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Original Research

The effect of gold nanoparticle on renal function in rats

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Abstract

Objective(s): This study aimed to address the gold nanoparticle(GNP)-dose and exposure duration effect on the kidney function of rats: *in vivo.*
Materials and Methods: A total of 32 healthy male Wistar rats were used in this study. Animals were randomly divided into groups, three GNP-treated groups and control group. Group 1, 2 and 3 received. /5 cc of solution containing 5, 10,100 ppm Au via IP injection for 7 successive days, respectively. The control group was treated with 0.5% normal saline. Several biochemical parameters such as BUN (blood urea nitrogen), creatine and uric acid were evaluated at various time points (7 and 14 days). After 14 days, the tissue of kidney was collected and investigated.
Results: There was no significant difference between the control and the intervention group regarding the amount of creatine-BUN and uric acid. The amount of creatine-BUN and uric acid showed increase in all the groups [except group1 (creatine) and group 2 (uric acid)] in the 7 and 14 days after intervention compared to the control group, but this difference was not significant. Results of histopatological tissue kidney showed: in group 1 and 3, complete destruction of the proximal tubules and distal cortical, in group 2, almost complete destruction of proximal tubules and distal.
Conclusions: The induced histological alterations might be an indication of injured renal tubules due to GNPs toxicity that become unable to deal with the accumulated residues resulting from metabolic and structural disturbances caused by these NPs.

Keywords: Gold nanoparticle (GNP), Rats, Renal function, Urea

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Introduction

Gold nanoparticles are great scientific achievements of nanotechnology and have many biological applications. They can be used as carriers for drug delivery for gene therapy (1). There are different reports on the nature of toxicity of these nanoparticles that depends on the modification changes such as the level of absorption performance, form, and the diameter size of spherical shape (2, 3).Abdlhalim et al. in 2011 a,b and 2012 a,b, and Abdelhalim and Jarrar in 2011,a, b, c, and d reported that biological accumulation of gold nanoparticles in different tissues of rats (liver, lung, heart, and kidney) depend on particle size and the duration of treatment. After the oral, inhalation or skin absorption of gold nanoparticles the distribution of these nanoparticles in the body are done via circulatory system. It is shown that the distribution of gold nanoparticles depend on the size and the amount of treatment. The smallest particles have the widest distribution in tissues (after 3 days of injection) (4-11). It was observed that nanoparticles with 10 or 20 nm can cause occasional glomerular congestion in rats that were treated for 7 days. While this process for nanoparticles with 50 nm was not observed, neither was mesangial proliferation or glomerular membrane thickening observed. But the renal cells in the glomeruli were torn, which reflects the impact of nanoparticles on cell adhesion and bonding interactions (11). Inumaro et al. stated that oxidative stress is an important factor in cell rupture. Also cell necrosis in proximal tubule was observed (12). The appearance change of these cells shows the interaction of gold nanoparticles with enzymes and proteins in renal tissue. This content expresses the antioxidant defensive mechanism, that leads to produce and accumulation of active oxygen which also causes cell swelling, and mitochondrial damage that causes necrosis and apoptosis (13). There are few studies on the effect of treatment duration and size of gold nanoparticles on rat's renal function. The aim of the present study was to investigate 7 days treatment with gold nanoparticles with spherical shape and size of 5-10 nm on the tissue and renal function of male rats.

Materials and Methods

Characterization of Au particles

100 ml of colloidal gold nanoparticles from Tehran Notrino that are manufactured in Spain were bought with the following specifications: 5-10 nm diameter, spherical shape, 99.9% purity, mineral in nature, moist synthesis method (alteration) solid after synthesis and wet and soluble during work, pale pink with 100 ppm concentration. Electron microscope image TEM of the nanoparticles is shown below.

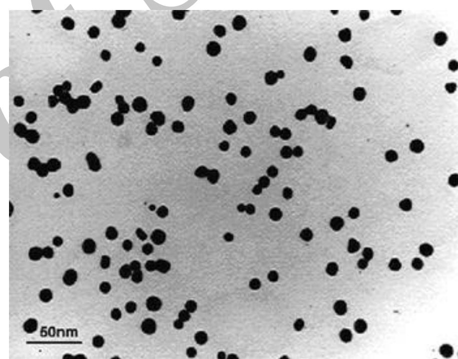

Figure 1. TEM image of gold nanoparticle with 5-10 nm diameters, spherical shape, 99.9% purity, mineral in nature, moist synthesis method (alteration) solid after synthesis and wet and soluble during work (right), figure of absorbance of gold nanoparticle (left).

