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COMPARISON OF TOXIC EFFECTS METAL OXIDE NANOPARTICLES OF GOLD, COPPER, TITANIUM, AND SILVER IN WISTAR RAT

DOUDI M[1] AND SETORKI M[2*]

1: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

2: Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

***Corresponding Author: doctor.setorgi@gmail.com**

ABSTRACT

Few studies regarding the toxic effects and pathological comparison of metal oxide nanoparticles of gold, copper, titanium, and silver have been performed on laboratory animals. The purpose of this study was to compare the toxic effects of these nanoparticles. Four hundred Wistar rats were randomly divided into13 groups: control (treated with 0.5 mL normal saline) and twelve experimental groups. . Group 2-10 were injected with various doses of nanoparticle TiO_2, nanocu, nanosilver (10, 100, and 300 ppm, group 2-10 respectively) and group 11-13 received a daily dose of solution containing 5, 10,100 ppm nanogold respectively, for 7 successive days .The effects of nanoparticles on serum levels of glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) were evaluated at 2, 7 and 14 days. In this study, liver enzymes in all concentrations of Au groups were larger than nanoparticles of copper, silver, and titanium dioxide. Furthermore, the mean of serum glutamic-pyruvic transaminase was greater in Ag 100 ppm than TiO_2 100 ppm. The results showed that gold nanoparticles (diameter 5–10 nm) compared with nanosilver, titanium and copper (diameter 10–15 nm) and silver nanoparticles compared with nanotitanium showed more toxicity.

Keywords: Titanium Dioxide, Serum Glutamic-Pyruvic Transaminase, Serum Glutamic Oxaloacetic Transaminase, Wistar

INTRODUCTION

An increasing number of studies are dedicated to various aspects of the biological activity of nanoparticles, including their toxicity, which is almost on par with the rapid developments in the field of nanotechnology. There is an inspiring hope that in the future a valid proof can be obtained to assess the health risks, it is not only linked with the production and indiscriminate use of nanoparticles, but the interference of nanoparticles in air pollution, the environment and the workplace in many traditional industries are also relevant. This research has focused on the effects of metal oxide of four nanoparticles, along with other predetermined related features and reliability, and it can serve as a model for the study of general patterns of adverse impact of nanoparticles on organisms **[1, 2]**.

Studies showed that in the conventional nanometer range, the relationship between particle size and toxicity are complex and unique, which may be due to differences in toxicokinetics, which is maintained by the physiological mechanisms responsible for the elimination and control of nanoparticles with different diameters, their unequal penetration through biological barriers and finally their unequal dissolution is controlled. The purpose of this study was to compare the toxic effects of metal oxide nanoparticles of gold, copper, titanium, and silver.

Gold nanoparticles are a tremendous achievement in science of nanotechnology, which is used in various fields of medicine, various industries such as agriculture, food packaging and to treat certain diseases **[1, 2]**. However, before these nanoparticles are used in actual clinical settings, knowledge about their potential toxicity and side-effects are necessary **[1, 2]**.

Today, the use of silver nanoparticles in different sciences, especially medicine can be seen around the world. For example, it is used in hospitals for external wounds and burns **[3]**. It is estimated that in the health sector and protecting the health, of all the nanomaterials, nanosilver is at the highest level of commercial **[4]**.

Copper nanoparticles, are among the nanoparticles that are industrially produced and are commercially available. Recently, copper nanoparticles are used as additives to oils, polymers, plastics, metallic coatings, etc **[5]**. Copper nanoparticles like other metal nanoparticles enter the environment and the human body in different ways (spillage during shipping, effluent, and handling).

Titanium dioxide, due to its excellent optical performance and electrical properties has a wide range of applications in many areas and, it is often considered to be physiologically ineffective to humans. Nevertheless, titanium dioxide when inhaled can be harmful and cause adverse health effects in rodents **[6]**.

Numerous studies have examined the toxicity of different samples of silver nanoparticles **[7-11]**. Comparing the results of these studies and the similar effects obtained by gold nanoparticles **[12-15]** suggested that gold nanoparticles compared with silver nanoparticles have less toxicity.

Few studies regarding the comparison of toxic and pathological effects of titanium dioxide nanoparticles and copper have been performed on laboratory animals. For example, the results of Jong et al. in 2011 showed that copper nanoparticles have a stronger inflammatory response associated with an increase in total cells and neutrophils, as well as increased activity of lactate dehydrogenase (LDH) compared with iron oxide, titanium dioxide, and silver **[16]**.

