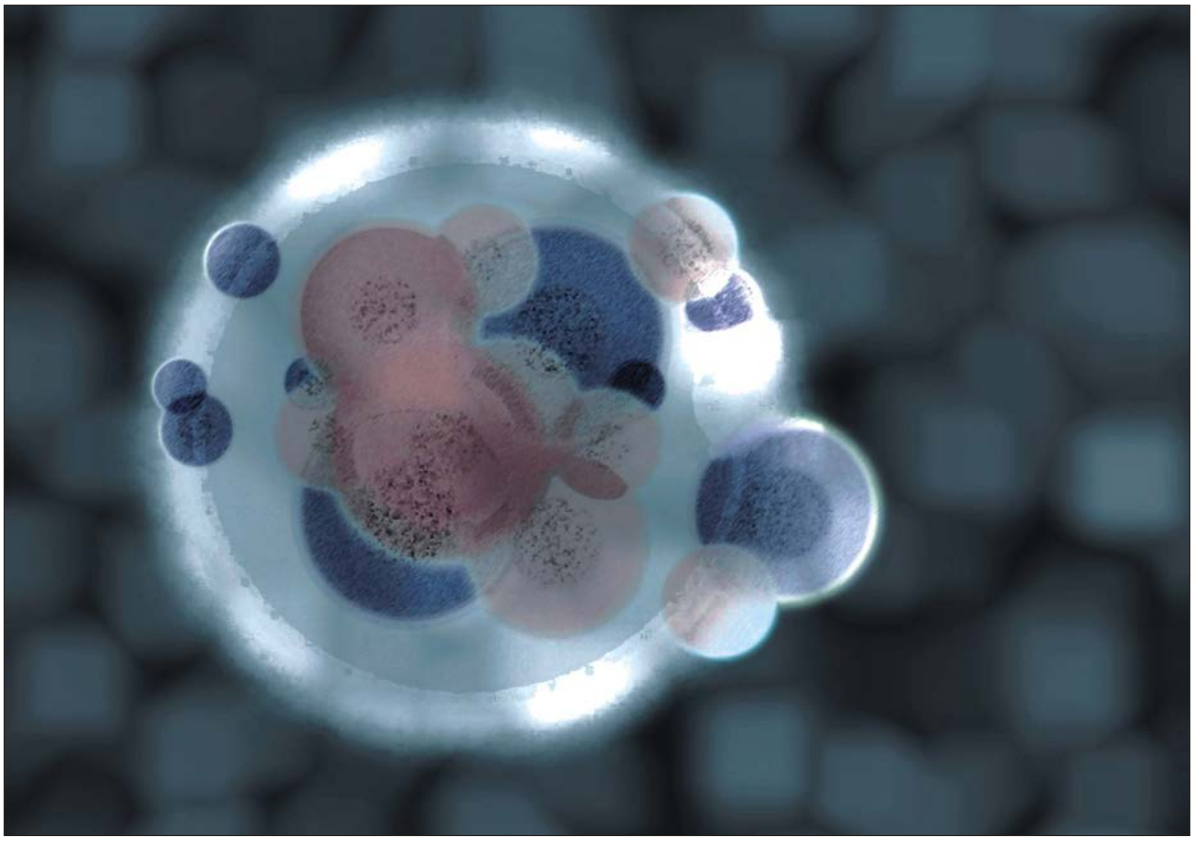

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Acute Effect of Nanosilver to Function and Tissue Liver of Rat after Intraperitioneal Injection

