Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Biology

Elixir Appl. Biology 59 (2013) 15587-15589

ISSN: 2229-712X

Investigation of the silver nanoparticle (Ag NPs) effects on the fertility potential of rats

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ARTICLE INFO

Article history:
Received: 27 April 2013;
Received in revised form:
3 June 2013;
Accepted: 8 June 2013;

Keywords
Fertility potential,
Silver nanoparticles,
Sperm mobility,
Sperm morphology,
Wistar rat.

ABSTRACT

Nanotechnology is rapidly growing science of producing and utilizing nano-sized particles that measure in nanometers. Now-a-day we are using nanoproducts in various field. Of these silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. But it has toxic effect in biological systems, because of active oxygen production. They can also easily pass the cell membranes, blood-brain barrier as well as blood- testis barrier. In this study we try to investigate the effect of silver nanoparticles (Ag NPs) on the sperms quality in rats. In this study 75 male rats (body weight 150±20 gram, 4week year old) were used which were divided into 5 groups (1 control group and 4 experimental groups), with 15 rats in each group. Different dosages of Ag NPs (25, 50, 100, 200 mg/kg) were administered to the experimental rats in a period of spermatogenesis (35 days). After this time interval they were killed by spinal cord severing method, their epididymides were separated and in order to analyze the mobility of the sperms, a homogenous solution was prepared in the ham's medium. Moreover in order to study the morphology of sperms we used the Giemsa stained samples. Finally statistical data was analyzed by T-Test and SPSS software. Results showed by ($p<0/05$) administration of silver nanoparticle has a significant effect on the reduction of sperm mobility and its natural morphology ($p<0/05$). The results showed that Ag NPs decreased sperm mobility and worse natural morphology, so can affect fertility potential of lab animals.

Introduction

The term of nanotechnology was first proposed by Norio Tanigochi the science Professor of Tokyo University. He used this term in order to describe the making of accurate instruments whose dimensional scope is in nanometers. According to the definition by scientific societies related to nanotechnology a nanoparticle is known as a particle with the dimensions of 1 to 100 nanometers (Aaseth et al. 1981). In the nanotechnology the increase of the surface ratio to the volume of nanoparticles, strongly increases their reaction potential. Furthermore the increase of the particles surface changes the surface pressure and results in changing of distances between particles and creates space between atoms of particles. Therefore the natural characteristics such as color, stability and resistance will also change.

In general nanotechnology is the production of the material, instrument and new system which by taking control in the molecular and atomic level and to use their characteristics in the scale of nano (Paull et al. 2003). Meanwhile the important and special characteristics of Ag NPs such as quick effect, high stability and anti-microbial characteristics makes it useful in the most field of industries such as medical equipment and home appliances (Sarkar et al. 2006). But the effect and the reaction of these particles against cells and organs is not clear. Compared to the high amount of efforts made in order to show the desirable characteristics of Ag NPs, on the other hand very few efforts have been made to evaluate the potential undesirable effects of these particles. Recently a lot of concerns have been reported about the effect of these materials on human health and the environment and that some nanoparticles produce reactive oxygen species (ROS) (Yoshida et al. 2004) which cause toxicity in the labs (Brandt et al. 2005). They can even easily pass the cell membranes (Borm et al. 2004), blood-brain barrier as well as bloodtestis barrier (Mcauliffe et al. 2007). The researchers also showed that long exposure to colloidal silver or sediments of silver salts causes skin related diseases such as argyria and argyrosis(Aaseth et al. 1981, Chen et al. 2007). The studies on the different animals show that the inhaling, swallowing or injections of Ag NPs can sediment in the skin or lungs and moves from the primary sediment sites to the secondary sites such as liver, pancreas, kidneys, muscles, brain, ovaries and testicles (Panyala et al. 2007).

Epididymis is a part organ that extends from the efferent duct in the testicles. While sperms pass through the epididymis they get physiologically mature by secretions from the glandular layer of epididymis. Based on the instructions of WHO and the microscopic studies on the sperm fluid of the end area of epididymis 4 types of sperm mobility can be seen:

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1. Type A: they have a mobility of approximately 25 micrometer per second.
2. Type B: they have a mobility of approximately 15 micrometer per second.
3. Type C: if the sperm are mobile but do not go forward, they have in-place movement.
4. Type D: these sperm are immobile and do not have any movement (Momeni H R 2009).

Generally in this study it is tried to show the effects of Ag NPs on the mobility and morphology of sperms and the sexual hormones.

Materials and Methods:

Animals:

The animals used in this study were male wistar rats at the age of approximately 35 days and weight of about 150 grams which were provided from the lab animal center of, payam-e-noor University of Yazd. The mice are kept in special cages and have been provided by the necessary amount of food and water. Their care situations included room temperature of 23-25 centigrades with the humidity of 55-60% and the light period was divided in to 12 hours of light and 12 hours of darkness. Then they are divided in to 5 groups of 15 mice in each. Control group: In this group the care situations were similar to the other groups with the difference that the oral administration of nanoparticles was not done. Group 1 with the dosage of 25 mg/kg, group 2 with the dosage of 50 mg/kg, group 3 with the dosage of 100 mg/kg, group 4 with the dosage of 200 mg/kg of Ag NPs, for 35 days (one period of spermatogenesis) with the time interval of once every 12 hours. Ag NPs were administered orally through Gavage and after this phase anatomic dissection were performed.

