Nanomedicine Journal

Received: Jan. 28, 2014; Accepted: Mar. 12, 2014
Vol. 1, No. 4, Summer 2014, page 248-257

Online ISSN 2322-5904
http://nmj.mums.ac.ir

Original Research

The acute liver injury in rat caused by gold nanoparticles

Monir Doudi[1], Mahbubeh Setorki[2]*

[1]Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[2]Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

Abstract

Objective(s): Gold nanoparticles (GNPs) command a great deal of attention for biomedical applications nowadays. The data about the degree of toxicity and the accumulation of gold nanoparticles in-vivo is not enough to judge.
Materials and Methods: A total of 32 healthy male Wistar rats were randomly divided into 4 including: three GNP-treated and one control group. Groups 1, 2 and 3 received 0.5 cc of a solution containing 5, 10, and 100 ppm Au daily via intraperitoneal (IP) injection for 7 days, respectively. The control group was treated with 0.5 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 2, 7 and 14 days after the last injection. After 14 days, all the rats were sacrificed and liver, lung tissues were separated and evaluated.
Results: SGOT two days after intervention was significantly greater in the group 2 than the control group. In liver histological assessment, in group 1, basophils were observed around the central veins, in group 2 fading and no observation of central veins was seen, and in group 3 hepatic damage was noticed. The lung histological results showed severe vascular hyperemia in group 1, air sacs damage in group 2, and complete air sacs destruction in group 3.
Conclusion: The results showed extreme changes in the histopathology of lung and liver tissues caused by spherical nanogold with 5-10 nm size in all of three treatment groups.

Keywords: Fibrous, Gold nanoparticle, Hypertrophy

***Corresponding Author:** *Mahbubeh Setorki; Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran.*
Email:doctor.setorgi@gmail.com

Introduction

Gold nanoparticles (GNPs) are a tremendous scientific achievement of nanotechnology, and are used in various fields of medicine and different industries such as agriculture, livestock, food packaging, household appliances, cosmetic, and disease treatment (1, 2). These particles are also used in DNA detection. GNPs are active as catalysts in a number of commercially important reactions, and have a suitable surface chemistry for the bonding of sulfur molecules such as thiol, which leads to settlement from lower to higher locations in useful and desired structures (1, 2).

For example, a significant synergistic effect of ultrasound combined with Au-PpIX (protoporphyrin IX) was observed; it reduced tumor relative volume and increased average animal survival time (3). Studies on GNPs have shown that the most frequent reason for their toxicity is accumulation in extracellular spaces and its solubility in intracellular spaces (4). In another study was observed that dissolved GNPs activates it activates a kind of released oxygen in the intercellular space and reduces the function of living cells. In conclusion, we can say that these released oxygen from GNPs action, prevent the proper functioning of living cells, and thus, slow down and stop the process of growth (5). In a study by Chen in 2009, GNPs with dimensions of 3, 5, 8, 50, 12, and 100 nm with the amount of 8 mg/kg were injected to Balb/C rats(6). The results showed that sizes 3, 5, 50, and 100 nm were not fatal. They also showed that nanogold accumulation in liver and spleen by the reticuloendothelial system (part of the immune system associated with complex components to detect, get, and filter foreign and micro antigens) can lead to hepatic and splenic toxicity (6). Cho et al. studied the toxicity of 13 nm GNPs in rats.

They found that these nanoparticles accumulate in the liver after injection, stimulate inflammation, and cause cell death in liver tissues (7).

De Jong et al. injected 10, 50, 100, and 250 nm GNPs with concentrations of 77, and 108 mcg to rats. They observed that after 24 hours the rats died.

They measured the concentration of gold in different organs. The results indicated that the smallest size (10 nm) of nanogold was seen in blood, spleen, liver, testicles, lung, and brain. The larger sizes were seen in the liver and spleen (8).

There is less information about the genotoxicity of GNPs, and it is rather contradictory. Some data indicates that nanogold causes genome instability through oxidative stress (9).

However, other experimenters have established that neither nano (2 nm or 20 nm), nor microparticles (200 nm) of gold possess genotoxicity either in vitro or in rats exposed to three consecutive intravenous or a single intratracheal administration (10, 11).

