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In Vivo Effect of Different Doses of Silver Nanoparticles on the Seminiferous Tubules in Albino Rats: Histopathological Study

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K E Y W O R D S	ABSTRACT	
Nanoparticles, Silver nanoparticles, Albino male rat, Seminiferous tubules.	The study aims to investigate the effects of silver nanoparticles (Ag NPs) on the seminiferous tubules in Albino rats. Several in vitro studies have been performed in different cell models, using various nanoparticles. Pure and spherical AgNPs with an average size of 30 nm, was injected into two groups of male albino rats (6 rats for each group) in different doses. Histopathological changes in testis tissues were showed a harmful effect of the silver nanoparticles, manifested by reducing the number of spermatogenic cells, and a decrease in the number of leyidg's cells (group 1), and hypotrophy in seminiferous and enlargement in interstitial spaces in group 2.	

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1. Introduction

Recently, nanotechnology rapid proceeds in diverse scientific branches repaired different effects on all parts of human, animal, environment and industrial in life [1]. Laser ablation of solid targets in the liquids is the perfect new physical process for the production of nanoparticles NPs [2,3]. Silver nanoparticle was substances used in nano-formulation. It is used for a long duration in jewelry, monetary currency, utensils, dental alloy, and photography [4]. On the other side, excessive using nanoparticles may be hazardous to human health and the environment. One of the most commonly used metal nanoparticles is silver nanoparticles (AgNPs), which have a wide area of applications included chemical catalysts, solar energy absorption coatings, and antimicrobial agents. AgNPs have a potential for bactericidal effects, inhibitory and as well as retard growth of mold, harmful germs and spores [1].

The characteristics of nps are important to determine the toxicity like size and surface area [4]. In contact with the human body, nanoparticles can endure a series of operations like reacting and binding with proteins, deposition, phagocytosis, translocation, and clearance [5]. Further, nanoparticles can reduce a spectrum of tissue restraint like activation of the cell, generation of reactive oxygen species (ROS) and the death cell [6,7].

A wide application of nanoparticles has been a lack of information about the effect of NPs on human health and the environment. It is believed that a chemical structure, morphology, particle size, and surface chemistry of NPs are key parameters that effectiveness their toxicity. On another side to optimize the fundamental influence of NP applications, it is to understand the interactions of NPs with biological systems [8].

In vitro studies in the male reproductive field performed in different cell models, using different NPs. The toxicity of different doses was observed in mouse spermatogonia [9]; for example, the affected of titanium dioxide and carbon black NPs studied in male mice causes proliferation and gene expression of mouse Leydig cells [10].

The LD50 according to the Organization for Economic Cooperation and Development (OECD) 425 guideline in mice treated with a dose of 5,000 mg/kg body weight [11].

The normal value of the silver medal in human blood is 0, 00028 ppm [12]. Thus, a field of Nanotoxicology still needs the needful information and clarifications to attain true risk assessment. The current search was planned to better examine the effect of Silver Nanoparticle on testis tissue in albino male mice.

2. Material and Methods

I. Experimental animals

Eighteen Albino male rats were randomly divided to three groups: C1 (control), T1 (Treated group1) injected by AgNPs at concentration 35μ m at a dose of (0.4 mg/kg.b wt/day), T2 (Treated group2) injected by AgNPs at concentration 35μ m at a dose of (0.6 mg/kg. b wt/day). Each group comprises six rats. Animals of each group are in different separated cages with sawdust bedding. All animals were sacrificed at the end of the experiment. The average weight of animals before the experiment was ranged (170-200) gm; the age of mature male rats was four months. Experimental animals were placed in a room with 25 °C room, 12h light: left free to take distal water and food, and were stabilized for two weeks before the start of the experiment.

Ag NPs have been obtained from the school of Applied Sciences, University of Technology, Iraq.