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Abstract: The use of nanosilver particles in medicine, due to its antibacterial and antifungal characteristics, dates back hundreds of years. However, several studies indicate that besides the antibacterial characteristics, these nanoparticles have high toxic effects on mammalian cells. For this study, 32 Wistar male rats were obtained. After 2 weeks of accommodation, they were randomly divided into groups; three nanosilver-treated rat groups and one Control Group (CG). The three treated groups were exposed to 5 cc of solution containing 5, 10 and 100 ppm nanosilver(Ag) (group 1, 2 and 3, respectively) via IP injection for 7 successive days. The control group was treated with 5 cc of normal saline solution with the same procedure. This study was performed to investigate the effect of different nanosilver concentrations on biochemical parameters such as Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic-pyruvic Transaminase (SGPT) at various time points (2, 7 and 14 days). After 14 days, the tissue of liver and lung were collected and investigated. There was no significant difference between SGPT and SGOT liver enzymes in different groups ($p>0.005$). However, the amount of SGOT enzyme had increased in the treatment groups compared to the control group but this difference was not significant. Liver histological findings in this study showed no pathological changes except the decrease in acidophilic property in treatment group 1; reduction in acidophilic property, hepatocyte hypertrophy, full sinusoids fading and hyperemia of the central veins in treatment group 2 and reduction of acidophilic properties, hepatocyte hypertrophy, fading and thinning of sinusoids and slightly enlarged central veins in treatment group 3. The histological findings of the lung in the group receiving a 10 ppm concentration of nanosilver were complete destruction of air sacs and bronchioles and disfiguring of the lung tissues from their normal form. Moreover, in the group receiving a concentration of 100 ppm they were sever and clear hyperemia, disfiguring of the air sacs from their normal form, abnormal condition of the bronchioles, vessel rupture and blood leakage to the bronchioles and air sacs. The histological findings in the group receiving a concentration of 300 ppm were complete destruction of air sacs and bronchioles and obvious hyperemia in the tissue (which indicates the impact of nanoparticles in this tissue, deformity in some places were so much that it was not possible to identify lung tissue). According to the findings it can be concluded that the nanosilver particles, which were used in the form of solution in citrate stabilizer, with spherical shape and 10 nm in size caused complete destruction of lung tissue and partially destroyed liver tissue.

Key words: Nanosilver particles, toxic, liver, lung

INTRODUCTION

Today, the use of nanosilver particles in different sciences especially medicine (to treat external wounds and burns) can be seen around the world. The antibacterial and antifungal characteristics of these particles has been proven (Hori *et al*., 2002; Lagaron *et al*., 2005). The ever-increasing number of studies devoted to various aspects of the biological activity of nanoparticles (NPs), including their toxicity, is almost on a par with the pace of developments in the area of nanotechnologies.

Chen *et al*. (2006) reported that different ways of exposure to nanoparticles such as skin contact, inhalation, intraperitoneal injection and etcetera can have different toxic effects. Certainly, it should be borne in mind that barriers through which particles penetrate into the blood from the lungs and from the peritoneal cavity are different anatomically and functionally and these differences can be reflected on NPs' toxicokinetics.

Some studies have shown that nanosilver significantly increases Reactive Oxygen Species (ROS). It

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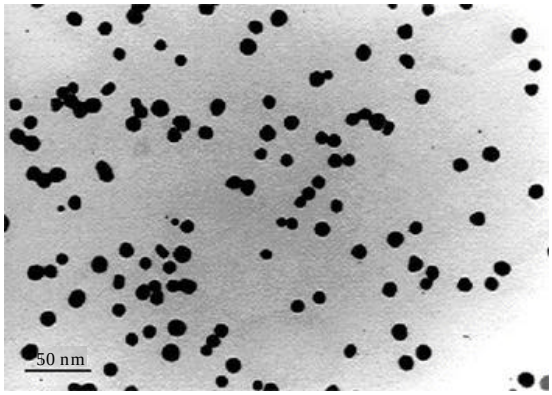

Fig. 1: TEM image of the nanosilver particle, 10-15 nm diameter, spherical shape, 99.9% purity, inorganic nature, wet synthesis method in liquid phase (alternation)

was also determined that nanosilver genomic effects will be inhibited in the presence of antioxidants, or free radical scavengers (Hackenberg *et al*., 2011; Kim *et al*., 2011; Asare *et al*., 2012; Karlsson *et al*., 2012). Considering the various emerging applications of nanosilver particles and the lack of investigations on their toxicity, this study was carried out to evaluate nanosilver particles potentials toxicity and its effects on some organs (liver and lung) in rats.