Due to the important uses of nanogold in biological and medical systems, the risk of toxic effects, and the limited number of studies on its toxic effects on animals, it seems that studies on the possible side effects of nanogold in invivo condition have particular importance.

This study aimed to examine the toxic effects of these nanoparticles on enzyme changes in liver, liver tissue, and lungs.

Materials and Methods

Characterization of Au particles

100 ml of colloidal nanogold from Tehran Neutrino Company, Iran, which provides from Spain, with specifications of 5-10 nm diameters, spherical shape, degree of purity of 99.9%, mineral nature, wet synthesis method in the liquid phase (alteration), object after synthesis, wet and soluble during work, and with the concentration of 100 ppm.

Electron microscopic image (TEM) of these nanoparticles is shown below.

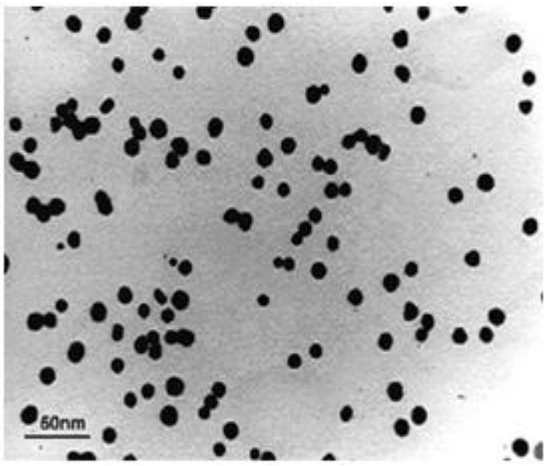

Figure 1. Electron microscopic image (TEM) of nanoparticles, 5-10 nm diameter, spherical shape, 99.9% degree of purity, mineral nature, and wet synthesis method in the liquid phase (alteration).

Animals, drug administration and samples collecting

A total of 32 healthy male Wistar rats obtained from the Animal Center of Shahrekord University, Iran. All animal handling and manipulation procedures were performed according to the guideline of the Animal Welfare Act and the experimental protocols were approved by the Office of Research Ethics Committee at University of Shahrekord.

The rats were nearly of the same age (12 weeks old) and weighing 225 ± 25 g. The rats were maintained on standard laboratory rodent diet pellets and were housed in humidity and temperature-controlled vent-ilated cages on a 12 hour day/night cycle. After two weeks of accommodation to the animal room, they were semi-randomly distributed into 4 groups of 8. Groups 1, 2 and 3 received a daily dose of solution containing 5, 10, and 100 ppm nanogold, respectively, via intraperitoneal (IP) injection for 7 days. Animals in group 4, the control group, received 0.5 cc normal saline with same procedure. The effects of GNPs on serum biochemical levels were evaluated at 2, 7 and 14 days after the last injection. Blood samples were collected from the eye vein by removing the eyeball quickly at time points. All animals (at 14th day) were anesthetized by diethylether and sacrificed. Serum was collected by centrifuging blood at 2,500 rpm for 10 minutes.

The tissues such as liver and lung were autopsied.

Fractions of tissues were kept in 10% (v/v) formalin for immediate histopathological examination.

Biochemical analysis of liver function

Whole blood was centrifuged twice at 3000 rpm for 10 minutes in order to separate serum. Using a biochemical autoanalyzer (Hitachi Automatic Analyzer 902, Roche, Germany), serum biochemical analysis was carried out. To evaluate the liver function, the levels of serum glutamate oxaloacetat transaminase (SGOT) and serum glutamate pyrvate transaminase (SGPT) were measured.

Histopathological examination

Histological observations were performed according to the standard laboratory procedures. Rats (four rat/treatment group) at the end of day 14th were dissected for histology. A small piece of lung and liver fixed in 10% (v/v) formalin was embedded in a paraffin block, sliced into 5 μm thicknesses and then placed onto glass slides. The section was stained with Hematoxylin–Eosin (HE) and examined by light microscopy.

