

Effect of Nanosilver Particles on Alanin Amino Transferase (ALT) Activity and White Blood Cells (WBC) Level in Male Wistar Rats, In Vivo Condition

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Article information	Abstract
<p>Article history: Received: 29 Jun 2011 Accepted: 29 Nov 2011 Available online: 13 Feb 2012</p>	<p>Background: Nanosilver is extensively used in medical equipment. Given the importance of white blood cells (WBC) in the immune system and liver cells in the body detoxification, this study has examined the effects of nanosilver particles on the white cell count and ALT liver enzyme in male Wistar rats.</p> <p>Materials and Methods: This experimental study was conducted on 40 adult male Wistar rats, provided from Pasteur Institute of Tehran. Nanosilver particles with concentrations of 50, 100, 200 and 400ppm were intraperitoneally injected to the treatment groups. Then, the mean of ALT enzyme concentration and white blood cell count of rats were compared in case and control groups at day 12 after treatment through ANOVA test.</p> <p>Results: The greatest effect of nanosilver particles on the ALT liver enzyme of rats was at concentration 50ppm after 3 days of injection ($p=0.002$). Also the greatest effect of nanosilver particles on white blood cells of rats was at concentration 400ppm after 12 days of injection ($p=0.0001$).</p> <p>Conclusion: The oxidative stress induced by intraperitoneally injection of nanosilver particles in rats causes severe irritation and immune system and increases the creation of white blood cells and liver damage in rats treated with nanosilver particles.</p>
<p>Keywords: Nanosilver particles White blood cells ALT Hepatocytes Rat</p>	
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Introduction

Pure silver has the highest coefficient of electrical emission and thermal transfer to other metals. Colloidal silver was first used by Lee in 1889 for medical use [1, 2]. In the old times, silver was used in World War I for wound dressing as disinfectant, but penicillin was substituted afterwards. Nowadays, the use of nanosilver particles can be seen around the world in various sciences; especially in medicine. For example, it can be used in hospitals for external wounds and burns [3, 4]. The use of Nano-technology, like other technologies, can have several disadvantages. For example, experts do not have enough information on whether nanosilver particles have destructive effects on tissue and cells of flesh.

The disposal of packaging makes it possible for nanoparticles to have serious consequences for the environment and human health. The particles being emitted in the environment, skin contact of researchers with these nanoparticles, and their various impacts on all organisms of human body, is a subject on which extensive and detailed research should be conducted [5].

On the other hand, various toxins and nanoparticles may change body immune system and blood cells using which may have disadvantages for the body. Considering that most toxins in the body enter the liver for detoxification, this study investigated liver enzyme changes and white blood cells changes after injection of nanosilver particles. The first step in detecting liver damage is to conduct a

simple blood test that indicates the presence of certain liver enzymes. Under normal conditions; these enzymes are within the liver cells, but when liver is damaged, these enzymes enter the blood stream. The most sensitive and most common liver diagnostic enzymes are alanine aminotransferases [6, 7]. This study has examined the physiological effects of nanosilver particles on the changes of this liver enzyme and the amount of white blood cells in rats. Given the importance and novelty of studies on nanobiotechnology and considering the effects of diameter and size of nanoparticles on their properties, this study used the first generation of nanosilver particles as colloidal, spherical and with a mean diameter of 4 nm for injection to the rats [1, 8].

Materials and Methods

This experimental study was performed on 40 adult male Wistar rats. These animals were purchased from Pasteur Institute of Tehran and were kept in the animal house of Islamic Azad University of Falavarjan for one month in order to prepare for the test. The animals were kept in the proper conditions and temperature of laboratory (temperature $22\pm 2^\circ\text{C}$) and enough room light (12 h light and 12 h dark). Laboratory animals with mean weight of $225\pm 25\text{g}$ were divided into five groups of eight.

These groups included control group that received 1ml saline so that the effect of shock of the injection in treated and control groups would be identical. One milliliter of

nanosilver particles with concentration 50ppm was injected to the second group. The third group was injected with 1ml of nanosilver particles with concentration 100 ppm. The fourth group was injected with 1ml of nanosilver particles with concentration 200ppm and the fifth group was injected with 1ml nanosilver particles with concentration 400 ppm. The injections were repeated for 5 consecutive days. The injection was intraperitoneally in all groups. On the days 3, 8 and 12, after treatment blood was taken from rats' corner of eye lids using the capillary tube.

