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Evaluation of hepatotoxic and genotoxic potential of silver nanoparticles in albino rats

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ABSTRACT

Silver nanoparticles (AgNPs) have wide medical applications regarding their antimicrobial effects. They are applied also in appliances such as refrigerators and washing machines. For assessment of toxicological potential of silver nanoparticles 20 mature female albino rats were divided into four groups (five rats per each). Animals were injected i/p by different doses of approximately 8.7 nm silver nanoparticles (1, 2 and 4 mg/kg b.w) daily for 28 days in addition to control group which were injected by deionized water only. Indicators of oxidative stress in liver tissue, determination of silver nanoparticles tissue concentration, description of hepatic histopathological alterations and detection of possible chromosomal aberrations in bone marrow were carried out. Results revealed various hepatic histopathological lesions that were dose dependent. The effect of Ag-NPs on hepatic malondialdhyde (MDA) and glutathione (GSH) levels were variable in different treated groups compared with the control. The tissue residues of silver nanoparticles were found in hepatic tissue and related to original treated dose. Finally, silver nanoparticles induced variable chromosomal aberrations that were dose dependent. Conclusion: Silver nanoparticles had the ability for inducing various hepatic histopathological alterations indicating hepatocytotoxicity presumably by oxidative stress, in addition to the induction of chromosomal aberrations in bone marrow cells denoting the genotoxicity of nanosilver particles.

1. Introduction

Nanomaterials are any materials that have at least one dimension <100 nm and they can be divided into two large groups; ultrafine nano sized particles that are not intentionally produced and engineered and nanoparticles that are produced in a controlled and engineered way (Oberdörster et al., 2005). One of the widely used nanomaterials is nano silver, its particles have a size ranging from 1 to 100 nm. Silver nanoparticles represent a prominent nanoproduct with potential applications in medicine and hygiene because of the antibacterial effects (Lok et al., 2006; Kim et al., 2007; Ayala-Núñez et al., 2009) antiviral actions (Elechiguerra et al., 2005; Mehrbod et al., 2009) and antifungal activity (Kim et al., 2008a). They also promote wound healing by playing a role in cytokine modulation (Wong et al., 2009). The use of silver nanoparticles is not only restricted on medical application but also extended to various issues related to environment and consumer products. There are many applications like disinfection of drinking water (Li et al., 2008; Lv et al., 2009), swimming pools anti-fouling (Yang et al., 2009a) and as a promising antibacterial additive to water-based paints (Holtz et al., 2012). The problem is that nasal and throat sprays, or contraceptive foams which contain nanosilver might leave remarkable residue in the human body; Also coatings of surfaces in contact with the human skin (textiles) or food will increase human exposure and uptake of nanosilver (Quadros and Marr, 2010; Hansen et al., 2008; Yang et al., 2009b). Several researchers have studied the hepatotoxic effect of silver

Abbreviations: AgNPs, silver nanoparticles; ANOVA, analysis of variance; GSH, reduced glutathione; kV, kilo volt; MDA, malondialdhyde; PAS, periodic acid Schiff; PVP, polyvinyl pyrrolidone; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TEM, transmission electron microscopy; UV–vis, ultraviolet–visible light; XRD, x-ray diffraction.

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nanoparticles via different routes and doses; hepatic alterations were induced by inhalation of silver nanoparticles in Sprague-Dawley rats for 28 days (Ji et al., 2007). On the other hand bile-duct hyperplasia, with or without necrosis, fibrosis, and/or pigmentation studied was observed after oral toxicity of silver nanoparticles (56 nm) over a period of 90 days in F344 male and female rats which were given different doses (30, 125 and 500 mg/kg) (Kim et al., 2010). Nanoparticles were detected in Kupffer cells lining, walls of the venous sinusoids, venous endothelial cells and in small foci of inflammatory reaction after intravenous injection of silver nanoparticles (AgNPs) with different sizes (20 nm and 100 nm) was for 28 days (De Jong et al., 2013). Although it is still questionable, whether the silver nanoparticles (AgNPs) cause damage to the genetic material of treated germinated onion root tips (*Allium cepa*) with different concentrations (10, 20, 40 and 50 ppm) (Babu et al., 2008), *in vitro* study demonstrated DNA damage in mammalian cells with exposure to silver nanoparticles (Cha et al., 2008; Asharani et al., 2009).

Our article investigates the hepatotoxicity and genotoxicity of silver nanoparticles in female albino rats via evaluating the hepatic histopathological alterations, determining the hepatic oxidative stress parameters and detecting the possible chromosomal aberrations occurring in bone marrow cells.