Statistical analysis

All data were analyzed using the statistical package for social sciences (version 19, SPSS Inc., Chicago, IL) software and were summarized and expressed as mean and standard deviation (mean ± SD). Multivariate analysis of variance (MAN-OVA) model was the used, using serum values at 2, 7 and 14 day after intervention as dependant variables. We compared the groups and the baseline value of serum was controlled.

We used Wilk's lambda or Roy Largest Root for total differences between the groups; P-value of each dependent variable was reported in last row of tables; and the Tukey was used for paired comparisons.

Less than 0.05 P-values were considered significant.

Results

According to MANOVA model and statistical test, P value = 0.032, F (12, 66.4) = 2.59, and Wilks' lambda = 0.433, mean serum SGOT enzyme was different between groups. Based on Dunnett's posttest, mean SGOT enzyme was significantly greater in the group receiving Au 100 ppm than the control group two days after intervention (P = 0.028) (Diagram 1). 14 days after the injection of Au 100 ppm, the liver damage returned.

Mean ± SD of SGPT enzyme in different times and divisions of study groups are shown in table 2. According to MANOVA statistical test, P value = 0.776, F (12, 66.4) = 0.667, and Wilks' lambda = 0.740, no difference was observed in mean SGPT enzyme of different groups. Although the amount of this enzyme was increased in all the treatment groups compared to the control group, this increase was not significant (Diagram 2).

Diagram 1: Comparison of the levels of Serum glutamate Oxaloacetat transaminase (SGOT) in four groups (Au of 5, 10, and 100 nm and control group).

Diagram 2: Comparison of the levels of Serum glutamate Serum glutamate pyrvate transaminase (SGPT) in four groups (Au of 5, 10, and 100 nm and control group).

Liver histopathological evaluation

The histological photomicrographs of the liver sections are shown in Figures 2, 3, and 4.

A. Treatment group 1, 5 ppm concentration of Au: According to figure 2, significant basophils are observed around the central veins, which can indicate hepatocyte damage and accumulation of cells in this area. It seems that acidophilic hepatocyte was slightly increased.

B. Treatment group 2, 10 ppm concentration of Au: According to figure 3, the main point in this tissue is the fading and no observation of central veins. Liver lobules are not detectable at all, and in some of the lobules the central veins are so small and hyperemic that they are not distinguishable in the first look. In addition, hepatocyte hypertrophy, Remak bundles, and thinning of sinusoids are other signs worth mentioning in this tissue.

C. Treatment group 3, 100 ppm concentration of Au.

According to figure 4, hepatic damage, Remak bundles, and active hyperemia between them, fading of lobular borders, and hyperemia in some of the central veins are the most important histopathology changes in these tissues.

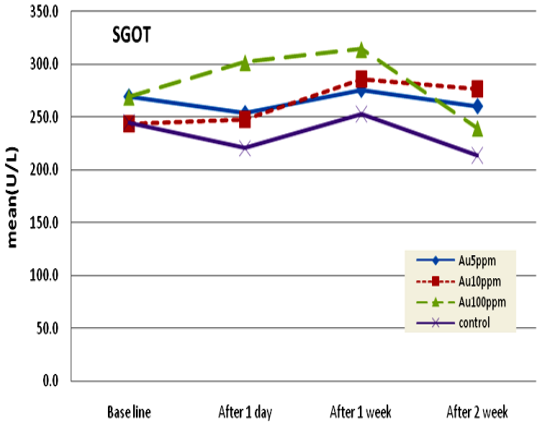

Figure 2. Light micrographs of sections in the liver of nanogold-treated rat received 5 ppm (group1), every day for 7 successive days demonstrating of changes histopathology.

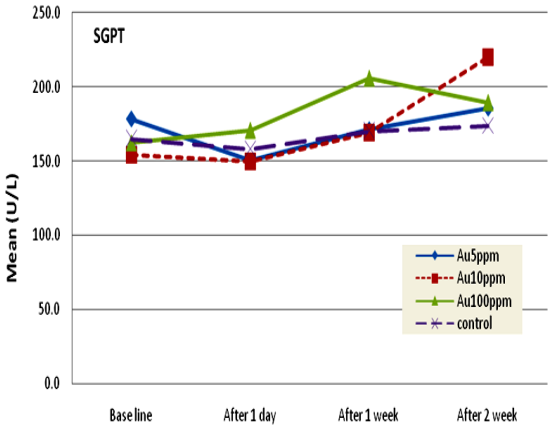

Figure 3. Light micrographs of sections in the liver of nanogold-treated rat received 10 ppm (Group 2), everyday for 7 days demonstrating of changes histopathology.