Making dilutions from stock solution

Mother solutions were prepared by the following method for gold nanoparticle:

15 ml gold nanoparticle with concentration of 100 ppm + 30 ml of sterile 2 times distilled water from Merck Germany = gold anoparticle with concentration of 5 ppm.

10 ml gold nanoparticle with concentration of 100 ppm + 10 ml of sterile 2 times distilled water from Merck Germany = gold nanoparticle with concentration of 10 ppm.

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Treatment animals

Wistar rat of 32 male (225± 25 g) were purchased from the Animal Center of Shahrekord University. Animals were housed in stainless steel cages in a ventilated animal room. Room temperature was maintained at 20 ± 2 _C, with relative humidity at 60 ± 10%, and a 12-h light/dark cycle. Distilled water and sterilized food for rat were available ad libitum. They were acclimated to this environment for 7 days prior to dosing. 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.

Animals were randomly divided into groups, three GNP-treated rat groups and one control group (CG). Group 1, 2 and 3 received. /5 cc of solution containing 5, 10,100 ppm Au via IP injection for 7 successive days, respectively. The control group was treated with 0.5% normal saline with same procedure.

Then, several biochemical parameters such as BUN,uric acid and urea were evaluated at various time points (7 and 14 days). Creatine (CR), blood urea nitrogen (BUN) and Uric acid were measured using enzymatic methods according to JAFFE. Urease-GLDH and TOOS, respectively. After 14 days, the tissue of kidney was collected and investigated.

Biochemical analysis of kidney function

Whole blood was centrifuged twice at 3000 rpm for 10 min in order to separate serum. Using a biochemical autoanalyzer (Hitachi Automatic Analyzer 902 , Roche), serum biochemical analysis was carried out. To evaluate the liver function, the levels of BUN (blood urea nitrogen), uric acid and urea were measured.

Histopathological examination

Histological observations were performed according to the standard laboratory procedures. Rats (four rat/treatment group) at the end of day 14 were dissected for histology. A small piece of kidney 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 by using the statistical package for social sciences (SPSS v.19) software and were summarized and expressed as mean (mean±S.E).

General Linear Models and manova method were used to compare the effectiveness of nanoparticles in three different levels of BUN, Cr, and UA factors with the control group.

Results

In the presented tables P1, P2, and P3 represent P-value respectively related to Dunnett test (comparing the relevant factor according to different levels of nanoparticles in comparison with the control group regarding the intervention time).

The last column also show the P-value related to the comparison of mean nanoparticle at the baseline, 7 and 14 days after intervention. If significant paired t-test is used to compare the mean related factor at baseline with 7 and 14 days after intervention, and the related P-value is given as subtitle. As observed there was no significant difference between intervention and control group regarding creatine, BUN and uric acid. Also among the intervention groups there was no significant difference.

In the treatment group Au 100, regarding the amount of BUN, there was a significant difference between the first and seventh day of the intervention (P =0.03).

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Table 1. Comparison level of creatine, BUN and uric acid in groups1, 2 and 3(Au 5, 10,100 ppm) with control group. P1, P2, and P3 represent P-value respectively related to Dunnett test (comparing the relevant factor according to different levels of nanoparticles in comparison with the control group regarding the intervention time). The last column also show the P-value related to the comparison of mean nanoparticle at the baseline, 7 and 14 days after intervention. *significant different with first day.

BUN	**first day**	**after one week**	**after two week**	**p(friedman)**
Au5 ppm	25.4 ± 4.3	30.1± 6.0	28.9± 7.1	**0.197**
Au10 ppm	24.8± 3.6	31.5± 4.5	28.3± 4.2	**0.072**
Au100 ppm	25.6± 2.9	33.4*± 4.4	27.8± 3.2	**0.030**
control	25± 1.2	28± 4.2	25.7± 1.8	**0.114**
p1	**0.991**	**0.712**	**0.372**	_
p2	**0.999**	**0.354**	**0.541**	_
p3	**0.962**	**0.087**	**0.690**	_

