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ORIGINAL ARTICLE

Silver Nanoparticles Effect on Testicular Tissue of Adult Albino Rats (Histological and Immunohistochemical Study)

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ABSTRACT

Background: Silver nanoparticles (AgNPs) are broadly used in many products either medical or non-medical due to their distinctive properties. This study aimed to study AgNPs induced testicular histological alteration in adult male albino rats.

Methods: Twenty healthy adult male albino rats were used in this study. They were divided into two groups; group I: control (negative and positive) and group II: AgNPs treated. The animals of the group II were injected intraperitoneally with a daily dose of 50 µg / kg AgNPs. After 4 weeks, all rats were anesthetized and their testes were dissected out carefully and processed for light and electron microscope examinations.

Results: H&E sections of AgNPs treated group revealed shrunken disorganized seminiferous tubules lying on irregular basement membrane with marked reduction in the thickness of their germinal epithelium and few spermatozoa in their lumina. Many cells exhibited deeply stained nuclei. The interstitium in-between the tubules were wide and contained homogenous vacuolated eosinophilic material. Ultrastructurely, many cells had irregular shaped nuclei and their cytoplasm contained disorganized mitochondria and many vacuoles.

Conclusions: AgNPs had adverse effects on the histological structure of the testis of adult albino rats.

Keywords: AgNPs; Testis; Bcl2; Rat; Structure

INTRODUCTION

In nanotechnology era, silver nanoparticles have appeared with various medical applications like surgical dressings and medical devices. Silver nanoparticles become one of the most used nanotechnology-derived nanomaterials due to their special features suitable for different purposes [1, 2]. More than one thousand products present in the markets containing NPs, 259 of them contained AgNPs. The use of AgNPs is mainly based on their antimicrobial properties [3, 4]. The relationship between silver nanotechnology and its potential toxic impact on man health is relatively new and some studies have showed that these NPs can be toxic to mammalian tissues. Interestingly, some studies have shown that these particles can induce genes changes with subsequently DNA damage and apoptosis in human cells. Moreover, studies on AgNPs routes of administration in rats and mice, whatever these routes were inhalation, ingestion or intra-peritoneal injection, AgNPs were detected in blood and caused toxicity in many organs [5]. Monitoring health evaluation of these particles has become vital for the safe use of nanomaterials in daily products and medicine; especially their possible harmful effect on reproduction and fertility, so this study aim to evaluate histological alteration induced by AgNPs on adult male albino rat's testis [6].

METHODS

Silver nanopowder with a particle size less than 100 nm and a 99.9% trace metals basis was purchased from Sigma-Aldrich Chemicals, Cairo, Egypt. Its Chemical Abstract Service Registration Number (CAS No) is 7440-22-4).

Animals and experimental design:

Twenty healthy adult male albino rats with average weight 200-250 gm were used in this experiment. They were housed in stainless steel cages at animal house of Faculty of Medicine, Zagazig University at room temperature, fed standard balanced diet and allowed water ad-libitum. All experimental procedures were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center (Cairo, Egypt). Rats were randomly divided into two groups; Group I (Control group): contained twelve rats further subdivided into two equal subgroups:
Subgroup Ia (negative control group): received no treatment till end of experiment.

And Subgroup Ib (positive control group): received 1 ml saline solution per day (Solvent of AgNPs) orally by gavage [7].

Group II (AgNPs-treated): contained eight rats; received AgNPs intraperitoneally at dose of 50 µg /kg/day dissolved in saline solution daily for 4 weeks. The dose was dissolved in 10 ml saline each rat received 1ml / day [7].

At the end of the experiment, all rats were anaesthetized with 50 mg/kg body weight of sodium phenobarbital through intra-peritoneal injection. Then, the testes were extracted and processed for histological study (light microscopic and electron microscopic examination).

Light microscopic study was done as the specimens were fixed overnight in Bouin's solution and were processed to prepare 5 µm thick paraffin sections for Haematoxylin and Eosin [8].

