Histomorphmetric Alterations Induced in the Testicular Tissues by Variable Sizes of Silver Nanoparticles

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Synopsis

Silver nanoparticles could induce deleterious impacts on the testicular tissues and spermatogenic process that could affect fertility and reproduction.

Abstract

OBJECTIVE: To investigate the histomorphmetric alterations induced in the testicular tissues by variable sizes of SNPs.

STUDY DESIGN: Male mice (BALB/C) were treated with SNPs (1mg/kg) using five different sizes (10, 20, 40, 60 and 100 nm) for 35 days. Testicular biopsies from all mice under study were subjected to histomorphological examinations.

RESULTS: Silver NPs (10 and 20 nm) had provoked morphometric changes in the testes of the subjected mice together with the following histological alterations: seminiferous tubules degeneration, spermatocytes cytoplasmic vacuolation, spermatocytes sloughing and spermatid giant cells formation. Larger SNPs (40, 60 and 100 nm) induced little or no testicular histomorphometric alterations.

CONCLUSION: The findings of the present work may indicate that SNPs sub-chronic exposure could induce deleterious impacts on the testicular tissues and spermatogenic process that could affect fertility and reproduction, with smaller SNPs are more toxic than the larger ones.

Keywords: silver nanoparticles**,** seminiferous tubules, spermatogenesis, spermatid giant cells, reproduction, morphometric alterations

Introduction

Silver NPs have unique optical, electrical and thermal properties and are being used widely in nanomedicine and consuming products owing to their unique antimicrobial, antifungal and antiviral properties[1-2]. These fine particles have been widely invested in alginate fibers, wound dressing, medical masks, gels, cosmetics, deodorants, shampoo, sunscreen, footwear and athletic shirts due to their ability to limit bacterial growth [3,4,5,6,7,8,9]. Moreover, SNPs are being used in biological and chemical sensors and in imaging of neural tissues[7]. These together gave SNPs potential promising as potent constituents of pharmaceutical, medical and industrial products [8].

The smaller nature of SNPS together with their high surface area to volume ratio enables these particles to enter the tissue components as biological molecules do [9]. In addition, these fine particles have large functional surface area with dimensions almost the same of the biological micromolecules with the possibility of being adsorbed on the surface of these molecules in the tissues and body fluids [6]. Nano studies indicated that SNPs toxicity might be related to the charge and functional groups on their surface together with their ability to bind or interact with the tissue and cell components leading to the production of hydrogen peroxide and reactive oxidative species (ROS) that could damage plasma membrane and cell organelles specially the mitochondria and cytoskeleton [10,11,12,13,14,15]. Some studies concluded that SNPs could demonstrate DNA damage, cell cycle disturbing, genotoxic and cytotoxic consequences and reduction in the metabolic activity due to their potential to release silver ions [16,17,18,19,20].

Silver NPs is becoming more reality in our lives that increased human exposure to variable forms of these particles with a high risk potential on human health. Full attention is needed to be given towards safety of these fine particles with special attention towards size, composition and behavior of these particles. Silver NPs were found to induce histological and

histochemical alterations in the vital organs including liver and kidney [2,7,21,22,23,24,25,26,27]. Few studies are available on the reproductive toxicity of SNPs associated with the histological alterations induced by these particles on the testicular tissues. With this objective, the present study aims to determine the histomorphometric testicular alterations induced by different sizes of SNPs.

Materials and Methods

Animals and conditions

A total of 42 adult healthy male mice (BALB/C) were used throughout the present study. All mice were randomly divided into 6 groups (control group and five test groups) of 7 animals each, housed at room temperature (24 ± 1 °C) with 12 hr light-12 hr dark cycle and kept in Faculty of Medicine animal facility, The University of Jordan. The mice were provided with commercial pellets and tap water *ad libitum*.

Nanoparticles

Naked spherical SNPs (10, 20, 40, 60 and 100 nm; Sigma-Aldrich, USA with mass concentration of 0.02 mg/ml in aqueous citrate buffer) were used in the present study. The specified size of these NPs was confirmed by Transmission Electron Microscope techniques.