METHODS AND MATERIAL

Preparation of Solutions

The mother solution for gold nanoparticle, copper, titanium, and silver was prepared by the following methods.

Gold Nanoparticle

10 cc nanogold + 10 cc two times distilled water of Merck company = concentration of 10 ppm of nanogold.

15 cc nanogold + 30 cc two times distilled water of Merck company = concentration of 5 ppm of nanogold.

Nanoparticles of Copper, Titanium, and Silver

1 cc of nanoparticles + 10 cc distilled water = concentration of 10 ppm.

10 cc of nanoparticles + 100 cc distilled water = concentration of 100 ppm.

30 cc of nanoparticles + 300 cc distilled water = concentration of 300 ppm.

Animals and Treatment and blood biomarker assay

Wistar rat of **104** 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 procedures used in animal experiments were in compliance with the local ethics committee. Animals were randomly divided into thirteen groups: control group (treated with. /5 cc normal saline) and twelve experimental groups. Experimental groups were injected intraperitoneal with various doses of nanoparticle TiO2, nanocu, nanosilver (10, 100, and 300 ppm, group 2-10 respectively) and group 11-13 received a daily dose of solution containing 5, 10,100 ppm nanogold respectively , via IP injection for 7 successive days every day for 7 successive days. The control group was treated with same procedure (Intraperitoneal). Group 2-13 received. /5 cc of solution containing nanocu, Tio2, nanogold and nanosilver for 7 successive days, respectively. The symptom and mortality were observed and recorded carefully every day for 14 days. The effects of all nanoparticles on serum biochemical levels were evaluated at various time points (2, 7 and 14 days).

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.

Biochemical Analysis of Liver Function

Liver function was evaluated based on the serum levels of serum glutamate Oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT).All biochemical assays were performed using a clinical automatic chemistry analyzer (Hitachi Automatic Analyzer 902 , Roche).

RESULTS

Compare Gold and Copper Nanoparticles

Serum Glutamic Oxaloacetic Transaminase (SGOT)

In this study, with a randomized controlled trial, the effects of gold nanoparticles at concentrations of 5, 10, and 100 ppm, and copper nanoparticles at concentrations of 10, 100, and 300 ppm on serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) were investigated. Based on MANOVA statistical analysis, Wilk's lambda = 0.375, P-value = 0.002, mean SGOT vary between different groups. According to the Tukey test (Tukey honestly significant difference [HSD]) mean SGOT, 14 days after the intervention in Au 5 ppm group was significantly larger than Cu 300 ppm (P = 0.006) and in Au 10 ppm group it was larger than Cu 300 ppm and Cu 100 ppm groups (P < 0.010). However, based on Dunnett test, Au 10 ppm was significantly different from the control group (P = 0.027). **Figure 1** shows mean and standard deviation of SGOT in different groups and at different times **(Figure 1)**.

SGPT

Figure 2 shows mean and standard deviation of SGPT in different groups and at different times. Based on MANOVA statistical test, with consideration of Roy's test = 0.323, P-value = 0.027, SGPT was different in different groups. According to the Tukey test (Tukey HSD), the mean SGPT, 14 days after intervention in Au 10 ppm group was greater than Cu 100 ppm group (P = 0.014).

Comparison of titanium and silver nanoparticles at concentrations of 10, 100, and 300 ppm

SGPT

Based on MANOVA statistical analysis, Wilks' lambda = 0.506, F (18, 130.5) = 1.97 and P = 0.015, mean SGPT varies between different groups. On the 2nd day after the intervention, based on Bonferroni test, mean SGPT in TiO 300 ppm group was significantly greater than TiO 100 ppm group (P = 0.007). Also in Ag 100 ppm group, it was greater than TiO 100 ppm group (P = 0.018). **Figure 3** shows the mean SGPT in groups and at different times **(Figure 3)**.

SGOT

Based on MANOVA statistical analysis, Roy's test = 0.451, F(6, 48) = 3.60, and P = 0.005, mean SGOT varies between different groups. According to on Bonferroni test, on the second day after intervention, mean SGOT in TiO 300 ppm group was greater than the control group (P = 0.019) **(Figure 4)**.

