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Original Research

Effects of intraperitoneal injection of gold nanoparticles in male mice

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Abstract

Objective(s): There is a rising use of gold nanoparticles (AuNPs) in goods and in the medical fields but there is concern about the toxicity of them. So in this study spherical AuNPs with 3 different concentrations were applied for investigating their effects *in vivo*.
***Materials and Methods*:** 40 male albino mice were randomly divided into sham, control, 25 ppm, 50 ppm, 100 ppm groups and were treated by intraperitoneal injection for period of 14 days. Blood was taken for measuring of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (SGOT and SGPT) enzyme levels and Complete Blood Count (CBC).
***Results*:** After the treatment and comparing groups with sham group, in 50 ppm group significant increases on RBC, HCT, HGB, MCHC and in 25 ppm group significant increase on MCHC and significant decrease on MCV and in 100 ppm group significant increase on MCHC were observed. Also in 50 ppm group an increase on SGOT enzyme level was observed. However, it was nonsignificant.
***Conclusion*:** By observing the abnormality on the RBC count and SGOT enzyme level in the 50 ppm group, we concluded a slight toxicity effect for AuNPs and the threat potential of their use in human.

Keywords: CBC, Gold nanoparticles, Male mice, SGOT, SGPT

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Introduction

As long as materials are at nanometer scale, their properties can be altered (1). Varied shapes of nanoparticles are including tube, dot, wire, cubic, fiber, and hollow capsule (2-4). We are currently witnessing rapid growth in the number of applications of engineered nanoparticles. Addition of useful effects of nanoparticles, they are as a potential hazard to human health. Several studies suggest some of the toxic effects of nanoparticles (5, 6).Among the prepared metal nanoparticles, gold is important. Gold nanoparticles have unique properties including their easy synthesis, stability, ability to selective combination with molecules such as peptides or proteins (7). AuNPs are used as carriers for drugs, gene delivery, sensors, target tumors, hyper thermal therapy and also in a wide range of cosmetic. The latest use of nanogold is gold salts that are used for medical and biological applications and in cancer treatment. Variable effects of AuNPs on liver and blood cells have been reported that is related to their shape, size, and surface coating, exposure dose, and administration methods. To detecting the safety of them for human use, toxicological examination in animals for choosing the safest condition and predicting for human should be done. Liver is the first target for absorbed materials from gastrointestinal tract before becoming systemic (8). One of the most important research areas is investigating the effect of gold nanoparticles on two important enzymes: SGOT and SGPT. The importance of these two enzymes is that they enable to do the relationship between lipids, amino acids and carbohydrates metabolism. SGOT is widespread in animal tissues such as skeletal muscle, heart, kidneys and particularly liver (9). SGPT is found in skeletal muscle, heart, kidney and particularly liver (10). So SGOT and SGPT enzymes are sensitive markers to different types of liver disease. Mostly when the liver becomes damaged, with increasing membrane permeability of the liver cells, these enzymes are released into the blood mainly (11).The next important step to determining the toxicity of gold nanoparticles is assessment of standard hematologic parameters, i.e. platelet count (PLT), hematocrit (HCT), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC),red blood cell count (RBC), red cell distribution width (RDW), and white blood cell count (WBC) (12). In some conditions translating adverse effects from animal to human is difficult but investigating of blood parameters in animals has a much predictive value for human toxicity (13). Therefore, the main objective of this study was to characterize the alterations of liver enzyme levels and blood cells count affected by AuNPs that have not yet been identified exactly to understand the abnormalities in relation with shape, diagonal, dose, time of exposure and administration methods of gold nanoparticles.

Materials and Methods

Synthesis of AuNPs

For producing colloid solution of AuNPs in this study, $Aucl_4$ was used as precursor and then was reduced by citrate. AuNps colloid solution with diameter of 10 nanometer and concentration 100 ppm were made. After that, require doses were prepared (25 & 50 & 100 ppm) by diluting the stock colloid with using deionized water.

Animals

40 male albino mice that had 5 weeks old and middle weight of 22.86 g were obtained from the Laboratory Animal Center (Ahvaz University, Iran). The mice were maintained on standard laboratory rodent diet pellets and comply with National Institutes of Health guide lines for the humane use of laboratory animals.
They had free access to food and water and were maintained on a 12 h dark/light cycle in a room with controlled

temperature (26-27 °C) and humidity (60±10 %).
They were randomly divided to 5 groups; 8 animals in each group (sham group, control group, 25 ppm group, 50 ppm group, 100 ppm group). After dividing of them, they were weighed.