Experimental Protocol

Following a period of stabilization (7 days), all members of all groups were exposed to intraperitoneal (i.p.) injection with a daily single dose of SNPs (0, 10, 20, 40, 60 and 100 nm) for 35 days.

All animals were handled and all experiments were conducted in accordance with the protocols approved by The University of Jordan Local Animal Care ethical committee while

the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Organ weight monitoring

The mice were killed by cervical dislocation and the testes of each dissected mouse were removed carefully, weighed in grams (absolute organ weight) on the dissection day. The relative testes weight for each mouse was calculated according to the following equation of Aniagu *et al* [28]:

Relative organ weight = [Absolute testes weight (g)/ Body weight of mouse (g)]x100.

Organ index

From the obtained data, the amount of change on the relative ratio of the testes (T_x) of mice subjected to different sizes of SNPs for 35 days was calculated according to the following equation[29]:

$$T_x = \frac{\text{Average weight of the experimental testes/ Average weight of the experimental animals}}{\text{Weight of the control testes/ Average weight of the control animals}}$$

Histological processing

Biopsies from the left testis from each mouse of all groups were cut out rapidly, fixed in 10% neutral buffered formalin and then dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the tissue samples in 2 changes of chloroform before being impregnated with 2 changes of melted paraffin wax, embedded and blocked out. Testicular sections (4-5 μm) from all experimental mice of all groups were applied for hematoxylin and eosin (H&E) stain according to Bancroft and Stevens[30]. The

histological processing and histological examination of all testicular samples was carried out in the Toxicological Unit, Zoology Department, King Saud University.

TUNEL staining

Terminal dUTP nick end-labeling (TUNEL) assay was performed by using an *in situ* Tunel Apoptosis Detection Kit (GenScript, USA). Known positive control sections were used while negative control sections were incubated in the label solution only without terminal transferase.

Statistical analysis

The amount of change on the relative ratio of the organ weight to body weight of mice subjected to different sizes of SNPs for 35 days ± Standard deviation (S.D) for each group after treatment with different sizes of SNPs was calculated and expressed from the obtained data. The significant differences between SNPs treated groups and the control one was tested by student *t*-test where *P* values < 0.05 were considered statistically significant.

Results

Macroscopic alterations

No mortalities or signs of toxicity were detected in any of the experimental groups of the present study. Also, no macroscopic anomalies were seen in the appearance and behavior of mice subjected to the used different sizes of SNPs.

*Effect on testes morphology***:** Mice of all treated groups, except 20 nm, showed a slight decrease on the relative ratios of total testis weight to body weight and testis index (Table I & Figure 1). The percentage absolute testes weights were not significantly (p-value >0.05) affected in all treated mice (Figure 2).

Table I *Change on the relative ratio of total testes weight to body weight of mice subjected to different sizes of SNPs for 35 days.*

Dose	Average total testis weight(g)	Average body weight (g)	Relative testis weight	Testis index (T_x)
Control group	0.25 ± 0.02	30.83 ±1.18	0.83±0.28	1.0
10 nm	0.24 ± 0.02	31.03 ± 2.21	0.79±0.10	0.95
20 nm	0.23± 0.03	27.77 ± 3.96	0.83±0.18	1.0
40 nm	0.22 ± 0.05	29.57 ± 3.31	0.75±0.15	0.90
60 nm	0.23 ± 0.03	31.27 ± 2.77	0.74±0.11	0.89
100 nm	0.22 ± 0.01	29.51 ± 1.69	0.74±0.14	0.89