UA	**first day**	**after one week**	**after two week**	**p(friedman)**
Au5 ppm	4.36 ± 0.13	4.29 ± 0.16	4.50± 0.26	**0.066**
Au 10 ppm	4.39 ± 0.15	4.48 ± 0.43	4.36 ± 0.12	**0.565**
Au100 ppm	4.34 ± 0.12	4.48 ± 0.37	4.41 ± 0.14	**0.962**
control	4.31 ± 0.11	4.21 ± 0.16	4.40 ± 0.17	**0.422**
p1	**0.774**	**0.928**	**0.181**	_
p2	**0.519**	**0.332**	**0.995**	_
p3	**0.961**	**0.332**	**0.763**	_

Cr	**first day**	**after one**	**after two week**	**p(friedman)**
Au5 ppm	0.79 ± 0.20	0.66 ± 0.19	0.66 ± 0.16	**0.163**
Au 10 ppm	0.93 ± 0.38	0.7 ± 0.16	0.55 ± 0.12	**0.122**
Au100 ppm	0.78 ± 0.21	0.68 ± 0.21	0.59 ± 0.28	**0.051**
control	0.64 ± 0.09	0.68 ± 0.17	0.48 ± 0.10	**0.051**
p1	**0.476**	**0.998**	**0.110**	_
p2	**0.065**	**0.986**	**0.737**	_
p3	**0.543**	**0.996**	**0.547**	_

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Kidney histopathological evaluation

The histological photomicrographs of the kidney sections are shown in Figure 2, 3, and 4, respectively.

Treatment group 1, 5 ppm: complete destruction of the proximal tubules and distal cortical, glomerular network condescending, increased volume of capsules in renal corpuscle, with hypertrophied cells lining the tubules were the most significant pathological changes in this tissue.

Treatment group 2, 10 ppm: almost complete destruction of proximal tubules and distal are observed. But renal corpuscles are almost normal. Active hyperemia in medullary tubules is also clearly seen.

Treatment group 3, 100ppm: complete destruction of the proximal tubules and distal, severe hyperemia in the cortex and medullar implies the impact of nano particles.

Discussion

This study aimed to address the GNP dose and exposure duration effects of GNPs on the kidney function of rats. In this study the amount of creatine-BUN and ureic acid showed increase in all the groups except Au 10 ppm uric acid group and Au 5 ppm creatine group in the 7 and 14 days after

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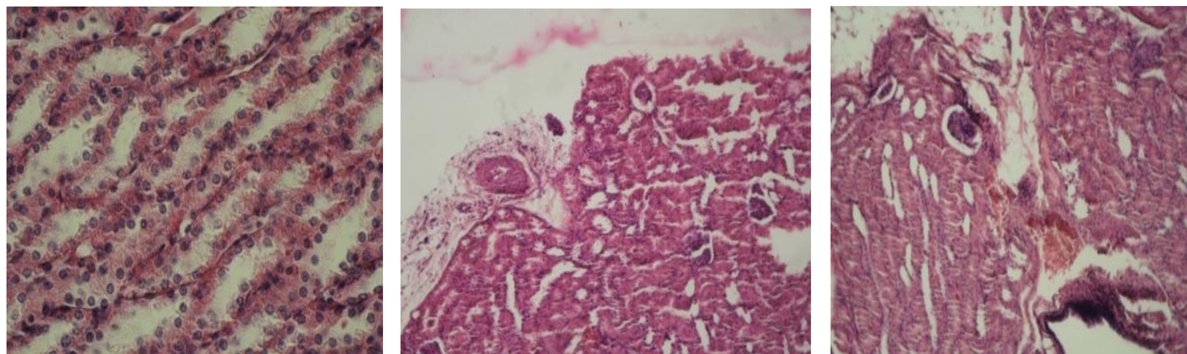

Figure 2. Light micrographs of sections in the kidney of: GNPstreated rat received 5ppm for 7 days.