Immunohistochemical study was done as paraffin-embedded sections were immunohistochemically stained using the avidin–biotin peroxidase system for the detection of Bcl2 (CAS No. 85878, Sigma-Aldrich, Steinheim, Germany). Serial sections were deparaffinized on positively charged slides. The primary rabbit polyclonal antibody for Bcl2 was ready-to-use. After several washes with phosphate buffer saline (PBS), slides were incubated for two hours with secondary anti-rabbit antibody versal kits diluted 1:200 for 30 minutes. [9]. For electron microscope preparation; testicular specimens were fixed and processed then ultrathin sections were obtained and stained [10]. The copper grids were examined and photographed using a JEOL JEM 2100 electron microscope (Jeol Ltd, Tokyo, Japan) in Electron Microscope Research Laboratory (EMRL) of Faculty of Agriculture, El Mansoura University, Egypt.

Sections stained with H&E. were morphometrically analyzed in department of Histology, Faculty of medicine- Zagazig University using the Fiji Image J (1.51n, NIH, USA).

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS software (version 16.0, Chicago, USA) via using one-way analysis of variance (one-way ANOVA) (Tukey test). The obtained data from morphometrical analysis of germinal epithelial height and optical density of Bcl2 immunoreactions in both germinal epihelium and Leydig cells were presented as mean±SD and analyzed statistically using (ANOVA). The probability values (P) less than 0.05 were considered statistically significant and highly statistically significant when P value <0.001and non-significant when P value >0.05 [11].

RESULTS

Light microscopic results: Examination of control rats of subgroups Ia and Ib revealed nearly similar histological results; so, only results of the control subgroup Ia were presented. Examination of sections from control subgroup Ia stained by H&E showed packed seminiferous tubules. Their lumina were patent contained clumps of sperms. The interstitium in-between the tubules were narrow and contained clusters of interstitial cells and blood capillaries. Seminiferous tubules were lined by stratified germinal epithelium formed of several types of cells; spermatogonia, primary spermatocytes, spermatids and Sertoli cells and there were spermatozoa in the lumina of the tubules. The interstitium exhibited groups of Leydig cells with oval nuclei and acidophilic cytoplasm and blood capillaries (Fig.1a, b). AgNPs treated group showed testicular parenchyma with shrunken disorganized seminiferous tubules with few spermatozoa in their lumena. The interstitium in-between the tubules contained eosinophilic material. There was marked reduction in thickness of germinal epithelium with separation in-between cells. Germ cells are few and many cells exhibit deeply stained nuclei (Fig.1c, d).

Immuno-histochemical stained sections for Bcl2 protein of control group showed strong positive cytoplasmic immunoreaction in most cells of the germinal epithelium and in the Leydig cells (Fig.2 a). However, in AgNPs treated group it showed weak cytoplasmic immunoreaction in few cells of germinal epithelium and in the Leydig cells (Fig.2 b).Examination of ultrathin sections from the testis of control group showed that the seminiferous tubules were surrounded by regular basement membrane and ensheathed by flattened myoid cell. Spermatogonia had rounded nuclei with marginated heterochromatin. Sertoli cells appeared with euchromatic nucleus and prominent nucleolus. Primary spermatocytes appeared with large rounded nuclei and electron dense clumps of heterochromatin. Their cytoplasm showed mitochondria (Fig.3a). Spermatids appeared with large ovoid euchromatic nuclei. Flattened Golgi saccules appeared near one pole of the nucleus and peripherally situated mitochondria in cytoplasm (Fig.3b). Cross sections in the tails of sperms showed end pieces had central axoneme formed of nine doublets of microtubules and two central singlets. The axoneme was surrounded by cell membranes (Fig.3 c).

Electron microscopic examination of the testis of AgNPs treated subgroup showed spermatogonia with heterochromatic nucleus partially separated from surrounding cells. Primary spermatocytes had irregular shaped nuclei and their cytoplasm contained mitochondria. Sertoli cells had indented nuclei with prominent nucleoli. Their cytoplasm contained mitochondria with disrupted cristae.

Little spaces were also noticed in-between cells (Fig.4a). Late spermatids appeared with ovoid nuclei and abnormally arranged mitochondria. Cytoplasmic vacuole was also noticed (Fig.4b). Cross sections of middle pieces of mature sperms showed some pieces with swollen mitochondrial sheath and irregular cell membrane (Fig. 4c)

Morphometric results: Statistical analysis of the mean values of the epithelial height of seminiferous tubule in random fields showed a significant decrease in AgNPs group compared to the control group (Table 1).

