural functions. Its nutritional deficiency leads to muscular dystrophy, endemic fatal cardiomyopathy (Keshan disease), and chronic degenerative diseases in humans that could be prevented by selenium supplementation when used alone or in combination **(Rayman, 2002)**. The most important metabolic roles of selenium in mammalian cell are due to its function in the active site of many antioxidant enzymes, e.g., thioredoxin reductase, glutathione (GPx) and GR **(Flora et al., 2002).**
Genital organs weights were among of the criteria used to evaluate the reproductive capacity of the tested selenium nanoparticles on rats. Our current data showed that selenium nanoparticles significantly increased the testes and epididymis weights and volume as compared to selenium and control groups. On the other hand, there were no significant differences in weight and volume of testis and epididymis among the selenium group as compared with control **(Figure 2)**.
Useful information on male reproductive capacity of laboratory animals can be obtained by measuring weights and the volume of testis, and epididymis **(Doul et al., 1980).** Generally, maintenance of weights of reproductive organs and accessory glands depends on testosterone level **(Jana et al., 2003)**. The present study showed that selenium nanoparticles caused a significant increase in the male reproductive organs and improve the testicular functions.
In our experiment, we have observed that nano-selenium treated rats exhibited a significant increasing in sperm count, motility and vitality than that of control and selenium groups **(Table 2)**. Increased number of sperm in rat's epididymis is due the increase of cell population in the seminiferous tubules and increased sperm vitality and motility after selenium nanoparticles adminsteration. The testicular size and sperm parameters is controlled by testosterone hormone level. **Sajjadian et al., (2014)** stated that increase the testicular antioxidant activity is associated with increase testosterone level.
Our results showed that Nano- selenium administration increased the testicular antioxidant activity and defense mechanism through increasing the testicular GSH concentration, decreases MDA concentration, increase catalase and SOD activity as compared to control and selenium groups **(Table 3)**. Moreover, Nano- selenium administration caused a significant increase of GR activity and TAC as compared to control and selenium groups **(Table 4)** while

no changes have been seen in NO concentration in both Nano – selenium and selenium group as compared to control group.

One of the most accepted hypotheses of Se antioxidant activity is the ability of Se to keep the glutathione in the reduced form where glutathione has the ability to detoxify the ROS. The action of Se is achieved by the selenium-containing enzyme GSH-Px protects cells against ROS **(Köhrle and Gärtner 2009)**.
More interestingly, a marked induction of MT by Se and Nano Se was observed in the present study **(Figure 1)**. The rat MT multi-gene family consists of four known members that are located on chromosome 8. MT-1 and MT-2 exist in all tissues examined, and are the predominant forms of metallothionein protein in the body; while expression of MT-3 and MT-4 is restricted in specific organs **(Klaassen et al., 1999)**. MT does not only play a role in sequestrating heavy metals especially cadmium from critical organelles, but is a free radical scavenger to protect against oxidative stress **(Liu et al., 2009)** and also, it has strong antioxidant properties **(Park et al., 2011; Weng et al., 2011)**. Thus the induction of MT -1 produces general protective functions against toxic stimuli such as chemical-induced oxidative damage **(Aydin et al., 2010; Smina et al., 2011)**. From our finding, we can hypothesized that, one of the most recent mechanisms of nano- selenium is the induction of MT-1 in testicular tissue.
Our current study show that administration of Selenium in the nano size (Nano Se) posses a more potent antioxidant activity as the GSH content, GR and catalase activity and TAC were higher in Nano Se group as compared to ordinary Se and control groups. Added to that, there was a significant decreases in MDA concentration in Nano Se as compared to Se and control groups. Also, MT – 1 expression significantly increases in Nano Se group as compared to Se and control groups.
The significant difference between Nano Se and Se could be contributed to the ability of the nanoparticles to offer several pharmacokinetic advantages, such as specific drug delivery, high metabolic stability, high membrane permeability, improved bioavailability, and long duration of action. The physicochemical properties of nanoparticles, such as size, surface charge, and hydrophobicity, affect their mucosal absorption characteristics, and smaller nanoparticles show higher transcellular uptake than do larger ones **(Petros and DeSimone, 2010; Roger et al., 2010)**. Moreover, selenium nanoparticles also show high biological activity and good absorptive ability due to the interaction between the nanoparticles and −NH2, C=O, −COO, and −C−N− groups of proteins **(Zhang et al., 2004).** Studies on the biological activities of selenium and its nanoforms revealed that hollow spherical nanoparticles of selenium have strong antioxidant properties **(Gao et al., 2002)** Similar studies declared that nano-selenium has the ability to act as an antioxidant with reduced risk of ordinary selenium toxicity **(Wang et al., 2007).**
Our biochemical findings were supported by the histopathological studies which confirmed that section of testes of Nano – selenium group showed a densely filled seminiferous tubules and healthy Sertoli cells attached to the basement membrane as compared to control group and selenium group **(Figure 4)**

Conclusions

The findings of this study showed that nano selenium may improve sperm count, motility and vitality in mice treated with nano selenium particles. Therefore, the antioxidant effects of nano selenium may be a major reason for its positive impact on spermatic parameters. However, further studies are required to define its exact mechanism of action.

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