The Effect of different Concentrations of Silver Nanoparticles on Enzyme Activity and Liver Tissue of Adult Male Wistar Rats in-vivo Condition

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Abstract. Nanotechnology as a branch of science which is related to nano materials helps in overcoming the limitations of size and can change the outlook of the world regarding science. Silver nano particles, due to their strong antimicrobial properties, are widely applicable in different industries. So, research on nano silver properties and its harmful effects on human and environment is critical. The aim of the present study is testing the harmful properties of Silver nano particles, in the size 18 to 32 nm, on enzymes and tissues of liver in Wistar rats. At first 50 adult male Wistar rats were picked and concentrations 5, 10, 20 and 40 ppm of Silver nano particle were tested on AST, ALT, ALP and GGT enzymes. Results showed that only 40 ppm of silver nano particle possesses a meaningful effect on ALT enzyme, and none of the other concentrations has meaningful effect on AST, ALP and GGT. Moreover, histopathology test from liver tissue revealed no damage in liver cells. As nano silver shows harmful effects on a broad range of cells, predetermined dosages of nano silver in limited amounts are suggested.

Keywords: Silver Nanoparticles, Nanotoxicology, ALT, AST, GPX, ALP

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials like cosmetics, ICT, food and feed, environmental health and agricultural productions at the nanoscale level (Wijnhoven et al., 2009). The term “Nano” is a Greek word synonymous to dwarf meaning extremely small (Rai et al., 2009) which is used to indicate one billionth of a meter or 10⁻⁹. The term Nanotechnology was coined by Professor Norio Taniguchi in Tokyo Science University in the year 1974 to explain precision manufacturing of materials at the nanometer level.

Nanotechnology as a branch of science which is related to nano materials helps in overcoming the limitations of size and can change the outlook of the world regarding science (Kim et al., 2007). By manipulating materials at the atomic level, nanotechnology offers to achieve unique properties for various desired applications. It is noticeable that most of the nature’s creations occur at the nanoscale regime too (Ghosh et al., 2010). Because of its widespread application, the commercial nanotechnology industry is predicted to increase significantly to $3 trillion by 2015 (Ahamed et al., 2010). The effects of nanosilver on tissues which were investigated in significant and analytical experiments showed the damages in the tissues. These damages were caused by the increase of free radicals and stimulation of oxidative stress; however, more immunological and genetical investigations will clear the biological effects of nanoparticles (Ranjbar Sardari et al., 2011). Nano silver has biological properties which are significant for consumer products, food technology (e.g., food processing equipment, packaging materials, food storage), textiles/fabrics (e.g., antimicrobial clothing), and medical applications (e.g., wound care products, implantable medical devices). In addition, nano silver has unique optical and physical properties that are not present in bulk silver, and which are claimed to have great potential for medical applications (e.g., diagnostics, drug delivery, and imaging). As a destructive effect of nano silver on human health, the respiratory system represents a major port of entrance for nano silver. Sprays containing nano silver are already available on the market, indicating that this is a relevant exposure route.

The distribution and disposition of nano silver in the respiratory tract depends on various factors
including particle size and breathing force. In addition, due to the small diameter of the nano silver, Brownian diffusion also determines deposition, resulting in a deep penetration of nano silver in the lungs and dispersion to the high lung surface area presented in the alveolar area. If nanoparticles are absorbed by the gastrointestinal tract, they will be transported directly to the liver through the portal vein. In general, the liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. However, no evidence exists for metabolism of nano silver by enzymes in the liver and the rest of the body. The widest and best known use of silver preparations in medicine is as preferred antimicrobial agents for treatment of serious burns (Monafo and Freedman, 1987; Pruitt et al., 1998; Hoffman, 1984; Miller et al., 1990; Klasen, 2000). Atopical cream that contains 1% silver sulfadiazine plus 0.2% chlorhexidine digluconate in a water immiscible cream base is the most widely used product for human use and veterinary medicine, marketed as Silvazine in the USA (by Marion-Hoechst-Russell Laboratories, Kansas City, MO, USA) and as Flammazine in other countries, largely in the UK (Smith and Nelson Company; Roche), Canada and continental Europe. From the initial use of silver sulfadiazine creams, there has been more recent incorporation of the silver sulfadiazine directly into bandages used on burned skin surfaces and similar large open wounds (Wright et al., 1998; Klaus et al., 1999).