ALT serum concentration was measured by biochemical kits and Spectrophotometry. Then, the mean of ALT serum concentration in rats of treated and control groups were compared. The amount of white blood cells was measured by cell counter. The average concentration of white blood cells was compared in treated and control groups of rats. Moreover, the nanosilver particles with average diameter of 4 nm were purchased from Nanonasab Pars Company. Nanosilver particles were diluted using serial dilution method. In order to compare the average white blood cells in control group with each treated group, *t*-test was used in each concentration of Nanosilver after treatments. The significant difference between samples was considered 1% and 5%. Also, in order to compare the rate of changes of white blood cells in all treated groups after treatment, ANOVA statistical test was used in different concentrations of Nanosilver. Considering that before treatment, the amount of white blood cells in rats did not differ significantly, the design used in this study was completely randomized. Meanwhile, software SPSS-8 was used for the statistical evaluation of data [9, 10].

Results

Figure 1 shows that the average ALT of serum in the control group is equal to 80.25 ± 23 , which increased to 96.33 ± 20 , 3 days after injection of nanosilver particles of 50ppm, the difference was statistically significant ($p=0.002$) (Fig. 1). Among the various concentrations of nanosilver particles, the most appropriate concentration to change liver enzyme level in treated groups was 50 ppm (Fig. 2). The average WBC in the control group and treated group was compared with nanoparticles, 12 days after the treatment (Table 1).

The number of white blood cells of rats in the control group is equal to $16.25 \times 10^3 \pm 1.02 \times 10^3$ that increased to $17.55 \times 10^3 \pm 3.83 \times 10^6$, 12 days after the injection in group of 50 ppm, which was not statistically significant due to the high level of standard deviation ($p=0.24$). In the group of 100 ppm, this number increased to $19.65 \times 10^3 \pm 1.40 \times 10^3$ on the day 12, which was a significant difference ($p=0.042$). In the group of 200 ppm, white blood cells were equal to $21.43 \times 10^3 \pm 1.68 \times 10^3$ on the day 12 after the treatment.

This difference was significant compared to the control group ($p=0.031$). In group of 400ppm, white blood cells were equal to $25.19 \times 10^3 \pm 1.80 \times 10^3$ on the day 12 after the treatment. This difference was significant compared to the control group ($p=0.002$) (Fig. 3). The treatment was on the day 12 after treatment with nanosilver particles at concentrations 50, 100, 200, and 400ppm. Figure 4 shows

that with increase of concentration of nanosilver particles, the level of white cell count will have a significantly nonlinear increase. Pearson's correlation coefficient is $r=0.991$, which indicates a significant direct correlation between the above two factors (Fig. 4). However, the effects of nanosilver particles on the amount of serum ALT do not depend on concentration, and the greatest increasing effect exists in the concentration of 50ppm (Fig. 5).

The number of white blood cells of rats in the control group equals to $16.25 \times 10^3 \pm 1.02 \times 10^3$ that increased to $17.55 \times 10^3 \pm 3.83 \times 10^6$, 12 days after the injection in group of 50 ppm, which was not statistically significant due to the high level of standard deviation (Table 1). In the group of 100 ppm, this number increased to $19.65 \times 10^3 \pm 1.40 \times 10^3$ on the day 12, which was a significant difference ($p<0.05$) (Fig. 2). In the group of 200 ppm, white blood cells were equal to $21.43 \times 10^3 \pm 1.68 \times 10^3$ on the day 12 after the treatment. This difference is significant in comparison with control group ($p<0.01$) (Fig. 3). In group of 400ppm, white blood cells were equal to $25.19 \times 10^3 \pm 1.80 \times 10^3$ on the day 12 after the treatment. This difference was highly significant compared to the control group ($p<0.01$) (Fig. 4). The treatment was on the day 12 after treatment with nanosilver particles at concentrations 50, 100, 200, and 400ppm. Figure 5 shows that with increase of concentration of nanosilver particles, the level of white cell count will have a significantly nonlinear increase. Pearson's correlation coefficient is $r=0.991$, which indicates a significant direct correlation between the above two factors (Fig. 5). Also, mean of serum ALT in the control group is equal to 80.25 ± 23 , which increased to 96.33 ± 20 , 3 days after injection of nanosilver particles of 50ppm. The difference was statistically significant ($p=0.05$). Among the various concentrations of nanosilver particles, the most appropriate concentration to change liver enzyme level in treated groups was 50 ppm.