CHARACTERIZATION OF SILVER PARTICLES

Two hundred fifty milliliter colloidal silver with the concentration of 1000 ppm from Tehran Neutrino Company, which provides from Spain, with specifications of 10-15 nm diameters, spherical shape, 99.9% purity, inorganic nature, resulted from wet synthesis method in liquid phase (alternation) was bought. Electronic microscope image TEM of the nanosilver particle is shown in Fig. 1.

PREPARATION OF MOTHER SOLUTION

Mother solution was prepared for nanosilver as follows:

- The 1 cc nanosilver with concentration of 1000 ppm+10 cc of sterile two times distilled water from Merck Germany = concentration of 10 ppm nanosilver
- The 10 cc nanosilver with concentration of 1000 ppm+100 cc of sterile two times distilled water from Merck Germany = concentration of 100 ppm nanosilver
- The 30 cc nanosilver with concentration of 1000 ppm 300 cc of sterile two times distilled water from Merck Germany = concentration of 300 ppm nanosilver

MATERIALS AND METHODS

A total of 32 healthy male Wistar rats of weighing (225±25 g) were used. Animals were randomly divided into groups, three nanosilver-treated rat groups and one Control Group (CG). Group 1, 2 and 3 received. /5 cc of solution containing 5, 10,100 ppm nanosilver (Ag) via IP injection for 7 successive days, respectively. The control group was treated with. /5 cc normal saline with same procedure. Then, several biochemical parameters such as Serum Glutamate Oxaloacetat Transaminase (SGOT) and Serum Glutamate Pyrvate Transaminase (SGPT) were evaluated at various time points (2, 7 and 14 days). After 14 days, the tissue of liver and lung was collected and investigated.

Biochemical assessment: Blood samples containing no anticoagulant agent were centrifuged at 3000 rpm for 10 min. Serum Glutamate Oxaloacetat Transaminase (SGOT) and Serum Glutamate Pyrvate Transaminase (SGPT) were measured using enzymatic methods according to the instructions provided by the manufacturer of the kits (Using a biochemical autoanalyzer: Hitachi Automatic Analyzer 902 , Roche).

Histopathological assessment: At the time of scheduled sacrifice, from each animal liver and lung were collected and stored in 10% buffered formalin to be fixed for histopathological examinations. The fixed tissue specimens were embedded in paraffin and 6 μm thick sections were provided and stained with hematoxylin and eosin (HE). Sections were then evaluated by light microscope.

Statistical analysis: All data were analyzed by using the statistical package for social sciences (SPSS v.19) software and were summarized and expressed as mean (Mean±SE). Statistical analysis of data was performed by Multivariate Analyses of Variance models, while serum values at 2, 7 and 14 day after intervention using as dependants variable. We compare between groups and baseline value of serum was controlled. We using wilk's lambda or Roy largest Root for totally differences between groups, p-value about each depended variables was reported in last row of tables, the Tukey was used to paired comparisons. p-value less than 0.05 were considered significant.

RESULTS

Biochemical analysis: Based on MANOVA statistical test and considering test statistics of p = 0.668, F (9, 60.9) = 0.743 and Wilks' lambda = 0.740, there was no difference regarding mean Serum Glutamic Oxaloacetic Transaminase (SGOT) enzyme in different groups. However, the amount of SGOT enzyme increased in the treatment groups compared to the control group, but this difference was not significant (Fig. 2). According to MANOVA model and test statistics of p = 0.250, F (9, 60.9) and Wilks' lambda = 0.650, the mean Serum Glutamic-pyruvic Transaminase (SGPT) enzyme was not different among the groups (Fig. 3).

Histopathological investigation

A-Liver: The histological photomicrographs of the liver sections are shown in Fig. 4, 5, 6, respectively.