Comparison of four nanoparticles of titanium, silver, copper, and gold

In this study, the effects of four nanoparticles of titanium, copper, and silver at concentrations of 10, 100, and 300 ppm and gold at concentrations of 5, 10, and 100 were performed on SGOT and SGPT.

SGOT

Based on MANOVA statistical models and SGOT control at baseline, statistics of Wilks' lambda = 0.590, F (12, 254.3) = 4.68 and P < 0.0001 were observed. Based on Bonferroni test, 2 days after intervention, mean SGOT in TiO group was significantly larger than the control group (P = 0.037). And also in the Cu nanoparticles group, it was larger than the control group (P = 0.010). Seven days after the intervention SGOT in Au group was larger than TiO_2 (P = 0.025). Mean SGOT, 14 days after intervention in the Au group was larger than Ag (P < 0.0001), greater than TiO (P = 0.001) and larger than Cu (P < 0.0001). Figure 5 corresponds to mean SGOT in different groups and at different times **(Figure 5)**.

SGPT

Based on MANOVA statistical models and mean SGPT control at baseline, statistics of Wilks' lambda = 0.782, F (12, 254.3) = 2.06 and P = 0.020 was observed. Based on Bonferroni test, 14 days after intervention, mean SGPT in Au group was larger than Ag group (P = 0.001) and greater than TiO_2 (P = 0.003) and greater than Cu group (P = 0.010).

Figure 6 corresponds to mean SGPT at different times in different groups **(Figure 6)**.

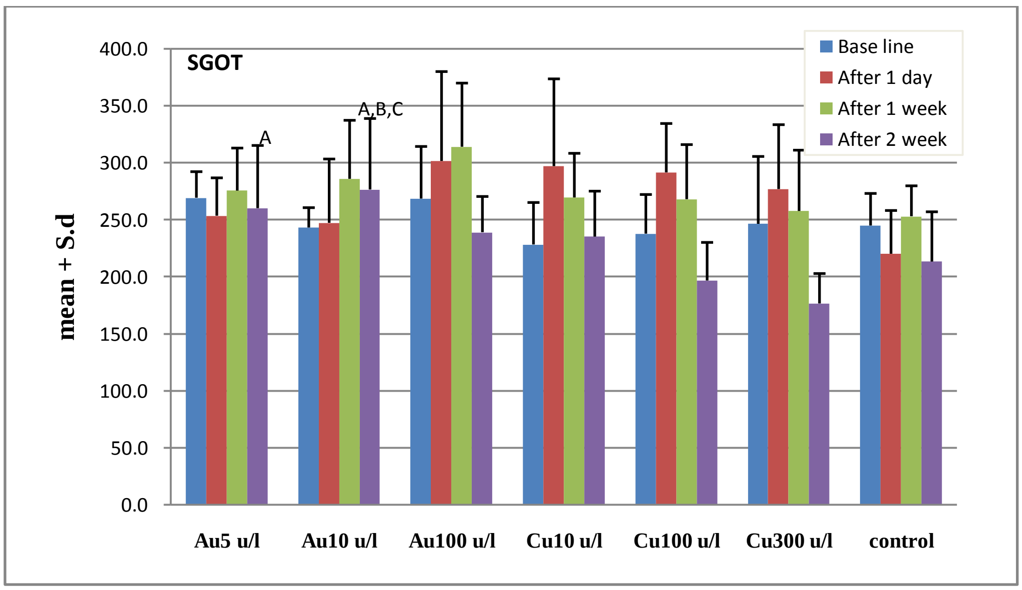

Figure 1: Comparison of Mean SGOT in Two Nanoparticles (Au and Cu).A: Difference with Cu 300 ppm, B: Difference With Cu 100 ppm, C: Differences With the Control Group

