Original Article

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Effects of Silver Nanoparticles on Lipid Peroxidation and Quality of Sperm Parameters in Male Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Considering the role of silver nanoparticles in inducing the production of free radicals, these compounds may lead to pathological effects and oxidative stress in the seminal fluid, resulting in the reduced quality of sperm parameters. Therefore, in this study, we aimed to assess the toxic effects of silver nanoparticles on sperm parameters and lipid peroxidation of sperm membranes in male rats.

METHODS: This experimental study was conducted on 24 Syrian adult male rats, which were randomly divided into one control and three study groups. The rats in the study groups were orally administered silver nanoparticles at concentrations of 0.07 µg (group A), 0.14 µg (group B), and 0.28 µg (group C), respectively per day for a period of five weeks. Then, lipid peroxidation of sperm membranes was analyzed, using thiobarbituric acid assay. Additionally, microscopic examinations were performed to evaluate sperm parameters.

FINDINGS: Based on the findings, the mean quality of sperm parameters such as sperm count and motility significantly declined in the study groups, particularly group B (77.69±16.96%; $16\pm2.6\times10^6$/ml) and group C (72.79±14.52%; $13.31\pm2.1\times10^6$/ml), compared to the control group (89.06±10.97; $23.57\pm3.46\times10^6$/ml) ($p<0.05$). Also, in terms of malondialdehyde concentration, a significant difference was reported among group A (0.25±0.01), group B (0.32±0.02), group C (0.35±0.01), and the control group (0.24±0.01) ($p<0.001$).

CONCLUSION: The results of the present study confirmed that silver nanoparticles through enhancing lipid peroxidation could reduce the quality of sperm parameters in a dose-dependent manner.

KEY WORDS: *Silver Nanoparticles, Sperm Parameters, Lipid Peroxidation, Male Rats.*

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Introduction

In nanotechnology, use of silver nanoparticles is one of the most invaluable methods, with a wide range of applications in different fields such as biology and medicine (1). Silver nanoparticles possess antibacterial, antifungal, anti-viral, and anti-protozoan properties. Therefore, by using nanoparticles, we can create antimicrobial surfaces to eliminate infections (2). Nanoparticles are able to bind to the bacterial cell membrane and penetrate into the cells. The contributing factor for nanoparticle-membrane binding is the presence of sulfur proteins.

This binding not only leads to cellular death through altering the cellular morphology, membrane permeability, cellular respiration, and cell division, but also destroys the bacteria by penetrating into the cells and reacting with sulfur and phosphorus compounds (e.g., DNA) (1, 3-5). Silver nanoparticles can also affect viruses through binding to sulfhydryl groups of viral surface glycoproteins and prevent their adhesion to the host cell. In fact, silver nanoparticles can influence all types of DNA, RNA, coated, and uncoated viruses (6, 7). This advantage has increased the application of nanoparticles in the treatment of diseases with a bacterial origin.

Despite the advantages and wide application of nanoparticles, their toxicity and side-effects should be further evaluated and monitored. So far, some of the toxic effects of nanomaterials have been revealed, resulting in some limitations in the application of these compounds. Among these toxic nanomaterials, carbon and fullerene nanomaterials with five- and six-sided rings of carbon can be named (8-10). According to recent studies, globular or circular carbon structures have high water solubility. This property can result in serious damages such as oxidation of fatty acids in fish brain, which is in fact indicative of biological degradation (11-13).

Through enhancing the production of free radicals, single-walled carbon nanotubes are able to reduce the effective concentration of antioxidants in cells. In fact, metal contamination in the production stage of carbon nanotubes could add to their toxicity (13, 14). On the other hand, several studies have shown that physical features of some nanotubes could be also involved in the development of pulmonary diseases and cancer (15-17). Due to their antimicrobial properties and production of free radicals, silver nanoparticles lead to oxidative stress and induce pathological effects in humans (18).

Many studies have shown that oxidative stress, caused by the increased production of free radicals and lipid peroxidation is one of the most important factors involved in the reduced performance and decreased quality of sperm parameters, which are closely related to infertility (19-25). Although several studies have demonstrated the negative impact of nanoparticles on different body organs, no comprehensive research has studied the effects of nanoparticles on the quality of sperm parameters, induced by the oxidation of sperm membrane lipids.