Table 1: Statistical analysis of height of germinal epithelium (µm) by one-way ANOVA test

Parameter	Control	AgNPs- treated
height of germinal epithelium (µm)	67.6±16.2	41.9±11.4

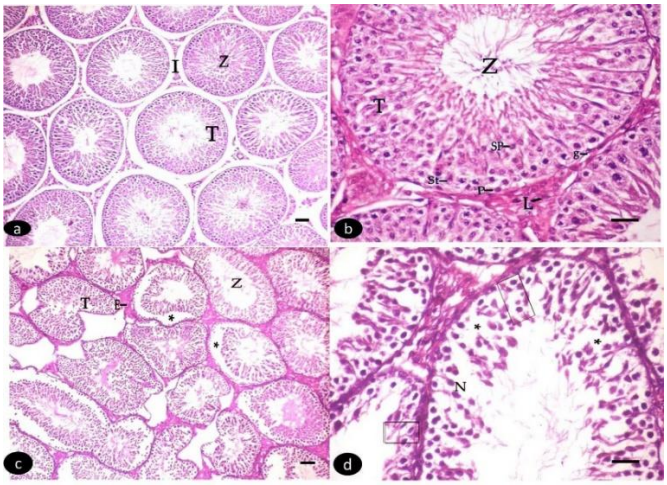

Figure (1): (a, b) H&E stained sections of control rat's testis: a) shows packed seminiferous tubules (T) separated by narrow interstitium containing clusters of interstitial cells (I). lumen contains clumps of spermatozoa (Z). b) Seminiferous tubule (T) lined by spermatogonia (g), primary spermatocytes (P), spermatids (SP) and supporting Sertoli cells (St). The lumen of the tubule contains spermatozoa (Z) and the interstitium contains Leydig cells (L) that have oval nuclei. (c, d) AgNPs treated testis c) shows many shrunken disorganized seminiferous tubules (T) with marked separation between the germ cells (star). Other tubules reveal reduction of in the number of spermatozoa in their lumina (Z). The interstitium in-between tubule contains homogenous eosinophilic material (E). d) Seminiferous tubules show marked reduction in the thickness of their germinal epithelium (rectangle) and separation between the germ cells (star). Many germ cells exhibit deeply stained nuclei (N). (H & E; a, c X100, b, d X 400, scale bar 30 µm).

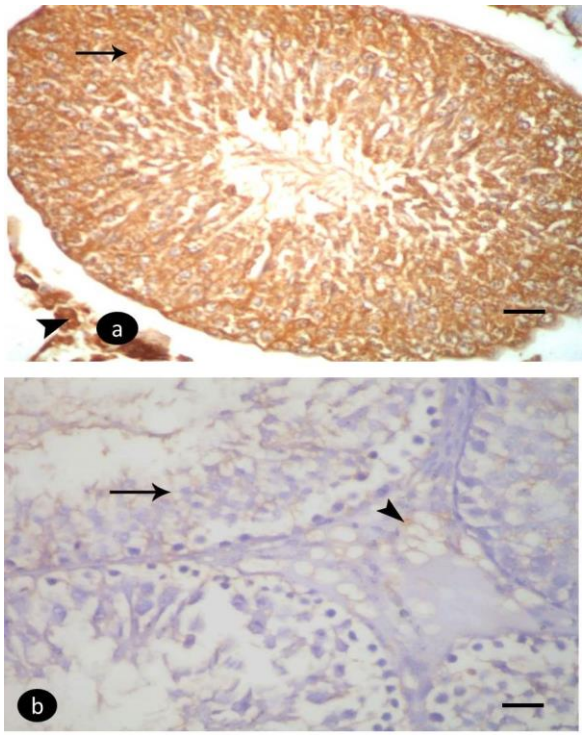

Figure 2: a) Immunohistochemical stained section of control group for Bcl2 protein shows strong positive cytoplasmic immunoreaction in most cells of germinal epithelium (arrow) and in the interstitial Leydig cells (arrow head). b) AgNPs treated group shows weak cytoplasmic immunoreaction in few germ cells (arrow) and in Leydig cells of the interstitium (arrow head) (Immunoperoxidase for Bcl2 protein x 400, scale bar 30 µm).