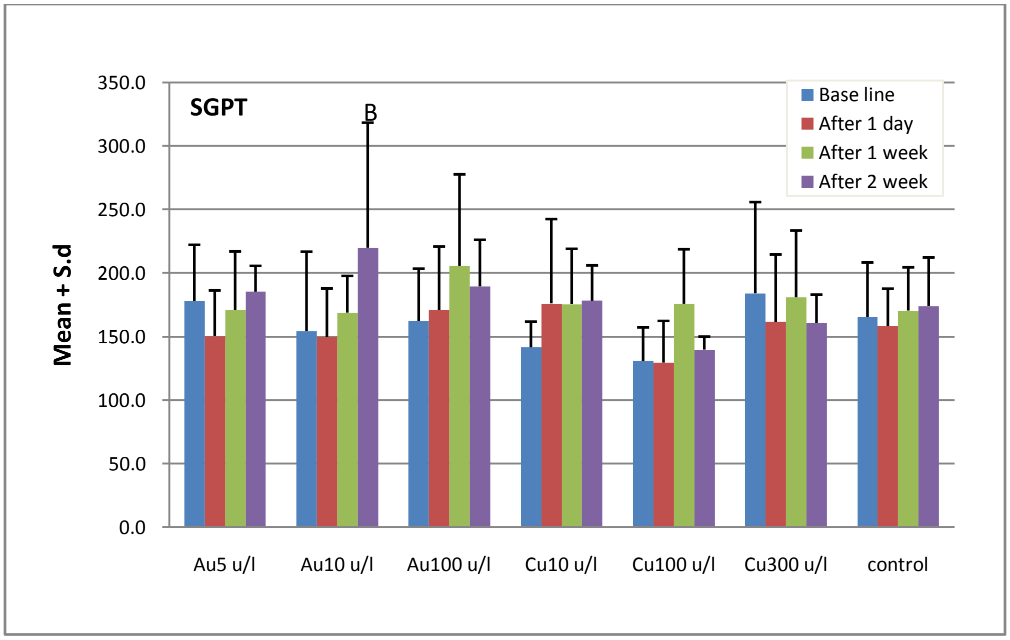

Figure 2: Comparison of Mean SGPT in Two Nanoparticles (Au and Cu). B: Difference with Cu 100 ppm

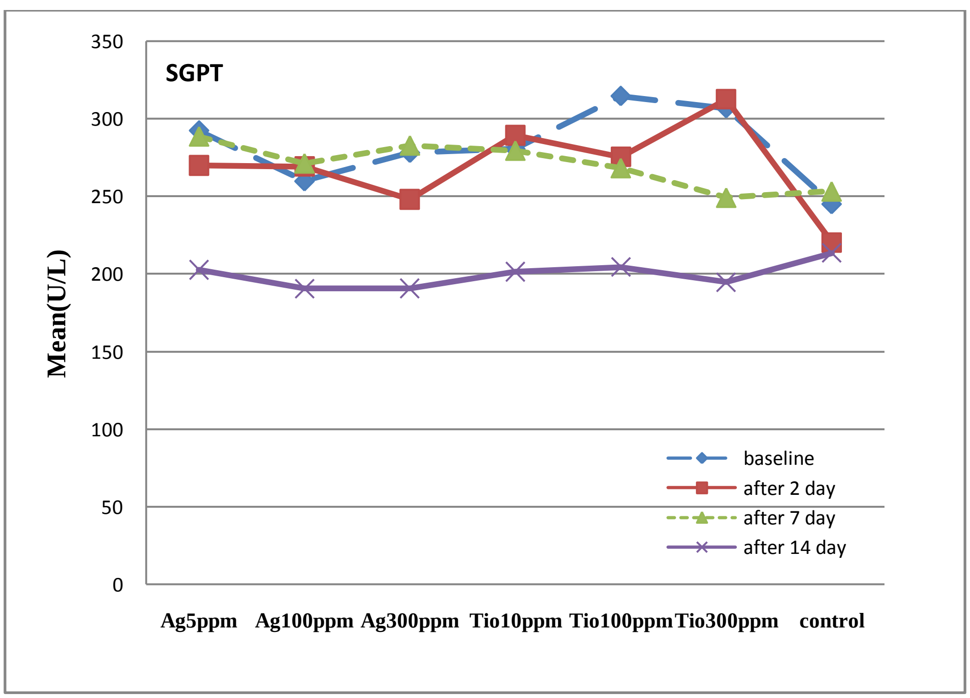

Figure 3:Comparison of Mean SGPT in Tio_2, Ag Nanoparticles Groups and Control Group