Considering the potential role of silver nanoparticles in enhanced production of free radicals, oversensitivity of sperm cells to the pathological effects of free radicals, and decreased level of antioxidants in the sperm cytoplasm, silver nanoparticles may be able to induce pathological effects, cause lipid peroxidation of sperm membranes, and consequently reduce fertilization.

Therefore, in this study, we aimed to evaluate the pathological effects of these nanoparticles on the level of lipid peroxidation and quality of sperm parameters in a rat model.

Method

Study samples: In this experimental study, 24 male rats were purchased from Pasteur Institute (Iran) and divided into one control and three study groups, each consisting of six rats. The rats were maintained under standard conditions, i.e., room temperature of 20-25°C, humidity of 55-60%, and a cycle of 12 hours of light and 12 hours of darkness. In a 35-day cycle of spermatogenesis, the rats were orally administrated different doses of silver nanoparticles via gavage on a daily basis. The study groups received 0.07 µg (group A), 0.14 µg (group B), and 0.28 µg (group C) of silver nanoparticle solutions, respectively (purity=99.99%;

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size: 20 nm; Pishgaman of Nanomaterials, Iran). The control group only received normal food and water.

Sampling of epididymal sperm: After anesthetizing the rats, the abdominal area was sterilized by 70% ethanol and incised. Next, the abdominal fat layers were opened with the help of forceps. The end of the epididymis was cut using sterilized scissors and placed in Ham's F10 culture medium. By placing the epididymis in the growth medium, the sperm cells started to float towards the growth medium through gentle squeezing and shaking. Afterwards, the extra segments of epididymis were removed from the medium. The culture medium, containing the sperm suspension, was incubated at 37°C under 5% CO_2 pressure for 30 min. Following the incubation, the samples were studied in terms of sperm parameters. The suspension containing the sperms was transferred to microtubes, using a sampler micropipette. The samples were centrifuged at 1000 g for 10 min. The supernatant was removed from the samples and immediately stored at -70°C for later malondialdehyde (MDA) measurements.

Analysis of sperm parameters: In order to determine the percentage of sperms with normal morphology, Papanicolaou's staining method was applied in line with a study by Park and colleagues (26). To study sperm morphology, slides were prepared for each sample and then fixated. Afterwards, Papanicolaou's method was utilized for staining the samples. The percentage of motile sperms was measured, using methods applied in previous research (27-29). Afterwards, 10 µl of each sample was placed at the center of the Makler chamber. After placing the cover on the chamber, a light microscope was used to count the sperms at a magnification of X4. In this evaluation, a total of 100 cells were counted, and the percentage of sperm motility was determined according to the World Health Organization (WHO) standards (30). Moreover, sperm viability was determined via cytoplasmic method and eosin staining (31,32). Approximately 10 ml of semen specimens from all groups was placed on sterile slides and mixed with 10 ml of 5% eosin. After mixing the stain with the samples, sterile cover slips were placed on the slides, and the samples were observed and measured using a light microscope at a magnification of X40. Considering the intactness of the cytoplasmic membrane, viable sperms could retain the eosin stain, which had penetrated into the cells; as a result, the sperms remained stained even after rinsing. On the contrary, non-viable sperms discarded the eosin stain due to their damaged membranes and appeared stainless under the microscope.

Measurement of lipid peroxidation of sperm membranes in the seminal fluid: In order to estimate the lipid peroxidation of sperm membranes, MDA concentration was measured in the seminal fluid of rats. MDA concentration was measured, using thiobarbituric acid reactive substance (TBARS) assay in line with a study by Tavilani and colleagues (33). Moreover, the MDA standard curve was depicted, using tetraethoxypropane (220.31 g/mol; Sigma Co.). Then, 100 ml of the examined samples was transferred to Eppendorf tubes and mixed with 500 ml of trichloroacetic acid. Subsequently, 10 ml of hydroxytoluene was added to the prepared solution and centrifuged at 3000 g for 10 min. Afterwards, 500 ml of the supernatant was removed from the solution, while adding 400 ml of TBARS (144.14 g/mol; Merck, Germany); the mixture was preserved at 95°C for one hour. The samples were stored and cooled down in a refrigerator for 15 min. Then, they were re-centrifuged at 4000 g for 10 min and the light absorption of the supernatant was analyzed by a spectrophotometer at a wavelength of 532 nm. Finally, MDA concentration was calculated, using the standard curve.