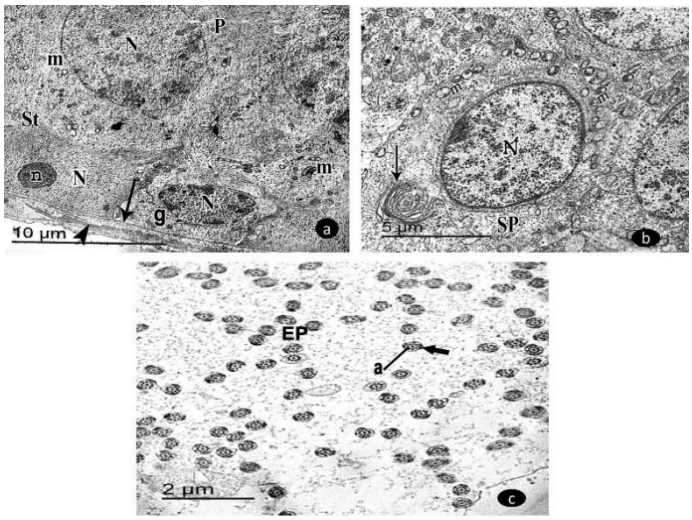

Figure 3: A transmission electron micrograph of control rat's testis shows a) A spermatogonium (g) with nucleus (N) contains marginated heterochromatin and a Sertoli cell (St) with electron dense nucleus (N), prominent nucleolus (n) and resting on thin regular basement membrane (arrow) enclosing flat myoid cell (arrow head). Primary spermatocytes (P) are also seen with large rounded nuclei (N) and electron dense clumps of heterochromatin. Their cytoplasm shows mitochondria (m) (TEM X9000). b) adluminal part of germinal epithelium containing a spermatid (SP) with oval euchromatic nuclei (N) and numerous peripherally arranged mitochondria (m) just beneath the cell membrane. Prominent Golgi cisternae (arrow) appear on one side of the nucleus (TEM X13400). c) Showing cross section in the end piece (EP) of sperm which consists of nine doublets of microtubules with two central singlets (a) surrounded by cell membrane (arrow) (TEM X22500).

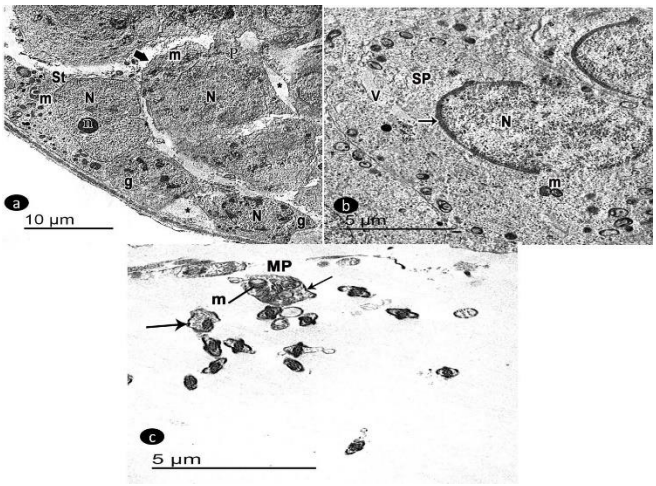

Figure 4: A transmission electron micrograph of AgNPs treated rat's testis shows a) spermatogonia (g) with heterochromatic nucleus (N). Primary spermatocyte (P) has irregular shaped nucleus (N) and their cytoplasm contained mitochondria (m) under irregular cell membrane (arrow). Sertoli cell (St) has indented nucleus (N) with prominent nucleolus (n). Its cytoplasm contained mitochondria with disrupted cristae (m). Many spaces were also noticed in-between cells (star) (TEMX5600). b) A late spermatid (SP) appears with ovoid nucleus (N) and abnormally arranged mitochondria (m). Cytoplasmic vacuole (V) was also noticed (TEMX14000). c) Cross sections of middle pieces of mature sperms (MP) showed some pieces with swollen mitochondrial sheath (m) and irregular cell membrane (arrow) (TEMX17000).