AuNPs treatment
The animals in sham group were fed just with normal food and water without any injection and control group was intraperitonealy injected with 0.3 ml of distilled water and other 3 experimental groups were intraperitonealy injected with 0.3 ml of 25 & 50 & 100 ppm of AuNps daily for period of 14 days.

Sample collection and biochemical assay
3 days after the last treatment, all animals were weighed and after that they were under fasting conditions for 12 hours and finally on the eighteenth day, they were anesthetized by intraperitoneal injection with ketamine and blood was taken for hematology and biochemistry tests.
CBC was analyzed by Sysmex K1000 and SGOT & SGPT enzymes were measured by Automatic Analyzer Hitachi 902.

Statistical analysis
All data was stored in SPSS for operating system of Microsoft .Co (version 19). Group comparisons were done using the analysis of variance test (ANOVA).
Significant differences between them were assessed by Dunnett and Student's t-test. All data was expressed as mean ± SEM. P-values less than 0.05 were considered to be significant.

Results
Control of AuNPs
The morphology and size of synthesized gold nanoparticles colloid solution were analyzed by transmission electron microscopy (TEM) and confirmed spherical shape and diameter of 10 nm (Figure 1).

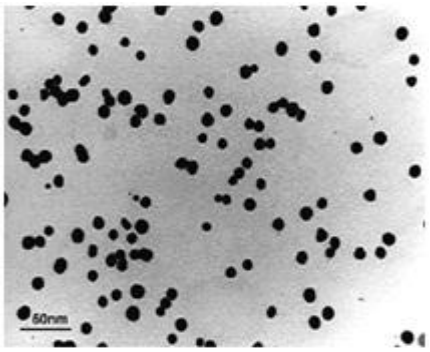

Figure1. TEM image evaluation of gold nanoparticles shows spherical 10 nm particles.

Animals' body weight and appearance
The general symptoms and weight alterations of male mice before and after the treatment of AuNps were ordinary and had non-significant differences. Furthermore, no mortality was observed during the experiment (Table1).

Table1. Comparing body weights of mice before and after treatment with AuNPs. No significant differences were observed between them.

Groups	Body weights	
	Before	After
Sham	21.0±0.4	21.8±2.1
Control	23.8±0.2	24.8±0.7
25ppm	23.6±1.4	24.6±1.7
50ppm	23.8±1.3	24.0±1.2
100ppm	22.1±0.8	25.8±1.2
Significant difference	P>0.05	

Enzymatic levels and hematologic parameters
After injection of AuNps, levels of SGOT and SGPT enzymes in experimental groups were compared versus sham group.

Table2. SGOT & SGPT levels in treated mice with AuNPs. No significant alterations were observed in treated groups in comparison with sham group.

Group	SGOT(IU/L)	SGPT(IU/L)
Sham	1965.0±700.0	250.0±100.0
Control	1076.0±132.2	118.0±12.2
25ppm	1238.2±167.1	158.2±20.1
50ppm	2151.5±591.2	158.4±42.0
100ppm	1467.7±258.3	202.9±55.3
Significant difference	P>0.05	P>0.05

Table3. Results of hematology in treated mice with AuNPs. Some significant alterations were observed in treated groups in comparison with sham group. (Stars indicate significant differences from sham group.

Group	WBC ($N\times10^3$/µL)	PLT ($N\times10^3$/µL)	RBC ($N\times10^6$/µL)	HCT (%)	HGB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW-CV (%)
Sham	5.3±1.8	1132.2±88.9	6.0±0.9	28.1±3.9	8.2±1.3	46.7±1.7	13.5±0.2	29.1±1.1	25.1±2.9
Control	3.9±0.8	810.2±94.5	7.9±0.1	32.7±0.7	11.0±0.3	41.4±1.6	14.0±0.5	32.0±0.6	20.1±0.9
25ppm	6.7±1.1	897.2±158.3	6.9±0.4	28.1±1.8	9.3±0.7	40.6±0.6**	13.5±0.2	33.1±0.6 **	23.9±1.4
50ppm	3.9±0.7	893.8±115.2	8.3±0.2**	35.5±1.1*	12.1±0.5**	43.0±0.8	14.6±0.3	34.0±0.6 ***	20.4±0.7
100ppm	6.3±1.1	832.5±127.9	7.3±0.2	32.9±1.2	10.5±0.4	44.2±1.1	14.3±0.3	32.5±0.6 **	22.5±1.7

*p<0.05, **p<0.01, ***p<0.001)

The statistical analysis did not show any significant increase or decrease ($p>0.05$). However, it is noticeable that in spite of decreases of these two enzymes in all groups, there is only an increase on SGOT enzyme level in 50 ppm group (Table 2).