Statistical analysis: Comparison of the mean level of sperm parameters and MDA concentration was performed, using ANOVA and Least Significant Difference (LSD) tests. A p-value less than 0.05 was considered statistically significant.

Results

Results of the analysis of sperm parameters: Based on the findings, there was no significant difference in the mean body weight or weight of the left testicle in rats after 35 days. As the results indicated, group C showed the lowest mean sperm count compared to the

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control group and study groups A and B. No significant difference was found in reduced sperm count between the control group and group A; however, the reduced sperm count in group B was significantly different from the control group (table 1). Compared to the control group, the lowest percentage of viable sperms was reported in groups B and C; also, the difference was statistically significant. As the findings revealed, the mean percentage of motile sperms in group C was significantly lower than other groups. On the other hand, the percentage of motile sperms in the control group was significantly higher than other groups. The highest percentage of immotile sperms was reported in groups B and C, compared to group A and the control group; however, there was no considerable difference between groups B and C. The percentage of sperms with abnormal morphology was higher in the study groups, particularly groups receiving higher doses of silver nanoparticles. The highest and lowest percentage of viable sperms was reported in the control group and group C, respectively (table 1). The mean TBARS concentration in the seminal fluid was significantly different between the groups ($p<0.001$). In fact, a rising trend was observed in the mean TBARS concentration from the control group to group C.

The mean TBARS concentration in group C (0.35±0.01 nmol/ml) was significantly higher than the control group (0.24±0.016 nmol/ml), group A (0.25±0.01 nmol/ml) ($p<0.001$), and group B (0.32±0.02 nmol/ml) ($p<0.05$). The mean TBARS concentration of the seminal fluid in group B was higher than the control group and group A ($p<0.001$). However, the difference between group A and the control group was not significant in terms of TBARS concentration (fig 1).

Table 1. Comparison of sperm parameters among different groups

Groups / Parameters	Control Mean±SD	A Mean±SD	B Mean±SD	C Mean±SD	P-value
Number of samples	6	6	6	6	-
Administered dose of silver nanoparticles (µg)	0	0.07	0.14	0.28	-
Body weight at the end of the study (g)	38.19±4.25	35.14±3.17	36.79±2.41	34.31±2.82	0.855
Weight of the left testicle by the end of the study (g)	0.12±0.01	0.11±0.02	0.11±0.02	0.09±0.01	0.399
Sperm count ($\times 10^6$/ml)	23.57±3.46	21.15±3.02*	16±2.6*	13.31±2.1**	<0.001
Percentage of motile sperms (%)	89.06±10.97	80.06±9.83	77.69±16.96**	72.79±14.52*	<0.05
Percentage of immotile sperms (%)	12.3±2.78	19.4±5.16	22.31±3.25*	27.21±3.12*	<0.001
Viable sperms (%)	66.23±5.13	57±6.29**	51.4±8.32**	7.51*±47.6	<0.01
Percentage of abnormal sperms (%)	28.17±4.47	37.33±1.27	35.83±4.4**	41.67±3.9*	<0.001

*p<0.01, **p<0.05

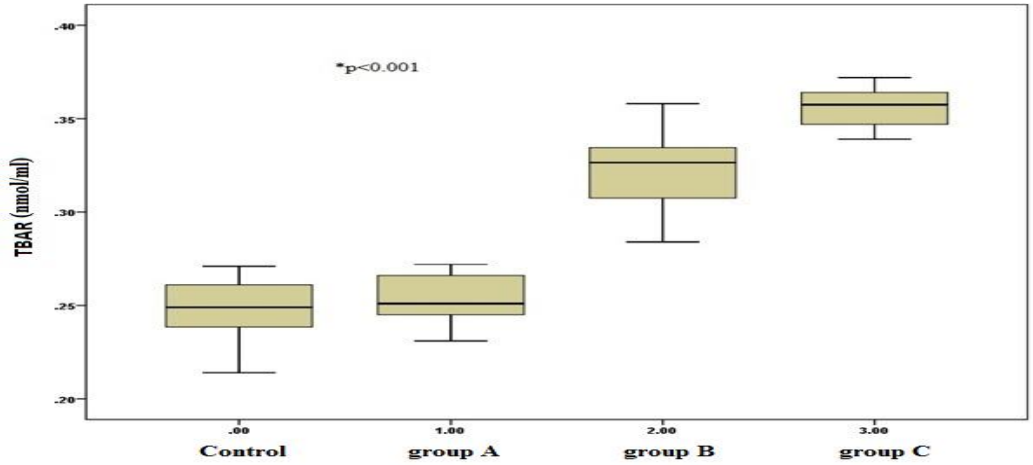

Figure 1. Comparison of the mean malondialdehyde (MDA) concentration between two groups. Group A: 0.07 µg of silver nanoparticles, Group B: 0.14 µg of silver nanoparticles, group C: 0.28 µg of silver nanoparticles.