DISCUSION

Silver nanoparticles have made a revolution in different medical applications ranging from silver based dressings, silver coated medical devices, nanogels, etc [1]. However, there is potential risk when released into the environment. Studies on AgNPs revealed that toxicity is dependent on various agents such as particle size, shape and coating agent [12]. There is still a limited understanding of the effects of AgNPs on spermatogenesis despite some studies reported that AgNP exposure was related to male reproductive toxicity in mammalian cells [13]. In our study, 20 healthy adult male albino rats were utilized. Albino rats are the most commonly used experimental animals in several biomedical researches, as they have been documented as very good model mammalian system [14].Light microscopic examination of AgNPs treated group revealed that most of seminiferous tubules were shrunken and disorganized. Similar results were found by Zhang et al [15] who mentioned that cytotoxicity of AgNPs is related to increased generation of reactive oxygen species (ROS), which play an important role in apoptosis induced by these particles. Also, there were marked separation and spaces between germinal epithelial cells with apparent diminished layers of germinal epithelium which was confirmed by highly significant decrease of the mean of epithelial height in AgNPs treated subgroup in comparison with the control group. Similar results were reported by Thakur et al [16] who used nanoparticles orally for 90 days and confirmed that degenerative alterations in seminiferous tubules showed that nanoparticles could directly inhibit spermatogenesis process.

Scattered cytoplasmic vacuoles were found within germ cells. These results were also confirmed by electron microscopic examination and were in accordance to Abdelhalim, [17] who explained that vacuolization designates a toxicity effect exhibited as a result of disturbances in membrane function, which results in massive influxes of water and sodium. In addition, Cellular swelling may be accompanied by cytoplasmic degeneration due to leakage of lysosomal hydrolytic enzymes. Also, there were few spermatozoa in their lumina. Ong et al [13] explained that as the decrease in germinal stem cells number may probably have badly affected sperm production and lead to debility in male fertility. Miresmaeili et al [18] refered that to release of spermatozoa to the mid duct of seminiferous tubules or the effect of nanoparticles on cell cycles and significant decrease of sperm precursor cells. The interstitium in-between the tubules was wide and contained homogenous eosinophilic material. These results were in agreement with Ahmed et al [19]. Amin et al [20] mentioned that interstitial tissue damage may be associated with AgNPs deposition in the tissue due to particle size. Shi et al [21] added that intracellular ROS caused by AgNPs act as essential mediators that lead to endothelial cell injury and dysfunction.Immuno-histochemical stained sections for Bcl2 protein in AgNPs treated group showed weak positive cytoplasmic immunoreaction in few cells of germinal epithelium and in the Leydig cells. This was

confirmed statistically by a highly significant decrease in the mean values of the optical density of immune reaction to Bcl2 in AgNPs group compared to the control group, which was going with Zielinska et al [22]. Gogvadze et al [23] added that ratio of Bax/Bcl-2 proteins plays a main role in mitochondrial outer-membrane permeabilization, release of cytochrome C into the cytosol and, consequently, initiation of apoptosis. Ultrastucturly, germ cells appeared with irregular shape nuclei and vacuolated mitochondria. Similar results were found by Zielinska et al [22] who considered degradation of cell organelles by AgNPs an example of necroptosis. Kumar et al [24] mentioned that oxidative injury to cell membranes results in great ionic imbalance, mitochondrial damage, and finally lysosomal activation. Electron microscopic examination of cross sections of middle piece of mature sperms of the same group showed swollen mitochondrial sheath and irregular cell membrane. Some pieces showed cytoplamic vacuoles. That was in agreement with Castellini et al [25]. Lu et al [26] added that the plasma membrane of spermatozoa has plentiful polyunsaturated fatty acids and the sperm cytoplasm is poor of these antioxidative enzymes so sperms are particularly vulnerable to the attack of free radicals that may induce lipid peroxidation increasing the defect in sperms.

CONCLUSIONS

the present study showed that AgNPs in adult albino rats had adverse effects on the histological structure of the testis especially the germinal epithelium and the sperms even when used in small doses. So, it is recommended to pay attention to the hazards of AgNPs and to limit their use to minimize their undesired effects. Moreover, more studies are needed concerning human application to evaluate their effect on reproductive system and hazards on male fertility.

Conflict of interest: There is no potential conflict of interest among the authors.

Financial disclosure: Non

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