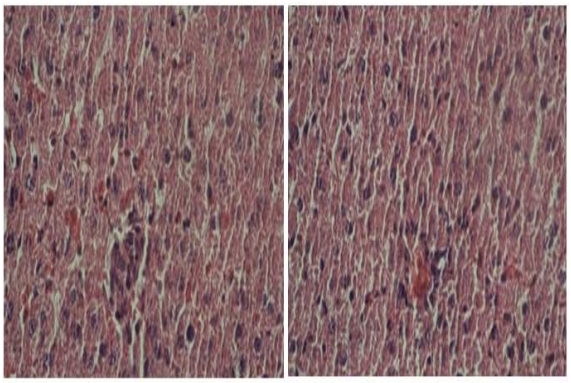

Figure 4. Light micrographs of sections in the liver of nanogold-treated rat received 100 ppm (group 3), everyday for 7 successive days demonstrating of changes histopathology.

No histopathological alteration was observed in the livers of control animals (Figure 5).

Lung histopathological evaluation the histological photomicrographs of the lung sections are shown in Figures 6, 7, and 8.

A. Treatment group 1, 5 ppm concentration of Au:

According to figure 6, severe vascular hyperemia, compressed air sacs, increase in fibrous tissues, and thickening of the air sacs are noteworthy facts about this tissue.

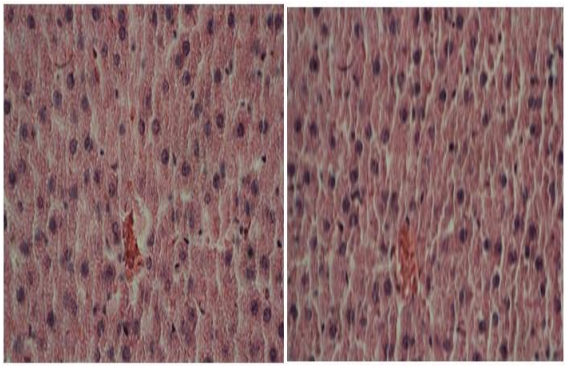

Figure 5. Light micrographs of sections in the liver of normal group.

B. Treatment group 2, 10 ppm concentration of Au:

According to figure 7, damage to air sacs, thickening of their walls, vasculature, and bronchial tears in some parts were observed. Moreover, fibrous was seen in some of the spaces between alveoli.

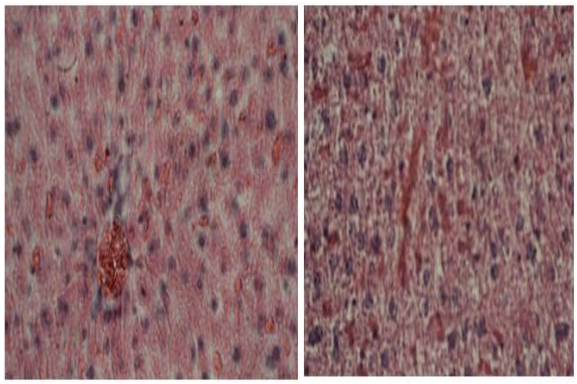

Figure 6. Light micrographs of sections in the lung of nanogold-treated rat received 5 ppm (group 1), everyday for 7 successive days demonstrating of changes histopathology.

C. Treatment group 3, 100 ppm concentration of Au:

The full impact of nanoparticles in the lung tissue is evident in figure 8. Complete air sac destruction, active hyperemia between air sacs, bronchiolar destruction, and disfiguring of the lung from its normal form are among the important pathological changes in these tissues.