Use of direct current electricity to accelerate the release of Ag (I) from the covering into the damaged tissue and then penetration into the tissue has been shown beneficial (Matylevich et al., 1996; Chu et al., 2000), although this appears without wide use. There were morphological changes in the rats which were exposed to nanoparticles (2 mg/kg dose); it showed significant changes in hair color. The evidence show morphological changes in spleen, kidney and liver of the experimental rat groups that were treated with silver nanoparticles when compared to the control group. Silver nanoparticles (70 nm) caused spleen color changes and atrophy in rat. The pathological results showed damages in kidney’s tissue, including necrosis of glomerular cells, Bowman capsule and proximal tubular in group. Proteinic sediment was seen in renal tubules, whereas inflammation of the parenchymal cells was observed in the liver, and nuclear duplication of some cells and intercellular space enlargement were observed in the hepatic lobule. Moreover, apoptosis around the central vein and blood between some cells were also observed (Ranjbar Sardari et al., 2011). Silver nanoparticles also cause histopathological changes in the liver, spleen and kidneys; which indicated the tendency of silver ions to binding thiol groups in livers, causing reduction reactions, transferring of glutathione to bile bladder and reducing the concentration of glutathione available for biochemical reduction reactions. It should be mentioned that reducing glutathione is necessary to remove peroxides (Hendi, 2010; Campen, 2003). So, different kinds of nanoparticles can be toxic in human and animal tissues (Miura and Shinohara, 2009). Our study aims at examine the harmful properties of silver nano particles, in the size 18 to 32 nm, on enzymes and tissues of liver in Wistar rats.

2. MATERIALS AND METHODS

2.1. Preparation of silver nanoparticles

In this study, the solution containing Ag nanoparticles with commercial name, NONO24460,was produced by Mobin chemical spadana Company. The concentration of Ag-NPs in this compound was 4000 ppm and it was in form of colloidal suspension. This compound keeps its stability in cultural medium. The size of this Ag-NPs was between 18 to 34 nm and Zeta potential of silver nanoparticles was -33.5 that showed the average stability of this compound (Figure 1and 2). All the applied concentrations have obtained by diluting different amount of the Ag-NPs solution with appropriate amount of distilled water.

2.2. Test group categorization:

50 adult male Wistar rats were used in this study. Their weights, at the beginning of study, were 200±25 g and their ages were between 2 to 3 months. The rats were kept at the laboratory temperature (25°C) and appropriate light (12h light and 12 h darkness). All the principles concerning the work with animals were observed carefully and their food was prepared from Navidan institution of animal food and their water was the drinking water used by citizens in the Isfahan city. All rats get adapted to the laboratory environment through spending 10 days in the environment. In this study, concentrations between 5 to 40 ppm of silver nano particles were selected as they possess antimicrobial property. At first, 5, 10, 20, and 40 ppm of nano particles were deionized using distilled water. The animals were randomly divided into 5 groups of 10 in each. The first group, served as control group, received only deionized distilled water and groups 2 to 5 received 5, 10, 20 and 40 ppm of silver nano particle, respectively. The rats, using Gavage, were fed silver nano particle for 30 days (Park et al., 2010).
2.3. Determination of serum AST, ALT, ALP and GGT activities

After the time of treatment, all rats were stupefied using Diethyl ether and then blood samples were taken from their hearts. Serum was prepared by aspiration of the clear yellowish liquid after clotting and centrifuged for 10 min at 3000 g in an MSC (Essex, UK) bench centrifuge. Estimation of enzyme activities was done using clear supernatant. In the next step the serum concentration of AST (Aspartate Transaminase), ALT (Alanine Transaminase), GGT (gamma glutamyltranspeptidase) and Alkaline phosphatase (ALP) had been evaluated using biochemical kits.