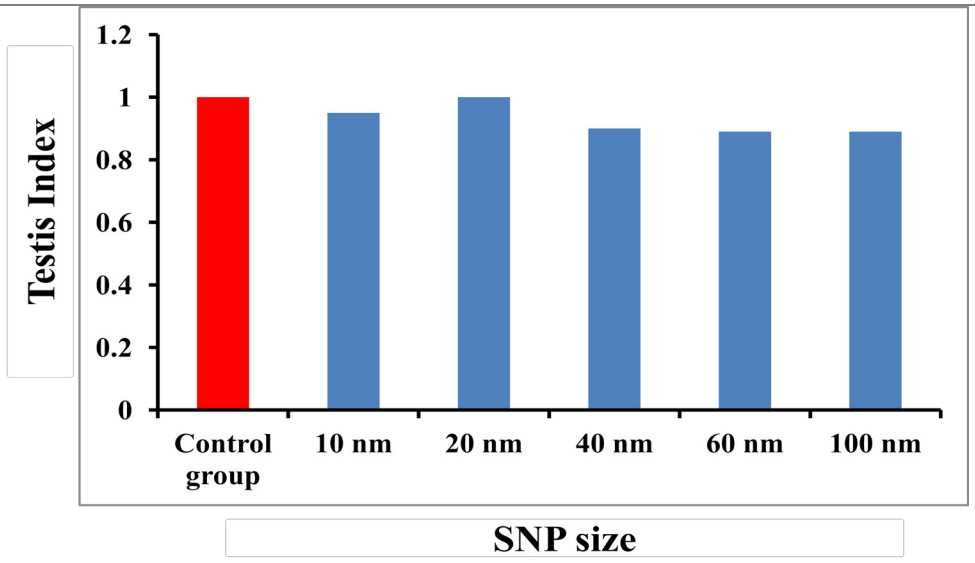


Figure 1 Testes index for control and SNPs treated groups

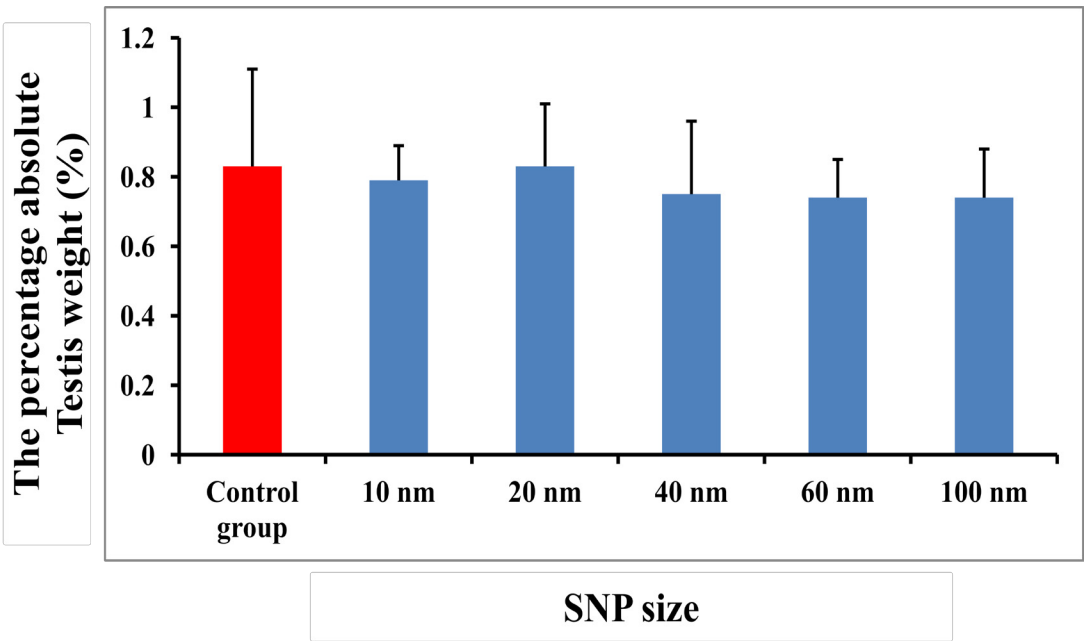
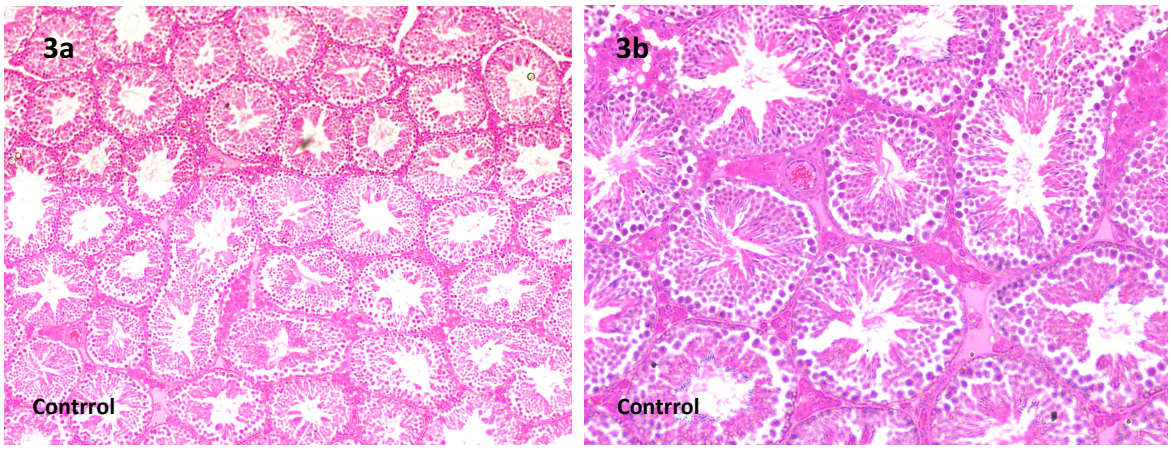