Treatment group 1, concentration of 10 ppm: Apart from the decrease in acidophilic property no other tangible and notable changes were observed (Fig. 4).

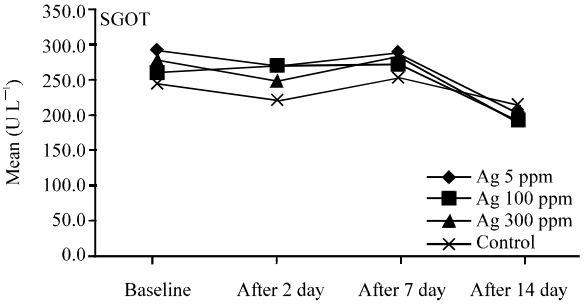

Fig. 2: Comparision the levels of serum glutamate oxaloacetat transaminase (SGOT) in four groups: (Ag10, 100, 300 nm and control group)

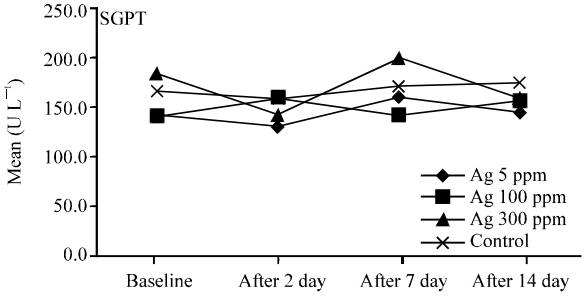

Fig. 3: Comparision the levels of serum glutamate serum glutamate pyrvate transaminase (SGPT) in four groups: (Ag10, 100, 300 nm and control group)

Treatment group 2, concentration of 100 ppm: Increase of acidophilic property is obvious, hepatocyte hypertrophy in a way that the sinusoids have almost disappeared, cells are normal and basophilic and central venues are clearly hyperemic (Fig. 5).

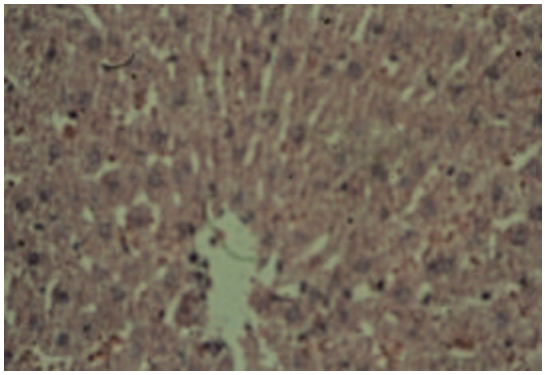

Fig. 4: Light micrographs of sections in the liver of nanosilver-treated rat received 10 ppm (group1), everyday for 7 successive days demonstrating of changes histopathology

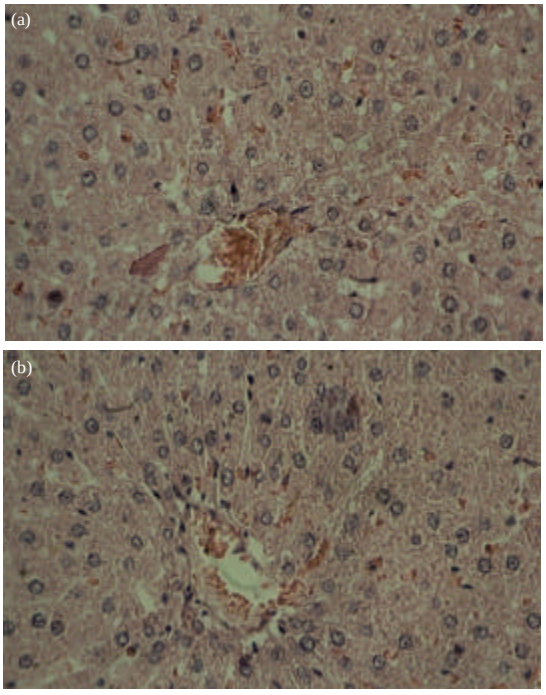

Fig. 5(a-b): Light micrographs of sections in the liver of nanosilver-treated rat received 100 ppm (group2), everyday for 7 successive days demonstrating of changes histopathology