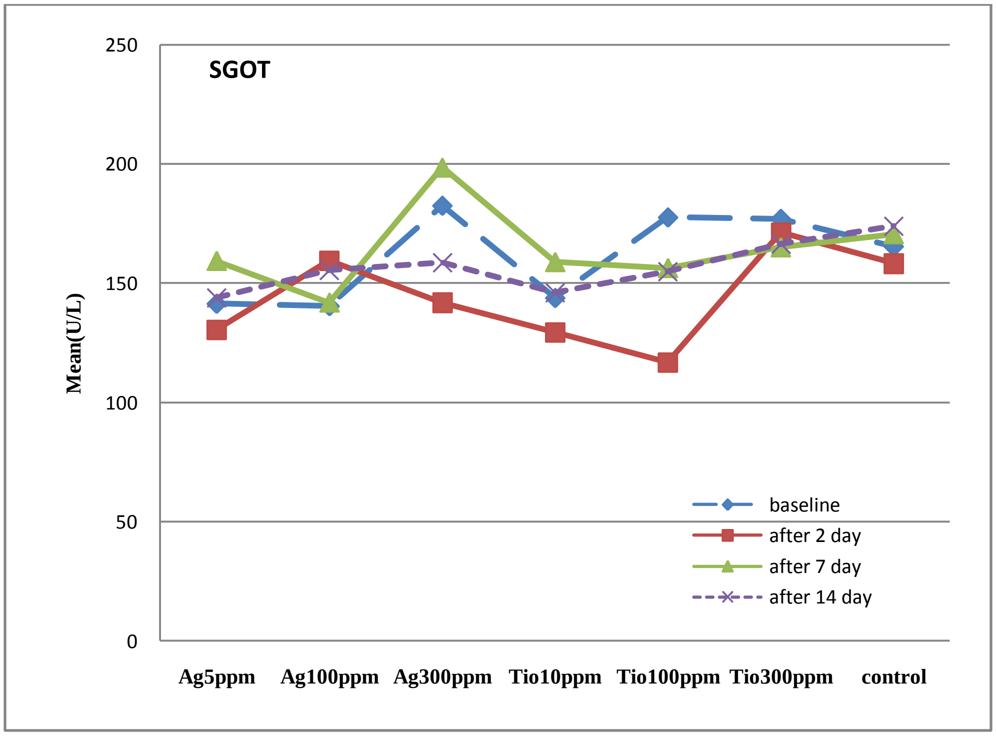

Figure 4: Comparison of Mean SGOT in Tio$_2$, Ag Nanoparticles Groups and Control Group

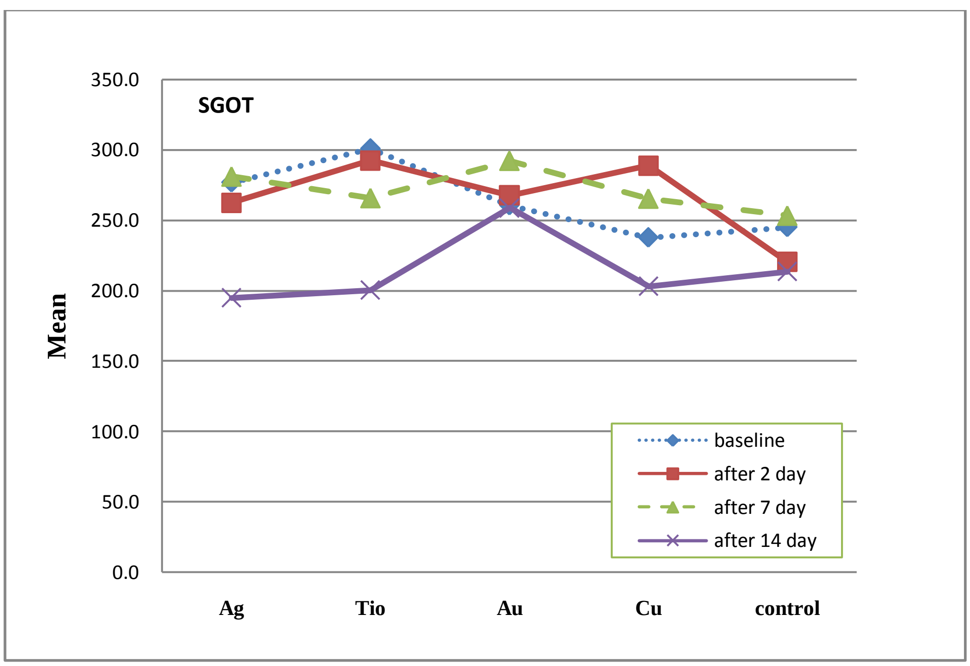

Figure 5: Comparison of Mean SGOT in Four Nanoparticles of Tio$_2$, Ag, Cu and Au