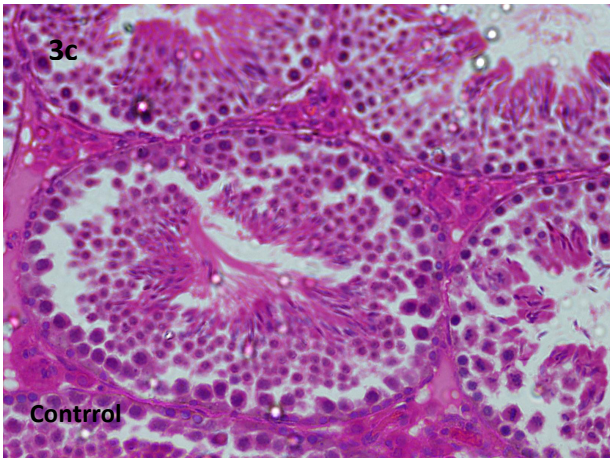
Figure 2 The percentage absolute total testes weight of control and SNPs treated mice.

Microscopic alterations

Control mice

Examination of the control mice testicular histological sections revealed normal intact seminiferous tubules at various stages of spermatogenesis together with normal intertubular tissues (Figures 3a-c).



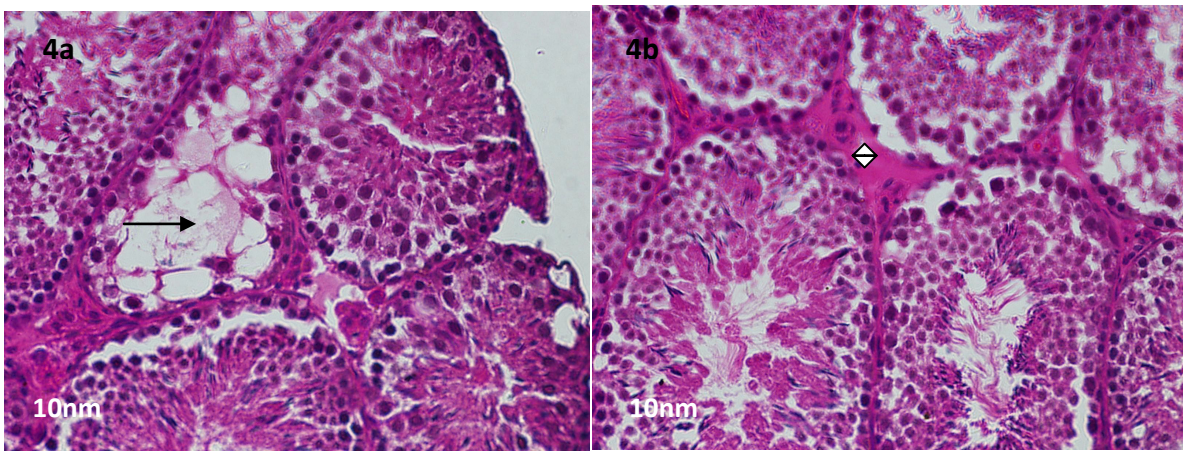


Figures (3a-c). Light photographs of sections in the testes of control mice demonstrating:

(a) Normal testicular histological structure. H&E stain, x 150. **(b)** Normal seminiferous tubules together with normal interstitial tissues. H&E stain, x 300. **(c)** Normal pattern of spermatogenesis. H&E stain, x 750.