II. Histopathology

A histological analysis over the toxicity of Silver Nanoparticles (SNPs) after fifty days was performed by checking changes of morphological that induced from silver nanoparticles, in the testes. An organ fixed with 10% formalin after that routine histological preparation was conducted the histological sections were cut at six micrometers in thickness by using rotator microtome and stained with Hematoxylin and Eosin (H&E) [13].

3. Results

In a present study interested in exploring the toxic effects of AgNPs on seminiferous tubules, Laydig cells. Experimental animals didn't show any significant changes in water/food consumption, as well, no death in the animal was observed through the treated time.

Histopathological examination of testis in a control group of rats showed normal spermatogenic cells, Leydig's cells among seminiferous tubules (Figure 1). Vacuoles in seminiferous tubules were also observed in the AgNPs treated groups. We test the possible effect toxicity of Ag NPs on rat seminiferous tubules. In the control group and treated groups on the significant difference was observed. T1 (Treated group1with AgNPs 0.4mg/kg body weight/day) shows reduce in the number of spermatogenic cells and a decrease in Leyidg's cell number (Figure 2). The present study was revealed, the histopathological changes in the seminiferous tubules in T2(Treated group2with AgNPs 0.6mg/kg body weight/day), relative hypotrophy in seminiferous, enlargement in interstitial spaces and decrease in the number of Leydig's cells (Figure 3), degeneration in the spermatogenic cells (Figure 4).

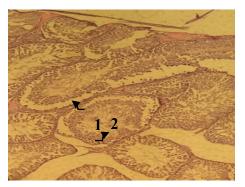


Figure 1: Rat seminiferous tubules, consisting of spermatogenic cells (1), Leydig's cells (2) among seminiferous tubules. Hematoxylin and Eosin (H&E) 200X (control group)

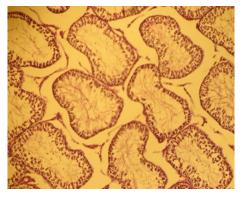


Figure 2: Rat seminiferous tubules, show reduce in the number of spermatogenic cells (1) and a decrease in leyidg's cells (2) number. H&E. 200X.Treated group1



Figure 3: Rat seminiferous tubules. Relative hypotrophy in seminiferous (1), enlargement in interstitial spaces (2) and a decrease in the number of Leydig's cells (3). Stained with H&E.200X. (Second treated group).



Figure 4: Rat seminiferous tubules, show degeneration in the spermatogenic cells (arrows). Stained with H&E.400X. (Second treated group).

4. Discussion

It is recognized that NPs can penetrate the reproductive tissue from biological barriers; therefore, they damage various cells: for instance, they could ahercell function and decrease sperm viability, as well as that embryo development [12]. However, a potential toxic effect of NPs on a reproductive tissue is conceivable and then insights are needed to explain this issue [13].

In vivo studies have shown reverse effects of CNPs on male reproductive systems of the mice (adult) [14] and after exposure during fetal life [15]. As well, rats exposed to diesel exhaust led to endocrine system disruption after birth and suppression of testicular function [16]. Another confirmation of potential toxic effects of NPs on spermatogenesis reported by [17] study the intravenous administration of silver nps in albino male rats, then observed a decreased count of epididymis sperm, increased levels of DNA damage in germ cells and seminiferous tubule morphometric changes.

Although *in vivo* testing will continue to provide the most related information on the human hazards [18].

Silver NPs, shown acute toxic effects on male reproductive organs .many research suggests that NPS inter a blood-testes barrier and deposited in testes, and caused adverse effects on sperm cells [19]. AgNPs can bind to different tissues and can cause potential toxic influence like cell activation, producing(ROS) reactive oxygen species, which are severed toxic to tissue, inflammation. Finally, all the processes gradually lead to the death of the cell [20].

5. Conclusion

This search is a trail to modify various toxicity tests to verify the toxic effects of silver nanoparticles.

6. Recommendations

1-further studies are required to explain any reverse effects and the necessity to support safe use of silver nanoparticles.

2- Study the effect of AgNPs on testosterone hormones.

3- Study the effect of AgNPs on seminal vesicles and prostate tissues.

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