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Discussion

Based on the analysis of sperm parameters, rats receiving silver nanoparticles showed a significantly lower sperm quality, compared to the control group. As a result, by increasing the dose of silver nanoparticles, the quality of sperm parameters, particularly normal morphology, viable sperm count, and percentage of motile sperms, decreased. On the contrary, the group receiving the highest concentration of silver nanoparticles (group C) showed the highest TBARS concentration, which could be indicative of the increased level of lipid peroxidation in rats. Based on the findings, silver nanoparticles not only could exert adverse effects on the quality of sperm parameters, but also could induce more severe pathological effects by increasing the concentration (dose-dependent properties).

It seems that one of the mechanisms involved in the effectiveness of silver nanoparticles in reducing sperm quality is enhanced oxidative stress, particularly lipid peroxidation, together with reduced effective concentration of antioxidants. Based on the current findings, it can be inferred that silver nanoparticles aggravate the adverse peroxidative effects in the seminal fluid, resulting in reduced sperm membrane fluidity and diminished sperm parameters. For this reason, the oxidative effects of silver nanoparticles and their pathological effects on the human body, especially on sperm quality and male fertility, should be assessed.

According to recent studies, silver nanoparticles at high doses (e.g., 200 mg/kg) can inhibit the clotting process by platelets and consequently increase the bleeding time. In addition, in previous research, the number of blood cells (i.e., red and white blood cells), hemoglobin concentration, as well as neutrophil and lymphocyte counts, has been reported to dramatically change (34-38).

Since few studies have examined the effects of nanoparticles (silver nanoparticles in particular) on fertility and sperm function, comparative analyses can be challenging. According to some previous studies, silver nanoparticles can block the growth of sperm cells and prevent sperm acrosome reactions (39-41). Moreover, analysis of the effect of titanium oxide nanoparticles on fertility of mice showed that these nanoparticles could lead to a substantial decline in the number of Leydig cells, sperm viability, and expression of some genes through inducing oxidative stress (42). In another study, the results indicated an increment in DNA oxidation and morphological changes of seminiferous tubules in groups treated with silver nanoparticles. However, no changes were observed in these groups regarding the count of sperms with abnormal morphology (41). In a study by Baki and colleagues, the number of Leydig cells in the study groups (particularly the group receiving 200 mg/kg of silver nanoparticles) significantly reduced. Furthermore, the concentration of testosterone remarkably reduced, while the level of luteinizing hormones significantly increased; however, no significant change was reported in the concentration of follicle-stimulating hormones. On the other hand, there was a significant decline in the number, morphology, and percentage of motile sperms, which was consistent with the results of the present research (43).

Therefore, the effects of silver nanoparticles on the quality of sperm parameters and male fertility could be considered as a fundamental and alarming issue for future generations. As demonstrated in our study, oxidative status of the seminal fluid in the studied samples could be involved in minimizing sperm quality. Since silver nanoparticles could increase the production of free radicals through several mechanisms, the percentage of sperms with mutated DNAs was speculated to increase. This finding is of great significance in procedures such as embryo microinjection, considering the possibility of unsuccessful in vitro fertilization and even multiple damages in future.

With the development of different techniques, the majority of infertile men may overcome their infertility without facing any particular problems. However, sperms with an abnormal morphology (e.g., sperms mutated in one or more specific genes) may affect the in vitro fertilization of the egg (e.g., through intra-cytoplasmic sperm injection). This may either lead to unsuccessful fertilization or induce adverse side-

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effects such as cancer in newborns and abnormal fetal development. Accordingly, evaluation of the application of silver nanoparticles in various scientific fields is of great significance. In fact, use of such technologies could boost men's reproductive abilities. The results of this study showed that silver nanoparticles could reduce the quality of sperm parameters in rats. One of the involved mechanisms in this process might be increased lipid peroxidation of sperm cells. Moreover, the toxic effects of silver nanoparticles were shown to be dose-dependent.

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