2.4. Histological studies

In the next step and after stupefying the rats using Diethyl ether and dying, their livers were separated and kept in Formalin 10%. The liver tissues were prepared through common histological methods and kept with paraffin blocks. The blocks were cut to 3-5 µm in diameter. These block pieces were put on glassy slides and then they were cleaned from paraffin and watered. Then, for microscopic checking, the slides were colored using Haematoxylin-Eosin (H&E) method. The structure of liver tissues in samples was checked regarding their morphological changes.

2.5. Statistical analysis

Data analysis was performed using ANOVA and Tukey test. Obtained results were represented as standard mean error, so that the difference among groups was measured p<0.05.

3. RESULTS AND DISCUSSIONS

Regarding the liver enzymes, the average amounts of AST serum was 220±5 in control group and this amount at the highest silver nano particle concentration (40 ppm) was almost 223±5 which indicate no meaningful difference in AST levels in all groups. In figure 3, another curve represents the level of ALT of serum in which its level was 96±2 in 40 ppm of silver nano particles leading to a meaningful effect when compared with control and other groups. Also other results proved that none of silver nano particle concentrations had meaningful effect on ALP and GGT (figure 3).
Checking the activity of AST and ALT enzymes is the first stage in examining the liver damages. The most sensitive and the most practical recognizing enzymes in liver are aminotransferases. These are aspartate aminotransferase. The enzymes are normally existed within the liver cells. When the liver gets damage, the cells flow the enzymes to blood and the increase in the level of the enzymes indicates a liver damage (Reitman and Frankel, 1957). Considering the meaningful difference in ALT enzyme, we in this study examined the liver histopathology in all treatment groups. The study showed us that no group had cellular damage and Sinusoids and Hepatocyte had no necrosis (figure 4).

Normally, an increase in AST and ALT indicates liver problem, but higher level of normal amounts of the enzymes should not lead to severe damage in liver cells and concentrations higher than 40 ppm of nano silver plus a more time interval for treatment may cause some damages.

A study in 2009 reported that after using different dosages of silver nano particles, some changes are made in the level of Lactate dehydrogenase (Saber and John, 2009). Another study proved that the effect of nano particles depends on the shape, size and diameter of the nano particles (Moudgi et al., 2006). So, one can conclude that spherical silver nano particles with 4 nanometer in diameter cannot change the enzymes activity. Studies on tissues revealed the most changes in enzymes activity using 400 ppm of silver nano particles. This experiment shows that high concentrations of silver nano particles cause some changes in liver tissues (AshaRani et al., 2009). Recently, it is reported that nano particles create free radicals and oxidative stress, and with stress oxidative mechanism (that is attacking free radicals to tissues), they can cause damage to organs and tissues (Akradi et al., 2012). Also results from a study in 2012 showed that silver nano particles cause membranous damage and decrease the activity of Superoxide dismutases (SOD) and Glutathione peroxidase (GPX) (ling song et al., 2012).

ALP is a hydrolytic enzyme which its activity is observed in alkaline pH and different forms of these enzymes are existed in blood. The enzyme is highly available in liver and bone and also available in some other tissues like kidney, placenta, bowel wall, thymus, lung and testicles (Soochan et al., 2012). The level of ALP serum of blood increases in pathologic conditions as well as in bone and liver damages. GGT is an enzyme existed in liver, bile ducts, kidney, prostate and spleen. Measuring this enzyme may help to recognize the liver and bile diseases.
4. CONCLUSION

Silver has long been using as a strong antimicrobial agent and in recent years its application has been increased vastly in various drugs and productions such as toys, cosmetics, refrigerators and so on. Today's, silver is used in its nano form which is extremely more effective compared with its larger particles. In present study, considering the level of ALP, GGT, AST and ALT enzymes and also histopathology of liver tissue, no damage was observed using 5 to 40 ppm. But regarding to the fact that silver nano particle has some harmful side effects on human health and environment, a very accurate examination is highly recommended. Also as nano silver shows harmful effects on a broad range of cells, predetermined dosages of nano silver in limited amounts are suggested.

REFERENCES


Gavanji et al.
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