Table 1. The effect of Ag NPs on sperms mobility

Experimental group(mg/kg)	Percentage of sperm A	Percentage of sperm B	Percentage of sperm C	Percentage of sperm D
Control	32±3.65	32±3.65	24.25±5.12	10.75±2.98
Experimental 25	28.5±3.10	28.5±3.10	28±6.05	12.5±2.08
Experimental 50	21.5±2.64	21.5±2.64	40.25±2.98	15.75±2.75
Experimental 100	20.75±3.59	20.75±3.59	37.25±6.39	18.25±3.30
Experimental 200	13.5±5.19	13.5±5.19	41±6.05	31±7.78

The test material and its preparation:

Ag NPs used in this study were got from the biochemical and biophysical research center of Tehran University. They were subject to ultrasound for about 10 minutes in order to prevent them from agglomerating. Then this material was solved in distilled water and was prepared in different concentrations of 25, 50,100,200 mg/kg. Epididymis sampling steps and the morphological examination of sperms: In this step after the Anastasia by chloroform, the rats were killed by spinal cord severing method and their abdomens were sterilized by 70% ethanol. In order to reach the abdominal cavity, the cutting was done in the abdominal area. Then in order to reach epididymis, fat layers have been removed by forceps. Then the end part of epididymis was separated and put in a petridish containing ham's medium. After the epididymis tissue was mashed in the ham's medium and a homogenous sperm solution was made, one 10μL drop of each sample was examined on a slide at 40X magnification and considering their mobility the percentage of different sperm types was examined. In order to examine the morphology of sperms, 10μL of sperm with 10μL of Giemsa stain (it destroys sperm mobility) were put on the macroscopic slide with at 40X, so that the percentage of normal and abnormal sperms were specified.

Statistical analysis:

For data analysis SPSS10 software was used and in order to specify the difference between the experimental and control groups the T-Test was performed.

Table 2. The effect of Ag NPs on sperms morphology

Experimental group(mg/kg)	Percentage of normal sperms
Control	76.20±7.22
Experimental 25	71.60±5.94
Experimental 50	64±4.74
Experimental 100	63±4.69
Experimental 200	51±6.20

Results:

These results showed a significant decrease in the percentage of rapid sperms of type A and also a significant reduction in the percentage of slow sperms of type B which was completely clear in the dosages of 100 and 200mg/kg.

The results about effect of Ag NPs dosage on the percentage of in-place sperms of type C showed a significant increase in the percentage of in-place sperms of type C and also a significant increase in the percentage of immobile sperms of type D in experimental group compared to control group and in the dosage of 200mg/kg the number of them have been increased. The results about the effect of dosage of Ag NPs on the sperms showed a significant change in the percentage of normal sperms in experimental group compared to control group which was related to the increase of the dose of nanoparticles.

Discussion:

Researches in the field of nanoparticles and nanotechnology are rapidly developing. For reasons such as its anti-bacterial characteristic, the use of Ag NPs is increasing. These nanoparticles enter the body through lungs by inhaling, digestive system, and skin (Phalen et al. 1973) and after they are located in the primary organs, are transferred to the main (secondary) sites such as liver, pancreas, brain and testicles (Megan et al. 2007, Phalen et al. 1973. Chen et al. 2003, Panyala et al. 2007). Researches show that these nanoparticles are able to pass through the cell membrane and hurt the target cells. Some researches have been done on effects of nanoparticles on cells and organs and their cytotoxic and genotoxic effects have been proved. The results of histopathology studies show the negative effect of Ag NPs (Takenaka et al. 2001) on the organs such as kidneys, liver (Hussain and Hess 2005), brain, and stem cells (Braydich – Stolle et al. 2005).

Few studies have been done about the effect of Ag NPs on epididymis but the results here showed compared to control group, the sperm mobility has a significant reduction in the experimental group which can be related to the effect of Ag NPs on the function of mitochondria. Studies done about the effect of Ag NPs on the function of mitochondria of different cells show decreased mitochondrial activity in all cases (Braydich – Stolle et al. 2005). Studies done by Braydich-Stoll on the C18-4 cell line showed that Ag NPs and Al NPs are able to pass the sperm membrane and can attach to the mitochondria and acrosome of the sperm (Braydich – Stolle et al. 2005).

Regarding the fact that the sperm mobility is gained in epididymis, studies show that the Ag NPs can effect epididymis and cause inflammation to it which plays a role in reduction of the sperm mobility (Manin et al. 2007). Nanoparticles increase ROS (Wang F. 2009) in cells which cause damages to the cilia

of the sperm and reduce the mobility of sperms. The results about percentage of normal sperms showed that, compared to the normal sperms in the control group, there is a significant decrease in the experimental group which is completely related to the dosage and in the dosage of 200 mg/kg the sperms with abnormal morphology reaches the highest percentage; since Ag NPs can react with DNA and cause inflammation and oxidative damage as well as disorder in cell function (Nel et al. 2006, Ji et al. 2011); this can be a result of the effect of Ag NPs on DNA and finally may cause genetic mutation and produce abnormal morphology.

Conclusion:

Ag NPs affect the morphology and the mobility of sperms as well as the mitochondrial function which reduces the sperm mobility which depend on the dosage of nanoparticle.

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