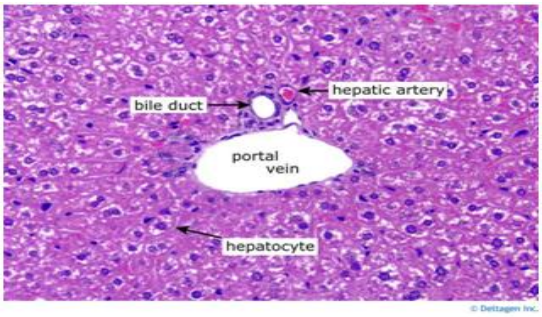

Figure 7. Light micrographs of sections in the lung of nanogold-treated rat received 10 ppm (group 2), everyday for 7 successive days demonstrating of changes histopathology.

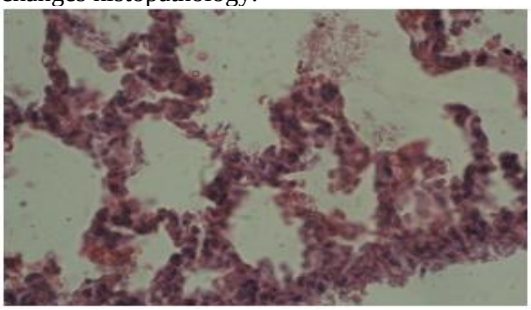

Figure 8. Light micrographs of sections in the lung of nanogold-treated rat received 100ppm (group3), everyday for 7 successive days demonstrating of changes histopathology.

No histopathological alteration was observed in the lungs of control animals (Figure 9).

Discussion

The aim of many studies in previous decades was on the understanding of interaction between different types of nanoparticles and cells, such as: function, size, form, and the chemical level of nanoparticles (12).

While gold (bulk) seems to be 'safe', but using nanogold requires experiments, for compatibility and its great impact on the environment (if these nanoparticles are

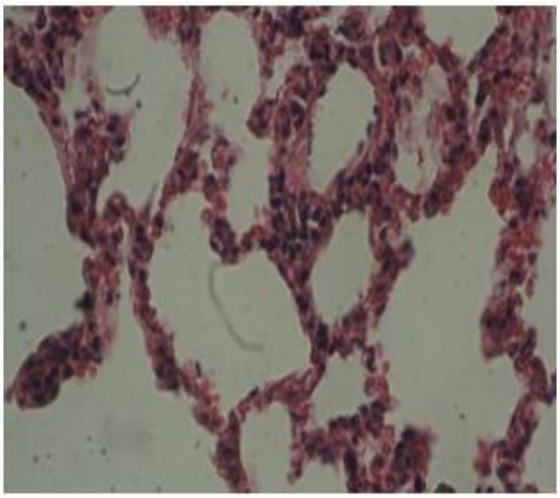

Figure9. Light micrographs of sections in the lung of normal.

in large amounts for in vivo usage) (13,14). GNPs can be considered as extraordinary molecular carriers for the targeting, intracellular trafficking and delivery of a huge array of biomolecules including DNA, RNA, proteins, peptides, drugs, genes and other molecules of therapeutic significance.

A number of researchers have studied the cellular uptake and cytotoxicity of these nanoparticles. The important issue is paying attention to the difference between cytotoxicity and cell damage. The nanoparticles which have less cytotoxicity or do not show any cytotoxicity (with standard tests) may still have the ability to cause severe cell damage. Cytotoxicity is also dependent on the type of used cell. However it is thought that the uptake and toxicity of nanoparticles are controllable and can be manipulated.

The results of this study showed that mean SGOT enzyme two days after intervention in the group receiving concentration of Au 100 ppm was significantly greater than the control group (P = 0.028). Fourteen days after the injection of Au 100 ppm the liver damage returned. Also between the mean SGPT enzymes of different groups no difference was observed (although the amount of this enzyme increased in all groups compared to control group but this increase was not significant).

Liver histological findings in the group receiving concentration of Au 5 ppm: basophils observed around the central veins (which indicate the hepatic damage and the accumulation of cells in this area), in the group receiving concentration of Au 10 ppm: fading and no observation of central veins, liver lobules not being detectable at all, the lobules of the central veins being small and hyperemic, hepatocyte hyper-trophy, Remak bundles, and in the group receiving concentration of Au 100 ppm: , hepatic damage, and Remak bundles, active hyperemia between them, fading of lobular borders, and hyperemia in some of the central veins were observed.