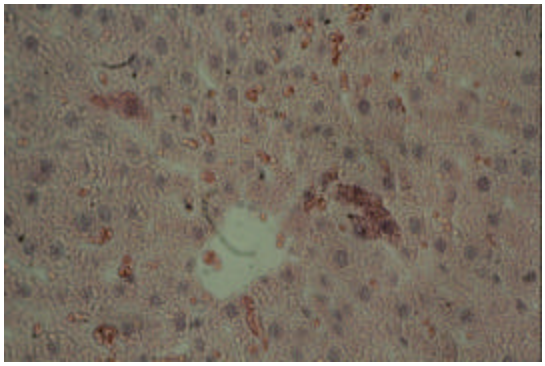

Fig. 6: Light micrographs of sections in the liver of nanosilver-treated rat received 300 ppm (group3), everyday for 7 successive days demonstrating of changes histopathology

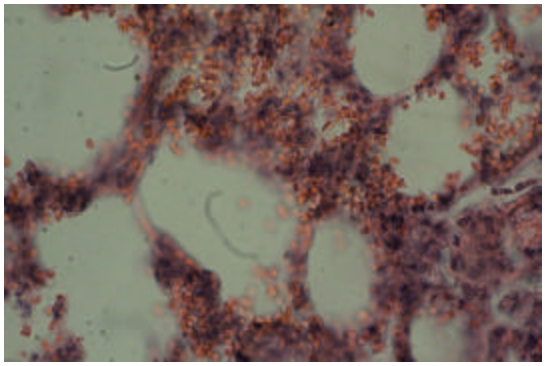

Fig. 8: Light micrographs of sections in the lung of nanosilver-treated rat received 100 ppm (group2), everyday for 7 successive days demonstrating of changes histopathology

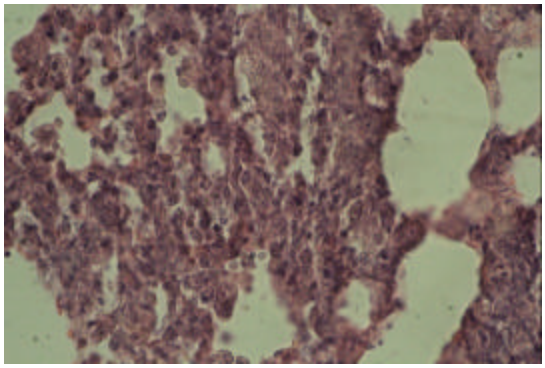

Fig. 7: Light micrographs of sections in the lung of nanosilver-treated rat received 10 ppm (group1), everyday for 7 successive days demonstrating of changes histopathology

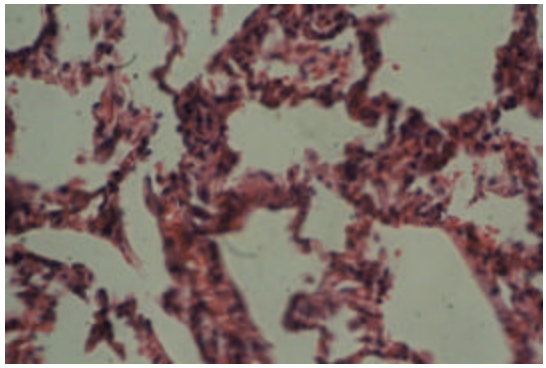

Fig. 9: Light micrographs of sections in the lung of nanosilver-treated rat received 300 ppm (group3), everyday for 7 successive days demonstrating of changes histopathology

Treatment group 3, concentration of 300 ppm: Decrease in the property of acidophilic, hepatic hypertrophy, fading and thinning of the sinusoids and enlargement of the central venous are the most notable pathological changes in the group (Fig. 6).