2. Materials and methods

2.1. Preparation of silver nanoparticles

Silver nanoparticles were prepared using chemical reduction method according to (Van Dong et al., 2012) with some modification. AgNPs were synthesized by using sodium borohydride ($NaBH_4$) and polyvinyl pyrrolidone (PVP) as reducing and stabilizing agents, respectively. First, 0.272 g of $AgNO_3$ was dissolved in 344 mL deionized water and then put on magnetic stirrer for 15 min. A mixture of 2.912 g of trisodium citrate and 0.504 g of polyvinyl pyrrolidone (PVP) was dissolved in 48 mL deionized water, stirred for 15 min and then added to the prepared $AgNO_3$ solution.

$NaBH_4$ solution is prepared by dissolving 1.89 g of sodium borohydride in 50 mL deionized water and is then put in refrigerator and 8 mL of freshly prepared cold aqueous solution of $NaBH_4$ was quickly added to the formed solution with continuous stirring for 30 min, the reduction reaction occurs with change of color to dark yellow and brown.

3. Characterization of silver nanoparticles

3.1. UV-Visible absorption spectroscopy

Absorption spectra were recorded using a double beam UV–Vis spectrophotometer (Cary 5000, Varian, Australia). The absorption spectra of diluted solutions of the prepared AgNPs in aqueous medium were recorded within the appropriate scan range (350–800 nm). The spectra of pure solvent were taken as a calibrating reference. Measurements were performed at room temperature.

3.2. Transmission electron Microscope (TEM)

The morphology of AgNPs and their particle sizes were examined using TEM (Tecnai, FEI, The Netherlands), operating at an accelerating voltage of 200 kV. A drop from a dilute sample solution was deposited on an amorphous carbon coated-copper grid and left to evaporate at room temperature forming a monolayer. Analysis of particle size diameters of the prepared AgNPs were estimated using the software program Image J over several shots of TEM images for the target sample (Ross and Dykstra, 2003).

3.3. Particle sizer

The particle size, size distribution were measured by zeta sizer (Malvern zeta sizer Nano ZS) based on the dynamic light scattering technique.

3.4. X-ray diffraction

Samples were air dried, powdered and used for XRD analysis. X-ray diffraction patterns were recorded in the scanning mode on an X'pert PRO PAN analytical instrument operated at 40 kV and a current of 30 mA with Cu $K\alpha$ radiation ($\vdash$ = 1.54 Å). The diffraction intensities were recorded from 35° to 79.93°, in 2θ angles. The diffraction intensities were compared with the standard JCPDS files. The software gave the information about the crystal structure of the particle (Bragg, 1914).

3.5. Experimental animal design and conditions

A total of 20 mature female albino rats were purchased from Research Institute of Ophthalmology and were kept in the Department of Animal Care and Management-Faculty of Veterinary Medicine, Cairo University. Rats were acclimated for 14 days before starting the experiment. During this period and the experiment period, rats were housed in plastic cages (5 rats per each cage) in room temperature and humidity in well-ventilated room with natural light. The rats were fed commercial pelleted feed and given water ad libitum. The rats were divided into four experimental groups 5 per each. The treated groups were injected i/p with different doses of silver nanoparticles (1, 2 and 4 mg/kg b.w) daily for 28 days. The control groups were injected with deionized water to exclude the factor of injection stress. Rats were weighed at the beginning of the study and weekly throughout the experimental period and the final body weights were also recorded at the end of the study. All procedures of using laboratory animals in this study met the regulations of Ethics of Research Committee at Faculty of Veterinary Medicine, Cairo University and received the approval number: Cu F Vet/F/PAT/2013/12.

3.6. Oxidative stress analysis

- Protein assay: Crude enzyme protein content was determined according to method described by (Bradford, 1976).
- MDA and GSH assay: Liver samples were kept frozen at −20 °C. and tissue homogenate was made in glass homogenizer using 10% (w/v) cold 1.15% KCl for (MDA) according to (Ding et al., 2002) and 5% 5-suphosalicylic acid for (GSH) according to (Anderson, 1985).
- Estimation of malondialdhyde (MDA) and GSH in liver was done according to (Albro et al., 1986) and (Ellman, 1959), respectively, and results expressed as nmol/mg protein.

3.7. Histopathological examination

The rats were observed throughout the experimental period; then the rats were euthanized using chloroform as anesthetic reagent and finally cervical dislocation was done after 28 days of exposure, Liver was removed carefully, weighed, and fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, then cleared with xylene and embedded in paraffin, sectioned at 5 μm thickness and stained by H & E stain, Masson's trichrome stain, periodic acid Schiff's (PAS) and Prussian blue stains and then examined microscopically (Bancroft and Gamble, 2008).