Mice exposed to 10 nm SNPs

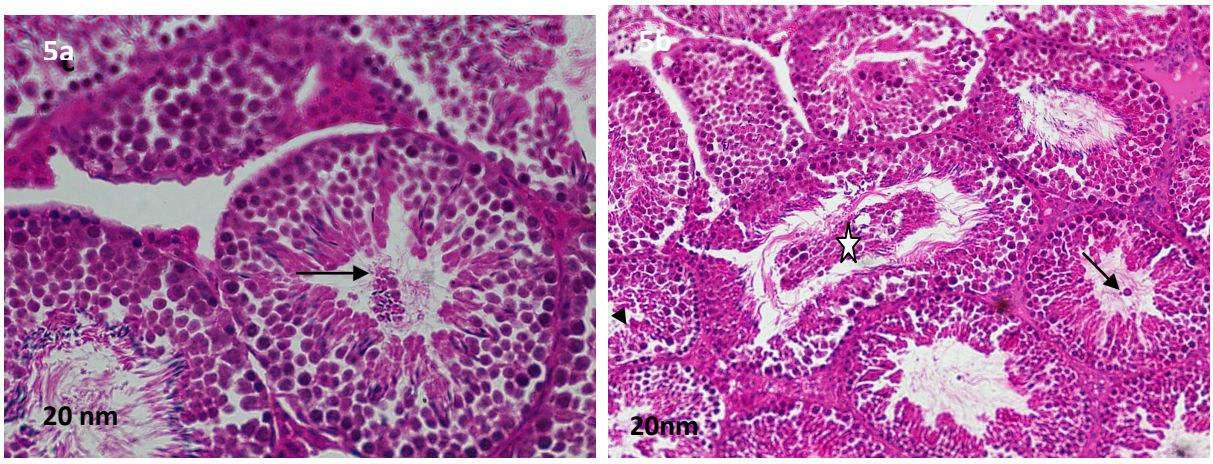
In comparison with the control group, the testes of mice treated with 10 nm SNPs for 35 days showed occasional seminiferous tubules ghost degeneration with spermatocytes cytoplasmic vacuolation (Figure 4a). Moreover, mild intertubular edema was also detected (Figure 4b).



Figures (4a-b). Light photographs of sections in the testis of mouse received 10 nm SNPs for 35 days demonstrating: **(a)** Degenerative spermatogenic cells with cytoplasmic vacuolation (arrow). H&E stain, x 450. **(b)** Mild intertubular edema (double triangles). H&E stain, x450.

Mice exposed to 20 nm SNPs

The testes of mice treated with 20 nm SNPs for 35 days had produced spermatocytes sloughing and occasional appearance of spermatid giant cells in the lumen of some seminiferous tubules. Spermatids detachment and accumulation of desquamated spermatocytes together with spermatids and cellular debris were also observed in lumen of the seminiferous tubules (Figures 5a & 5b).



Figures (5a-b). Light photographs of sections in the testis of mouse received 20 nm SNPs for 35 days demonstrating: **(a)** Spermatocytes sloughing (arrow). H&E stain, x 450.

(b) Accumulation of desquamated spermatocytes (star) and spermatid giant cell (arrow). H&E stain, x 300.