B-Lung: The histological photomicrographs of the lung sections are shown in Fig. 7, 8, 9, respectively.

Treatment group 1, concentration of 10 ppm: The impact of nanoparticles on the liver tissue is more evident. Destruction of air sacs, bronchioles and disfiguring of the lung tissue from its normal form are the most important pathological changes in this tissue (Fig. 7).

Treatment group 2, concentration of 100 ppm: The pathological changes observed are visible. Severe and clear hyperemia is visible in the tissue. Air sacs are disfigured and have mired. Abnormal condition is observed in the bronchioles and blood is seen in them, which indicates the rupture of vessel wall and blood leakage into the bronchioles and air sacs (Fig. 8).

Treatment group 3, concentration of 300 ppm: Complete destruction of air sacs and bronchioles. Hyperemia in the tissue indicates the effect of nanoparticles on it. Deformity in some places is so much that it is not possible to identify lung tissue (Fig. 9).

DISCUSSION

The evaluation of physiological and histopathology effects of nanosilver particles in different concentrations

on SGPT and SGOT liver enzymes was conducted in this study. The results indicated that there was no difference between mean SGPT and SGOT liver enzymes among the groups ($p>0.05$). Although the amount of SGOT enzyme had increased in the treatment groups compared to the control group, this difference was not significant.

The histological results of the liver tissue showed no pathological changes apart from decrease in acidophilic properties in treatment group 1, increase in acidophilic property, hepatocyte hypertrophy, fading of all sinusoids and hyperemic central venues in treatment group 2 and decrease in acidophilic property, hepatic hypertrophy, fading and thinning of the sinusoids and enlargement of the central venous in treatment group 3.

The liver tissue damage in high dosage of nanosilver particles (treatment groups 2 and 3), is probably caused by stimulation of the antioxidant system in these cells by nanosilver injection. Free radicals induced from nanosilver particles attack hepatocyte and cause the release of stored liver enzymes in them and enter the blood serum. Therefore, pathological changes in liver tissue might be due to the accumulation and sediment of these nanoparticles in the tissue.

As many reseachers showed that with diameter changes in the nanoparticles, their distribution in different tissues and effects will vary. The smaller the diameter of the nanoparticle, the higher its penetration into the cells and molecule effects on intracellular mechanisms (Wijnhoven *et al*., 2009).

A study by Koohi *et al*. (2011) on the effects of different sizes of nanosilver on the histopathologic changes of a rabbit, showed midzonal and periacinar necrosis, mononuclear infiltration portal, change in liver fat, congestion of liver and hyperemic central venous. The results of the study by Kim *et al*. (2007) showed changes in the level of alkaline phosphatase and cholesterol in rats related to a significantly high dosage of nanosilver. This suggests that exposure to more than 125 mg kg^{-1} nanosilver will result in slight damage to the liver. Histopathalogic evaluation showed high indices of hyperplasia in bile duct, with or without necrosis, fibrosis or pigmentation in animals that have been treated with nanosilver.

Due to the toxicity of silver, another study was conducted with reference to oxidative stress in cells. Results indicated a significant decrease in the level of glutathione (GSH), decrease in mitochondrial membrane potential and increase in ROS level, which indicated the cytotoxicity of silver (100-150 nm) in liver cells caused by oxidative stress (Hussain *et al*., 2006).

Since the liver is the main place for biological changes and it defends the body from foreign substances and xenobiotic, identifying the causes for liver toxicity and its damage is important. Liver damage from nanoparticles is classified based on histological damage such as inflammation or necrosis and these damages are characterized at the molecular level. Some of the most common mechanisms of injury of hepatocytes are established based on metabolic cytochrome P450 methods. Liver excretes the substances into bile; therefore, the biliary system is also exposed to nanoparticles. Studies show that various toxins with different mechanisms, including activation of cytochrome P450, activation of alcohol dehydrogenase, membrane lipid peroxidation, inhibition of protein synthesis, disruption of calcium homeostasis and activation of receptors enzymes pre-apoptotic, cause damage to liver cells (Oberdorster *et al*., 2005).