The statistical analyses after injection of AuNps and comparing hematology results in experimental groups versus sham group showed significant increase on HCT, RBC, HGB and MCHC in 50 ppm group and significant increase on MCHC in 25 & 100 ppm groups and significant decrease on MCV in 25 ppm group ($p<0.05$ or $p<0.01$ or $p<0.001$).

Other hematologic factor alterations such as WBC, PLT and RDW did not show any significant differences (Table3).

Discussion

During the period of this study with mentioned conditions, treatment of mice with gold nanoparticles for 14 days did not cause obvious adverse effects on growth and body weight.

Reductions and increases in body weight are valuable indicators in assessing the toxicity of an extract

The low decrease in weight after 4 days of treatment in all of the treated groups can suggest adverse effects of toxic substances on the animals (15).

Furthermore, no abnormal clinical signs were detected in treated groups and thus AuNPs could not induce any obvious or acute toxicity in mice in the condition of this study. AuNPs immediately enter into the liver via gastrointestinal tract to purify (16) but endothelial cells lining the blood vessels are a physical barrier to pass particles, but in some specific organs such as the liver, the punching endothelium has hole sizes greater than 100 nm and therefore used spherical gold nanoparticles with a diameter of 10 nm in the present study have had allowance for easy passage into the liver. SGOT and SGPT are two liver key enzymes (17-19) and increasing their levels in blood shows the damage of the liver (20-22).

In this study after injection of gold nanoparticles in animals at 3 doses, there is an increase of SGOT enzyme level in 50 ppm group that it shows detrimental effect of gold nanoparticles on hepatocytes at this dose and excessive secretion of SGOT to blood flow.

However, unlike the SGPT enzyme that is found mainly in the liver, SGOT enzyme is also present in other tissues (23-24) so definitely increasing of SGOT cannot be the reason of hepatocytes damage only.

Rezaei also studied liver enzyme changes in rats during 14 days by oral administration of titanium dioxide nanoparticles at 50 & 100 & 150 mg / kg doses and found increasing SGOT and SGPT at the highest dose and concluded the toxicity of titanium dioxide nanoparticles in this dose (25).

In this study, no significant differences about number of WBC in male mice suggests that a 14-days intraperitoneal inject of gold nanoparticles with diameter 10 nm at listed doses are not caused infection or inflammation in male mice because generally an increase on WBC count is a inflammatory normal physiological response to foreign substances into the blood (26) and decrease on WBC count can be created following the entry of excessive doses a toxic substance to the body and infection (27).

In the present study, daily intraperitoneal injection with 50 ppm of gold nanoparticles resulted in significant increase in the number of RBC.

Red blood cells after some steps influenced the erythropoietin hormone and are created from hematopoietic stem cells in the bone marrow into the blood stream and therefore probably 0.3 ml daily intraperitoneal injection of 10 nm in diameter at 50 ppm dose of gold nanoparticles for 14 days take effect on the erythropoietin hormone secreted by the kidney and hematopoietic system.

By the way, the group injected with 50 ppm of AuNPs, in addition to a significant increase in the number of RBC, also showed significant increases in HGB and HCT and MCHC levels that they mean polycythemia.

By the way in this study, results of MCV and RDW that were statistically with no significant differences (with the exception of 25 ppm group) define that sizes of red cells after the treatment have not changed and they are normocytic.

So it is understood that a 14-day intraperitoneal injection of spherical gold nanoparticles with diameter 10 nm and concentration 50 ppm has caused normocytic hyperemia.

Conclusion

AuNPs are used in the various fields and the toxicity of them is related to their characteristics and administration intraperitoneal injection of spherical gold methods.

So we demonstrated that nanoparticles with diameter 10 nm and concentration 50 ppm can specially taint on liver and red blood cell count and could make hyperemia that can result in blood concentration in male mice.

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