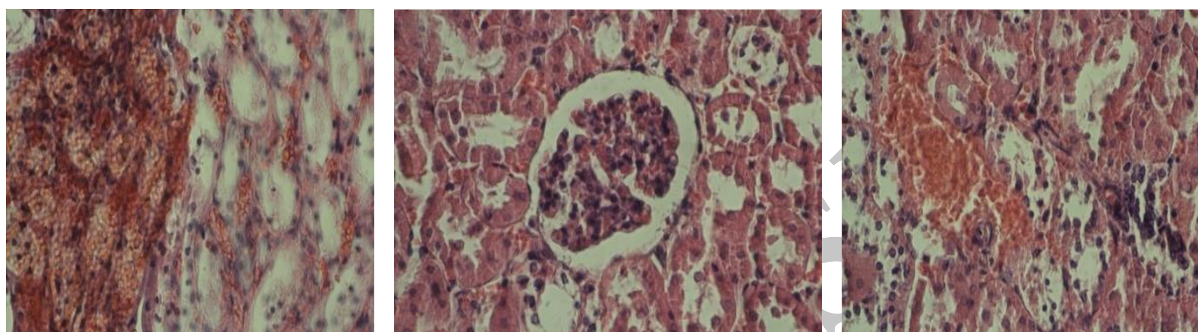

Figure 3. Light micrographs of sections in the kidney of: GNPstreated rat received 10 ppm for 7 days.

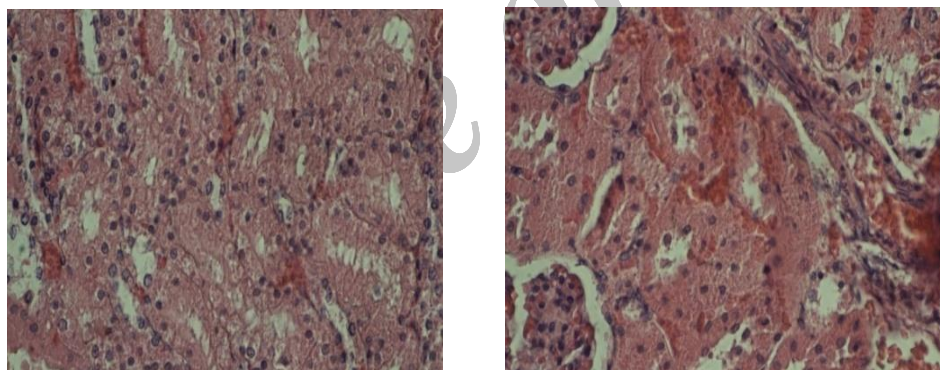

Figure 4. Light micrographs of sections in the kidney of: GNPstreated rat received 100 ppm for 7 days.

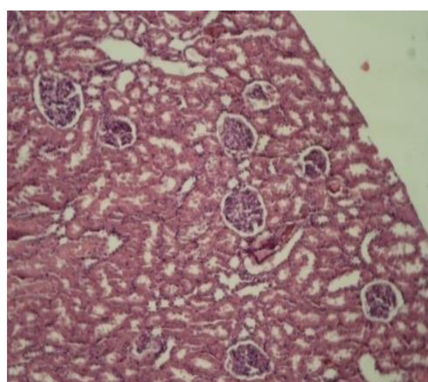

Figure 5. No histopathological alteration was observed in the kidney of control animals.

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intervention compared to the control group, but this difference was not significant. Histopathology results of the kidney indicated complete destruction of the proximal tubules and distal cortical, glomerular network condescending, hyperemia of the cortex and medullar was clearly seen in all the three treatment groups. As we know, the amount of creatine in the blood depends on the speed of renal glomeruli function, which indicates the renal performance. When creatine level is higher than the normal level it seriously disrupts renal function. Normal creatine levels may vary between different tested species, and while in some of them the level of creatine serum is in the normal range, may suffer a significant decline in renal function. Similarly, increases in creatine serum levels can also reveal chronic kidney disease (14). Creatine serum concentration depends on glomerular filtration rate. However, creatine serum is not entirely related to creatine clearance rate. When the glomerular filtration rate is 50% above normal levels, creatine serum amount rapidly increases, and when creatine level is high the renal function is damaged (15).

Regulation of urea by kidneys forms a crucial part of metabolism. In addition to the role that urea has as a carrier of nitrogenous waste, this combination has a part in mutual exchange that takes place in nephrones system. It allows the reabsorption of water and ions that are secretion for excretion in the urine. Uric acid is also excreted by the kidneys. Therefore during kidney failure its amount increases in the blood. One of the important factors of the toxicity of nanoparticles is investigating the uric acid.