To study the effect of nanoparticles on specific areas of the liver the following should be considered.

Enzyme systems, mitochondrial function, albumin synthesis, cell separation, protein or gen or membrane damage expression, mechanically special separated parts such as cell morphology, viability, membrane vulnerability, ATP volume, single valence protein bond, increased peroxisomal numbers and GSH volume are used. Nevertheless, biomarkers are always alive since they are associated with cell lines and are different from organs in terms of genotypic and phenotypic characteristics and have constraints. In addition, hepatocyte cultured cells show a single cell system and only provide information about the events which directly affect the cells (Worth and Balls, 2002; Dambach *et al*., 2005).

Enzymes such as SGOT and SGPT are the most important enzymes in liver cells and are nonfunctional enzymes in plasma. The amount of SGPT in the cytoplasm of liver cells is several times more than extracellular fluid. When the liver cell membranes are damaged, or when liver cells have died, the level of SGPT will increase in plasma and the amount of this increase is an indication of the degree of liver damage (Worth and Balls, 2002; Dambach *et al*., 2005).

SGOT exists in all body tissues and in mussel, skeletal and cardiac tissues, such as the liver, a large amount of this enzyme exists. It seems that the main mechanism of toxicity of nanoparticles is created by oxidative stress, which damages lipids, carbohydrates, protein and DNA (Worth and Balls, 2002; Dambach *et al*., 2005). The study of injection of nanosilver particles and its effect on liver tissue by Hsin *et al*., (2008) showed that it causes cell death (apoptosis) and increased, dose dependent, oxidative stress in the liver cells.

Nanosilver by oxidizing the oxygen atoms produces oxygen ion and by hydrolyzing water produces OH ions, which are both strong foundation and causes of oxidative stress. Another mechanism of nanosilver is related to the silver ion function in colloidal solution (Naghsh *et al*., 2012). In this mechanism the toxicity of silver is due to the conversion of metallic nanosilver particles to active silver ions.

The study by Naghsh *et al*. (2012) showed that liver damage caused by the injection of nanosilver particles to male rats can strongly stimulate the oxidant system in these cells. Most of the liver cell damage was caused in low concentrations (50 ppm) 3 days after the injection. In higher concentrations (100, 200 and 400 ppm) no significant changes in liver enzymes were observed following liver damage (Braydich-Stolle *et al*., 2005).

In the present study, nanosilver particles with concentration of 50 ppm (4 nm in diameter) penetrated into the liver cell membrane and ruptured it. The accumulation of lipid droplets was observed in the hepatocyte cytoplasm, inflammation around the vessels and portal space. Histological studies of silver staining in liver tissue showed the penetration of nanosilver particles in hepatocytes, which has eventually led to the formation of destructive vesicles and entry of substances which resulted from cell damage into the liver sinusoids. This study was similar to the study by Hussain *et al*. (2006) and Saito *et al*. (2003).

In the present study the histological lung results in the group receiving a concentration of 10 ppm of nanosilver particles showed the destruction of air sacs, bronchioles and disfiguring of the lung tissue from its normal form. In the group receiving a concentration of 100 ppm they showed severe and clear hyperemia, disfigurement of air sacs, abnormal condition in the bronchioles, the rupture of vessel wall and blood leakage into the bronchioles and air sacs. Moreover, in the group receiving a concentration of 300 ppm they showed complete destruction of air sacs and bronchioles and hyperemia in the tissue (which indicates the effect of nanoparticles on it) and deformity in some places is so much that it is not possible to identify lung tissue.

CONCLUSION

According to the above findings it can be concluded that nanosilver particles, which were used in the form of solution in citrate stabilizer, with spherical shape and 10 nm size showed complete destruction of lung tissue and also partially destroyed liver tissue.

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