Mice exposed to 40 nm SNPs

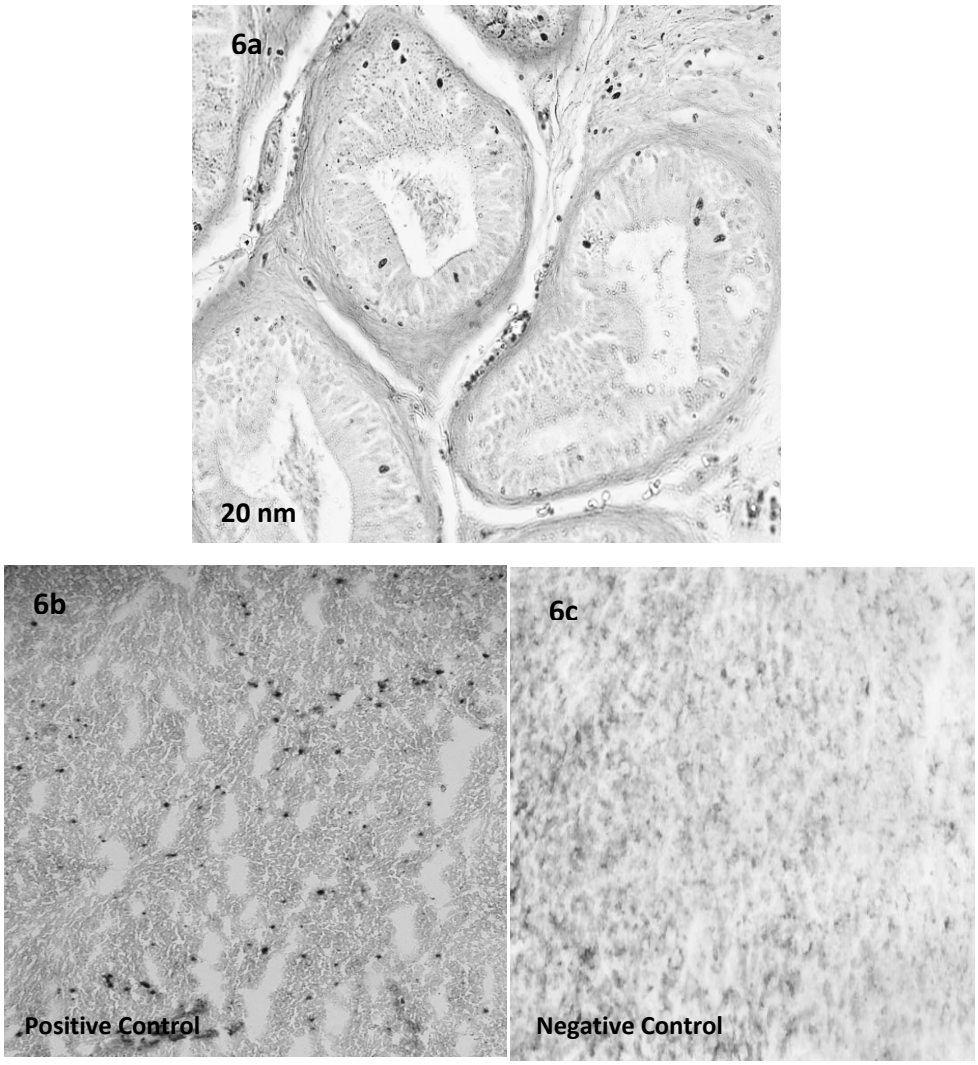
Mice treated with 40 nm SNPs for 35 day showed almost normal testicular tissues with occasional seminiferous tubules epithelial degeneration.

Mice exposed to 60 or 100 nm SNPs

The testicular tissues of mice exposed to 60 or 100 nm SNPs for 35 days showed almost normal testicular structures.

Apoptosis detection

In comparison with the control sections, sloughing spermatocytes in the testicular tissues of mice subjected to 10 or 20 nm SNPs for 35 days failed to stain by Tunel method (Figure 6).