Abdelhalim et al. in 2013 studied the effects of size and treatment duration of gold nanoparticles (3 days) on liver and renal function of rat. The creatine and urea level after treatment with 10 nm and 50 nm nanoparticles increased compared to the control group, but it was not significant. Non-significant changes in the creatine and urea levels might be related to the high clearance of gold nanoparticles through kidney or the short period of the treatment (14). These results were similar to the results of the present study (the difference lied in the size of nanoparticles: 5 nm and 100nm and duration of treatment: 7 days).

Lasagna Reeves et al. in 2010 found that after repeated injections of gold nanoparticles, a significant increase in the amount of nanoparticles was observed in the kidneys. In contrast, the concentration percentage of gold nanoparticles reduced (when nanoparticles dosage increased), this shows that the gold nanoparticles have sufficient clearance from the body. The amount of urea, creatine, bilirubin, and ALP in rats' blood serum was measurements of the kidney and liver function. Detailed analysis of these metabolites in the blood serum of treated animals with different dosages of gold nanoparticles in comparison with the control group showed no significant difference (16). These results were consistent with the founding of the present study.Abdelhalim and Jarrar in a, b, c, and d 2011 reported that treatment with different dosage of gold nanoparticles cloudy swelling, vacuolar degeneration, hyaline droplets and casts, karyorrhexis and karyolysis were observed. Mild hyperemia of the glomeruli and non-increased cells nor thickening of basal membrane was seen. These changes primarily in the cortex and complex proximal tubules were more observed than the distal tubules. The renal tubular necrosis, tubular dilation of the capillaries, hemorrhage and cellular inflammation were seen (8-11). The renal histological results were consistent with the present study.Katsnelson et al. studied the effects of gold nanoparticles (50 nm, 10 nm diameter, and 0.5 mg per mL of deionized water) on rats.

They measured the cell dimensions of malpeque capsules. They observed the external diameter, glomerular diameter and an increase in these diameters.

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But according to the negligible changes in diameters, and also because of the proximity measurin g index of the toxicity, such as kidney mass (renal weight based on ml onanimals weight based on g), blood creatine and urea levels for treatment and control groups, minimal toxicity effects were observed (17). Jang et al. also studied the effect of gold nanoparticles injection and their concentration on toxicity factors.

They also investigated indices of different organs.

Results indicated that gold nanoparticles at low concentrations did not show severe toxicity (reviews in terms of blood such as creatine and urea or in terms of weight), but at higher concentrations, significant changes were observed in organ indices (15). The toxicity of gold nanoparticles based on size, shape, surface coating, the effective dosage, and their functions have been studied. In general, in most of the reports major toxicity in the kidneys has not been observed. But it has been indicated that the size of particles play an important role in this regard (18).

Results from Nghiem et al. study also showed that gold nanoparticles without any binding agent did not show any toxicity. But when coating on gold nanoparticles is used, removing the connectors may cause toxicity in the blood and reduce the efficiency of kidney (19).

Balasubramanian et al. conducted studies on distribution of gold nanoparticles in the body. They found that in the initial period of injection, the concentration of gold nanoparticles is insignificant in the kidney and increases over time. This fact has an inverse relationship with the amount of gold in urine (20). Yang et al. stated that 13nm gold nanoparticles after 28 days existed with the amount of 1.5%-9.2% in the kidneys, while the amount is negligible in the urine. One reason that justifies this fact is that gold nanoparticles can gradually be covered with serum proteins that this matter can change shape, size, charge, and even their hydrodynamic diameter. Each protein with negative charge that joins the nanoparticles will be excreted by glomerular membrane. The concentration of gold nanoparticles may make their size inadequate to pass through the filter carrier (21). Terentyuk et al. in 2009 reported the proliferation of aptlyal cells of Bowman's capsule for 15 nm gold nanoparticles (22).

Conclusion

Findings of this study showed that gold nanoparticle after 14 days did not show any relatively significant changes in the statistical measurements of the kidney factors (urea, creatine and uric acid).

But with concentration of 100 ppm regarding the amount of BUN between the first and seventh day of treatment there was a statistically significant difference. Histopathological changes in concentrations of 5 ppm and 10 ppm showed significant changes.