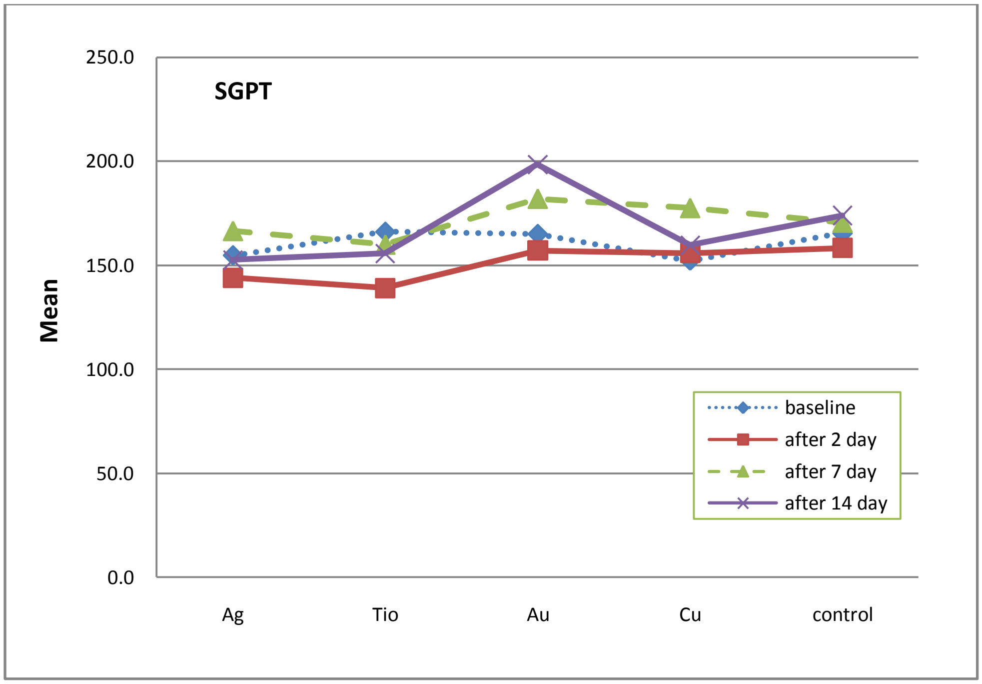

Figure 6: Comparison of mean SGPT in four nanoparticles of Tio$_2$, Ag, Cu and Au

DISCUSSION

The results showed that gold nanoparticles (diameter 5–10 nm) compared with nanosilver, titanium and copper (diameter 10–15 nm) and silver nanoparticle compared with nanotitanium had more toxicity.

Susan *et al*., showed that with changes in the diameter of nanoparticles and concentration distribution in body tissues they have different effects. As smaller the diameter of nanoparticles, their penetration into the cells will increase, and also their harmful effects **[17]**. Specific characteristics of nanoparticles, such as their small size, shape, surface area, and specific structure, are reasons for industrial and medical applications of nanoparticles **[18, 19]**. However, in recent years, evidence of the adverse impact of nanoparticles have been reported, including increased mortality, cardiovascular and respiratory diseases, asthma, and malignant diseases **[20]**. Recently, experimental evidence obtained, confirming that different nanoparticles, such as titanium dioxide nanoparticles **[21, 22]**, zinc oxide **[23]**, silicon dioxide **[24, 25]**, black and white carbon **[26]**, etc., are capable of producing destructive effects on DNA. However, it is worth mentioning that all authors have not received the same positive results for nanomaterial especially, when they are tested in vivo and not on two cells (e.g., on a crystalline form of titanium dioxide anatase) **[27]**. It seems that the main mechanism of toxicity of nanoparticles is through oxidative stress, which damages lipids, carbohydrates, proteins, and DNA **[28]**. Oxidative stress is a normal cellular process that exists in many aspects of cellular signal information. Additional oxidative stress can be harmful, this is why many studies have shown that the presence of nanoparticles increases oxidative stress, and to cope with the increased oxidative stress in cells, protective or destructive responses are formed **[29]**. Oxidation through lipid is very dangerous, because it leads to changes in the cell membrane properties that can disrupt the functioning of biological cells. When the particle size decreases, surface area increases and there is a possibility of creating atoms or molecules on the surface with a higher proportion. It is an important feature of matter in toxicology and causes penetration to the majority of cells and tissues.