Figures (6a-c). Light photographs of: (a) TUNEl stained testis section of mouse received 20 nm SNPs for 35 days demonstrating no apoptotic nuclei of the detaching germ cells. TUNEL assay, x 450

(b) Positive control section for apoptosis after incubation in TUNEL stain working solution demonstrating apoptotic nuclei stained dark brown. TUNEL assay, x 200

(c) Control section for apoptosis after incubation in the label solution only without terminal transferase to work as negative control. No positive stain is seen, x 200

Discussion

Silver nanoparticles are the most widely invested NPs in consumer products and medicine due to their unique properties[31]. These fine particles have been used in different sectors of the medical field including prevention, diagnosis and treatment. Nanosilver particles with the size of 9-50 nm, are being utilized in many products as antimicrobial agent, medical devices coating and as biosensor for disease diagnosis. The number of SNPs-containing products has grown from 300 in 2006 to 1300 by the beginning of 2014[32]. The routine access to SNPs clinical application includes wound dressing, nanosilver coated medical devices such as venous catheter, drainage catheters, contact lenses, dental instruments and biosensors. In addition, these particles are invested in nanosilver bone cement, - topical cream,, - meshes to bridge large wounds, and in numerous assays as a biological tags for quantitative detection.

The results of the present work showed that sub-chronic exposure to SNPs caused slight decrease on the relative ratios of total testis weight to body weight and testis index while the percentage absolute testes weights were not significantly affected in all treated mice. One study reported that chronic exposure to 56 nm SNPs for 90 days induced testis enlargement, in the left one particularly [23]. Together, these findings may support some reports indicated that SNPs could cross blood-testes barrier and accumulate in the testes [31,33].

The findings of the present study showed that 10 and 20 nm SNPs could induce testicular alterations in the form of spermatocyte sloughing and moderate seminiferous tubules degeneration. These findings are on line with a recent ultrastructural study by Thakur *et al* [34] where 20 nm SNPs induced disappearance of the normal testicular histological feature with structural damage including depletion and necrosis of spermatocytes and Ledydig cells. In addition, the results of the present work is consistent with report from the Norwegian Institute of Public Health which indicated testicular damage cells due to SNPs exposure [31]**.** Moreover,

particles size dependent cellular toxicity was reported by Kim *et al* [20], where 10 nm SNPs were more toxic than the larger sizes (50 and 100 nm).

Spermatocytes and spermatids sloughing as demonstrated by the present study might indicate that SNPs could induce cytotoxicity. The failure of detaching spermatocytes to stain with TUNEL stain might indicate that SNPs cause no DNA fragmentation and the detached germ cells are not apoptotic ones but might be resulted from the disruption of the physical interaction of these cell due to SNPs toxicity. On the other hand the induced seminiferous degeneration might indicate SNPs interference in the dynamic process of spermatogenesis. Several studies reported cytotoxicity and oxidative stress with exposure to SNPs. Sub-chronic exposure to these particles showed accumulation of these particles in the testicular tissues and induced germ cells death by SNPs oxidative stress [17,33]. Some studies revealed that SNPs could cause a decrease in the epididymal sperm count and DNA damage in spermatocytes together with change in seminiferous tubules morphometry [35].

The morphometric abnormalities and histological alterations in the testicular tissues as seen in the present work revealed that smaller SNPs (10 and 20 nm) were more toxic and induced more testicular damage than the larger ones (40, 60 and 100 nm). This finding is in agreement with other studies that considered the size of SNPs a key factor of the toxicity of these nanoparticles[36]. Smaller size silver NPs might have more impacts on the germ cells cytoskeleton and spermatogenesis than the larger ones with potential risk on male fertility and reproduction where smaller NPs have much greater surface area to volume and have longer circulating residue than the larger ones. In addition, some studies indicated that SNPs potential toxicity could be related to dissolution rate, reactive surface area and bioavailability where smaller particle release more silver ions due to their greater surface area to mass ratio[15]. Oxidative dissolution of smaller particles may produce more hydrogen peroxide, depletes

more dissolved oxygen, generates more intracellular reactive oxygen species (ROS) and leads to more cellular damage than the dissolution of the larger ones does.

The findings of the present study may indicate that SNPs sub-chronic exposure could induce toxicological effects on the testicular tissues and spermatogenic process with potential risks and possible consequences on fertility and reproduction. The results of the current work also indicate that different sizes of SNPs have different impacts on the testicular tissues where smaller SNPs are more toxic to the testes than the larger ones.

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