The lung histological results showed: severe vascular hyperemia, compressed air sacs, increase of fibrous tissues, and thickening of the air sacs (treatment group 1), air sacs damage, thickening of their walls, vascu-lature, bronchial tears in some parts, and fibrous in some of the spaces between the alveoli (treatment group 2), complete air sacs destructions, active hyperemia between them, bronchiolar destruction, and disfiguring of the lung from its normal form (treatment group 3).

There are various reports regarding the normal toxicity of these nanoparticles that depend on different modifications of these nanoparticles, surface functional attachment, form, size, and the diameter of these particles (15, 16).

Terentyukl *et al.* studied the functionalized distribution of GNPs in rat organs and tissues in vivo situation. Within 24 hours after intravenous injection they observed that the maximum concentration of gold was in the liver and spleen. This might be due to the fenestrated structure of capillaries in these organs. Although the endothelium of glomeruli in the kidney is fenestrated, the glomerular basal membrane forms a barrier which prevents the accumulation of nanoparticles. They also found that the changes in the internal organs depend on nanoparticle size. For example nanoparticles with 60 nm size have impact on the vein walls and lead to degeneration of vascular endothelial cells. 50 nm nanoparticles also have the same effect on internal organs. In the present study it was observed that in the liver GNPs resulted in severe hepatic degeneration and moderate blood congestion (17).

Ganeshchandra Sonavanea et al. examined the biological distribution of colloidal nanogold after intravenous injection in mice (18). They found that the sediment of nanogold in different tissues depends on the size of nanoparticles. 15 nm GNPs have the most sediment in blood, liver, spleen, and kidney, respectively (18).

Sadauskas et al. showed that the amount of nanogold decreases over time; this indicates the effective clearance of nanoparticles from the body (19). With cytopathology experi-ments on mice, the results showed that nanogold sediments in different organs without significant toxicity. The results of this study showed that nanogold are distributed in the liver in subcellular loca-tions including lysosomes/endosome vesicle, such as macrophage structures and kupffer cells (19).

The results of the study by Aggarwal et al. showed that kupffer cells easily uptake GNPs. This matter indicates that these cells are the kind of major body cells that are able to uptake nanogold. This is an evidence for reducing the load of these nanoparticles over time. In addition, the biological distribution of nanogold in different tissues depends on the interaction of these nanoparticles with plasma proteins (20).

Other studies showed that nanogold sediments quickly and consistently in liver, spleen, kidney, and testicles of the mice; liver is one of the first places for sediment while lungs, spleen, kidney, and blood are next (1). The amount of nanogold in lungs decreases after one week, while the delay in sediment of this nanoparticle is observed in the kidney. The majority of the genes that are affected due to the sediment of nanogold include those genes that are involved in lipid metabolism, detoxification, cell cycle, defen-sive responses, and circadian rhythms. The results from the study by Lanone and Boczkowski showed that nanogold precip-itates with the highest speed and amount in lungs and aorta of rats (21). For 13 nm sized colloidal gold beads, after intraperitoneal injection, the highest amount of gold was observed in the liver and spleen (22).

In another study by Niidom *et al*. it was determined that a few minutes after the intravenous injection of 65 ± 5 mm diameter of nanogold these nanoparticles significantly sedimented in the liver (23). Swelling hepato-cyte may be a result of dysfunction of cellular membrane due to the massive influx of water and sodium (due to the influence of nanogold). This will be accompanied by the release of hydrolytic lysosomal enzymes which will eventually lead to cytoplasmic degeneration and a mass of macromolecules (24-31).

Swollen vacuoles in the rat's hepatocyte cytoplasm, which have been influenced by nanogold, may show acute and subacute injury in the liver. These changes depend on the size of nanoparticles and the amount of exposure time. The smaller GNPs will have greater effect (24-31).

Jittiwat et al. performed a study on the biological distribution of nanogold and the

adjustment of gene expression in the liver and spleen after intravenous injection in rats. They found that microarray results of liver and spleen point to significant effects on gene related detoxification, lipid metabolism, cell cycle, defense response, and circadian rhythm. These results demonstrate that significant biological distribution of Au occurs in the body over 2 months after a single IV injection of AuNPs, accompanied by gene expression changes in target organs (1).