As we know, damage to liver enzymes can cause increase of enzymes in these organs due to the release of substance in the cell to the blood plasma. Enzymes such as oxaloacetate transaminase (AST), pyruvate transaminase (ALT), and alkaline phosphatase are important enzymes in the liver cells, and are among the non-functional enzymes in plasma [30]. Probably, the toxic effects of nanoparticles in this study, leads to increased production of free radicals and the beginning of reactive oxygen species (ROS) reactions, that causes damage to liver hepatocytes, and increases ALT and AST due to tissue destruction and releasing these enzymes.

Many reports have proven that nanosilver can easily be transported in the body. It is possible that the mechanisms of their toxicity are attributed to silver ions released from nanosilver or nanosilvers that have penetrated through the cell membrane **[6]**. Effects of silver ions are due to a number of biological events such as, sticking to the cell membrane, changes in membrane permeability, production of ROS, and deactivate cellular enzymes **[31]**.

Some studies have shown that silver nanoparticles not only increase the production of ROS considerably, but significant inhibition of genotoxic effects of these nanoparticles in the presence of antioxidants or free radical absorber is also evident. Only in one genotoxicity article, gold and silver nanoparticles are compared in parallel **[32]**. Research on liver cancer cells (HepG2) has been carried out, and showed that at concentrations above 100 mm nanosilver caused more DNA damage than gold nanoparticles.

Boris *et al.*, studies showed little difference, but noticeable, among some nanoparticles in relation to their biological distribution in vivo after intraperitoneal injection: in particular, it appears that gold nanoparticles, have a higher affinity for the liver and spleen, but lower affinity to the kidneys compared with nanosilver. Both differences are probably due to the lower solubility of gold nanoparticles. Overall, a review of the resources and some other sources indicate that gold nanoparticles are not only generally less toxic, but also have lower genotoxicity than nanosilver **[33]**. However, the difference between this study and other studies are due to differences in the diameter of nanosilver and gold nanoparticles.
Titanium dioxide has a high ratio of surface area to weight **[34]**, and has a wide redox activity **[35]**. These features are due to usual reasons in relation to adverse effects or intrinsic toxicity of these nanoparticles to human health and the environment. Due to these characteristics of titanium dioxide, a risk assessment of the potential adverse effects of nanoparticles on human health and environmental pollution are needed. Although it was found that nanoparticles of titanium dioxide or other nanoparticles can induce serious liver toxicity, the molecular mechanisms and pathogenesis, are not yet clear. When titanium dioxide nanoparticles stimulate hepatocyte, they can induce inhibitory proteins such as phosphorylated IkBs and break them down, and then inhibit nuclear factor kappa-B cells activity. Which ultimately leads to the transcription of pro-inflammatory cytokine genes and inflammatory in rat liver **[36]**.

In another study, different sizes of nanoparticles, such as silver Ag (100 and 150 nm), aluminum Al (30 and 103 nm), iron oxide Fe_3O_4 (30 and 47 nm), titanium dioxide TiO_2 (40 nm), in creating cytotoxic responses, due to the different sizes of nanoparticles and chemical composition of the core were evaluated. Results showed that in cells exposed to silver nanoparticles (10–50 µg/ml) LDH leakage significantly increased while the other tested nanoparticles (TiO_2, Al, and Fe_3O_4) only at higher doses (100 and 250 µg/ml) caused LDH leakage **[37]**.

Obviously, it should bear in mind that, barriers through which the nanoparticles penetrate into the bloodstream (via the lungs and peritoneal cavity) are anatomically and functionally different. And these differences can be referred to the toxicokinetics of nanoparticles. However, it is possible to assume that this fact does not cause a major bias in the estimate of the potential size of the particles or the chemical nature for such influence to distant organs.

Chen et al. reported that differences in exposures, such as inhalation and skin contact, and using variety of metal salts can cause different effects of toxicity. Therefore, further research in relation to the exposure methods and long-term effects of low levels of nanomaterials is needed **[38]**.

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