But in the concentration of 100 ppm complete destruction .Therefore it can be concluded that with increasing concentration of gold nanoparticles to the rats they are first absorbed by the kidneys through blood circulation and temporary effect on its performance (changes in the creatine and urea levels). But over time, these nanoparticles are excreted from this organ and the effects are gone. In higher concentrations (100 ppm) it not only affects the BUN level but it also completely destructs the kidney tissue.

So it can be said that application of small quantities of gold nanoparticles at low concentrations in the medical fields does not create serious hazards. But with the increase of concentration and aggregation of nanoparticles, they can cause irreparable damages to the function and tissues of the kidneys.

References

1. Gibson JD, Khanal BP, Zubarev ER. Paclitaxel-functionalized gold nanoparticles. J Am Chem Soc. 2007; 129: 11653–11661.
2. Takahashi H, Niidome Y, Niidome T, Kaneko K, Kawasaki H, Yamada S.

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Modification of gold nanorods using phosphatidylcholineto reduce cytotoxicity. Langmuir. 2006; 22 (1): 2–5.

3. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, Simon U, et al. Size-dependent cytotoxicity of gold nanoparticles. Small. 2007; 3: 1941–1949.
4. Abdelhalim MAK. Exposure to gold nanoparticles produces cardiac tissue damage that depends on the size and duration of exposure. Lipids Health Dis. 2011a; 10: 205.
5. 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. 2011b; 10: 233.
6. 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 Sci Ther. 2012a; 4 (3): 046– 050.
7. Abdelhalim MAK. Optimizing a novel method for synthesizing gold nanoparticles: biophysical studies. J Cancer Sci Ther. 2012; 4: 140-143.
8. Abdelhalim MAK, Jarrar BM. Gold nanoparticles administration induced prominent inflammatory, central vein intima disruption, fatty change and Kupffer cells hyperplasia. Lipids Health Dis. 2011a; 10: 133.
9. 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. 2011b; 10: 166.
10. 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. 2011c; 10: 163.
11. Abdelhalim MAK, Jarrar BM. The appearance of renal cells cytoplasmic degeneration and nuclear destruction might be an indication of GNPs toxicity. Lipids Health Dis. 2011d; 10, 147.
12. Inumaru J, Tanihar H, Umezawa K, Niwa S, Suzuki Y, Nakumura S, et al. Molecular mechanisms regulating dissociation of cell-cell junction of epithelial cells by oxidative stress. Gene Cell. 2009; 14(6):703-716.
13. Pandey G, Srivastava DN, Madhuri S. A standard hepatotoxic model produced by paracetamol in rat. Toxicol Int. 2008; 15(1):69-70.
14. Abdelhalim MAK, Abdelmottaleb Moussa SA. The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: *In vivo*. Saudi J Biol Sci. 2013; 20(2):177-181.
15. Zhang XD, Wu HY, Wu D, Wang YY, Chang JH, Zhai ZB, et al. Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int J Nanomed. 2010; 5:771-81.
16. Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Sadaqopa Ramanujam VM, et al. Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem Biophys Res Commun. 2010; 393: 649–655.
17. Katsnelson BA, Privalova LI, Gurvich VB, Makeyev OH, Shur VY, Beikin YB , et al. Comparative in vivo assessment of some adverse bioeffects of equidimensional gold and silver nanoparticles and the attenuation of nanosilver's effects with a complex of innocuous bioprotectors. Int J Mol Sci. 2013; 14(2): 2449-2483.
18. Mironava T, Hadjiargyrou M, Simon M, Jurukovski V, Rafailovich MH. Gold nanoparticles cellular toxicity and recovery: effect of size, concentration and exposure time. Nanotoxicology. 2010; 4(1):120-137.
19. Nghiem THL, Nguyen TT, Fort E, Nguyen TP, Nhung Hoang TM, Nguyen TQ. Capping and *in vivo* toxicity studies of gold nanoparticles. Adv Nat Sci Nanosci Nanotechnol. 2012; 3 015002.
20. Balasubramanian SK, Jittiwat J, 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.
21. Yang RS, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC, et al. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. Environ Health Perspect. 2007; 115(9): 1339-1343.
22. Terentyuk G, Maslyyakova G, Suleymanova L, Kogan B, Khlebtsov B, Akchurin G, et al. Tracking gold nanoparticles in the body. J Biomedical Optics. 2009; 14: 19-16.

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