Studies show that various toxins with different mechanisms including: activation of cytochrome p450, activation of alcohol dehydrogenase, lipid peroxidation membrane, protein synthesis inhabitation, disruption of calcium homeostasis, and activation of pre-apoptotic receptor enzymes cause damage to liver cells (32). It seems that the main mechanism of toxicity is through oxidative stress that causes damage to the lipids, carbohydrates, protein, and DNA. Probably the pathological changes inl iver tissue is caused by accumulation and deposition of nanoparticles in this tissue (33). On the other hand the entry of 5-10 nm gold nanoparticles in this study shows their passing through blood and air barrier and damaging the lung tissue. In other words, the nanoparticles after intrapritoneal injection pass through different cell walls and membranes, enter the bloodstream and go through different organs. Among the applic-ations of nanoparticles that help in biological and medical fields is the influence and accumulation of effective nanoparticles in cells and various body organs after its injection.

Conclusion

The results of the present study showed extreme changes in the histopathology of lung and liver tissues caused by spherical GNPs with 5-10 nm size in all the three treatment groups. The pathological changes (lung and liver tissue) in treatment group 3 (Au 100 ppm) was more intense compared to the other groups. Moreover, in high dosage of these nanoparticles (Au 100 ppm) we were faced with increased concentration of liver enzymes (SGOT).

On the other hand, worldwide use of Different GNPs requires more accurate studies on the effects of these nanoparticleswith different concentrations and shapes for further research on the usage of nano-technology.

Acknowledgements

All authors have contributed to the preparation of the manuscript and agree with the submitted manuscript content. The authors wish to thank Izeh and Falavargan University.

References

1. Manikandan J, Ong CN, Yu LE, Ong WY.Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats. Biomaterials. 2010; 31(8):2034-2042.
2. AlkilanyAM, Murphy CJ.Toxity and cellular uptake of gold nanoparticles: What we have learned so far? J Nanopart Res. 2010; 12(7): 2313-2333.
3. Shanei A,Sazgarnia A, TayyebiMeibodi N, Eshghi H, Hassanzadeh-Khayyat M, Esmaily H, et al. Sonodynamic therapy using protoporphyrin IX conjugated to gold nanoparticles: an in vivo study on a colon tumor model. Iran J Basic Med Sci. 2012; 15(2): 759-767.
4. Studer AM, Limbach LK, Van Duc L, Krumeich F, Athanassiou EK, Gerber LC, et al.Nanoparticle cytotoxicity depends on intracellular solubility:comparison of stabilized copper metal and degradable copper oxide nanoparticles. Toxicol Lett. 2010; 197(3):169-174.
5. Gunawan C, Teoh WY, Marquis CP, Amal R.Cytotoxic origin of copper(II) oxide nanoparticles: comparative studies with micron-sized particles,leachate and metal salts. ACS Nano. 2011; 5(9):7214-7225.
6. ChenYSh, Hung YCh, Liau I, Huang, GS. Assessment of the invivo toxicity of gold nanoparticles. Nanoscale Res Lett. 2009; 4(8): 858-864.
7. Cho WS, Cho MJ,Jeong J ET AL.Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles.

ToxicolApplPharmacol. 2009; 236(1):16-24.
8. De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE.Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials. 2008; 29(12):1912-1919.
9. Choi SY, Jeong S, Jang SH, Park J, Park JH, Ock KS, et al. In vitro toxicity of serum protein-adsorbed citrate-reduced gold nanoparticles in human lung adenocarcinoma cells. ToxicolIn Vitro. 2012; 26(2):229-237.
10. Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, Newport GD, et al. Brain microvessel endothelial cells responses to gold nanoparticles: In vitro pro-inflammatory mediators and permeability. Nanotoxicology. 2011; 5(4):479-492.
11. Li JJ, Lo SL, Ng CT, Gurung RL, Hartono D, Hande MP, et al. Genomic instability of gold nanoparticle treated human lung fibroblast cells. Biomaterials. 2011;32(23):5515-5523.
12. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. Small. 2008; 4(1):26-49.
13. Colvin, V. The Potential environmental impact of engineered nanomaterials. Nat Biotechnol. 2003; 21: 1166-1170.
14. Shukla R, Bansal V, Chaudhary M, Basu A, Bhonde RR, Sastry M. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: a microscopic overview. Langmuir. 2005; 21(23): 10644-10654.
15. Takahashi H, Niidome Y, Niidome T, Kaneko K, Kawasak H, Yamada S. Modification of gold nanorods using phosphatidylcholine to reduce cytotoxicity. Langmuir. 2006; 22(1): 2-5.
16. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, et al. Size-dependent cytotoxicity of gold nanoparticles. Small. 2007; 3(11): 1941-1949.
17. TerentyuklGS,Maslyakova GN, Suleymanova LV, KhlebtsovBN, Kogan BY,Akchurin GG, et al. Circulation and distribution of gold nanoparticles and induced alterations of tissue morphology at intravenous particle delivery. J Biophotonics. 2009; 2(5): 292-302.
18. Sonavane G, Tomoda K, Makino K.Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size. Colloids Surf B Biointerfaces. 2008; 66(2): 274-280.
19. Sadauskas E, Danscher G, Stoltenberg M, Vogel U, Larsen A, Wallin H. Protracted
elimination of gold nanoparticles from mouse liver. Nanomedicine. 2009; 5(2): 162-169.
20. Aggarwal P, Hall JB, McLeland CB,Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv Drug Deliv Rev. 2009; 61(6): 428-437.
21. Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. CurrMol Med. 2006; 6(6): 651-663.
22. Hillyer JF, Albrecht RM. Correlative instrumental neutron activation analysis, light microscopy, transmission electron microscopy, and X-ray microanalysis for qualitative and quantitative detection of colloidal gold spheres in biological specimens.MicroscMicroanal. 1999; 4(5): 481-490.
23. Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, Kawano T, et al. PEG-modified gold nanorods with a stealth character for in vivo application. J Control Release. 2006; 114(3): 343-347.
24. Abdelhalim MAK, Jarrar BM. Gold nanoparticles administration induced prominent inflammatory, central vein intima disruption, fatty change and Kupffer cells hyperplasia. Lipids Health Dis.2011; 10: 133.
25. Abdelhalim MAK, Jarrar BM. Gold nanoparticles induced cloudy swelling to hydropic degeneration, cytoplasmic hyaline vacuolation, polymorphism, binucleation, karyopyknosis, karyolysis, karyorrhexis and necrosis in the liver. Lipids Health Dis.2011; 10: 166.
26. Abdelhalim MAK, Jarrar BM. Renal tissue alterations were size-dependent with smaller ones induced more effects and related with time exposure of gold nanoparticles. Lipids Health Dis. 2011; 10: 163.
27. Abdelhalim MAK, Jarrar BM. The appearance of renal cells cytoplasmic degeneration and nuclear destruction might be an indication of GNPs toxicity. Lipids Health Dis.2011; 10: 147.
28. Abdelhalim MAK. Exposure to gold nanoparticles produces cardiac tissue damage that depends on the size and duration of exposure. Lipids Health Dis.2011; 10: 205.

29. Abdelhalim MAK. Exposure to gold nanoparticles produces pneumonia, fibrosis, chronic inflammatory cell infiltrates, congested and dilated blood vessels, and hemosiderin granule and emphysema foci. J Cancer SciTher.2012; 4(3): 046-050.
30. Abdelhalim MAK. Gold nanoparticles administration induces disarray of heart muscle, hemorrhagic, chronic inflammatory cells infiltrated by small lymphocytes, cytoplasmic vacuolization and congested and dilated blood vessels. Lipids Health Dis. 2011; 10: 233.
31. Abdelhalim MAK. Optimizing a novel method for synthesizing gold nanoparticles: biophysical studies. J Cancer SciTher. 2012; 4: 140-143.
32. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol. 2005; 2:8.
33. Damabach DM,Andrews BA,Moulin F. New technologies and screeninig strategies for hepatotoxicity: use of invitro models.ToxicolPathol. 2005; 33(1):17-26.