Table 1. Comparison of WBC in the control group and the group treated with nanosilver particles 12 days post treatment

Concentration of nanosilver	Controls	Treatments
12 day post treatment		
50ppm	$(16.25 \pm 1.02) \times 10^3$	$(17.55 \pm 3.83) \times 10^3$
100ppm	$(16.25 \pm 1.02) \times 10^3$	$(19.65 \pm 1.40) \times 10^3$
200ppm	$(16.25 \pm 1.02) \times 10^3$	$(21.43 \pm 1.68) \times 10^3$
400ppm	$(16.25 \pm 1.02) \times 10^3$	$(25.19 \pm 1.80) \times 10^3$

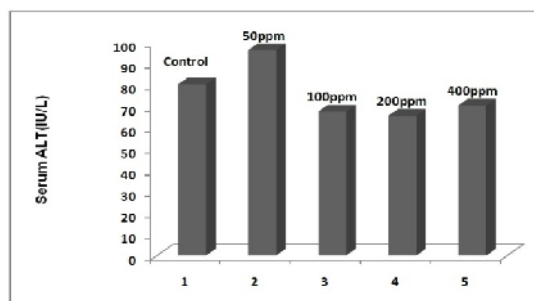


Figure 1. The comparison of ALT serum level average in control and treatment groups 3 day after treatment in different concentration of nanosilver

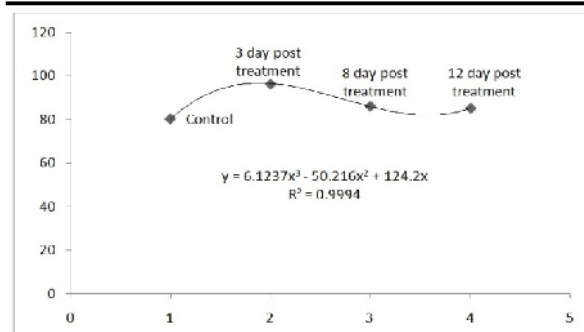


Figure 2. The comparison of ALT serum level average in control and treatment groups in different days after treatment of nanosilver

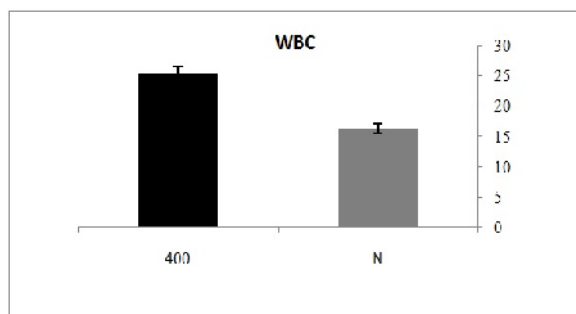


Figure 3. The comparison of WBC average in control and treatment groups in 400ppm concentration of nanosilver

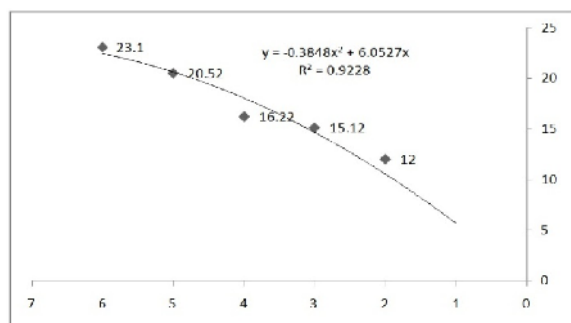


Figure 4. The comparison of WBC average 12 day after treatment in different concentration of nanosilver

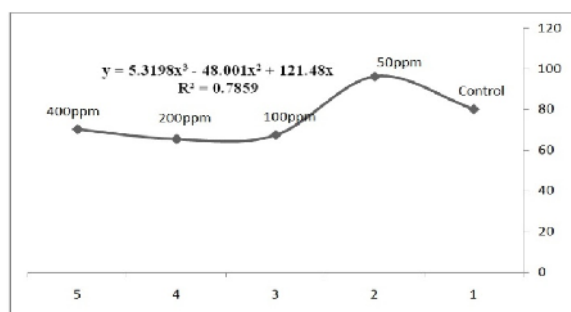


Figure 5. The optimum concentration of nanosilver in change of ALT serum level average in control and treatment groups 3 days after treatment of nanosilver

Discussion

In this study, the greatest damage to liver cells occurred at low concentrations, such as 50ppm, 3 days after injection,

while at higher concentrations such as 100, 200 and 400 ppm, no significant change was made at ALT serum enzyme levels after liver damage. The oxidative stress induced by intraperitoneally injection of nanosilver particles in rats causes severe irritation of white blood cells creation and subsequently, occurrence of a state of excessive increase in white blood cells in the rats treated with nanosilver particles [8, 9]. These effects were not significant at low concentrations such as 50 ppm on the day 12 after the injection, while it was significant at concentrations of 100 and 200 ppm on the day 12 after the injection. At concentrations above 400 ppm, Nanosilver particles caused an apparent increase in the number of white blood cells on the day 12 after the injection. The results of this project show that the effects of nanosilver particles on the amount of white blood cells are dose-dependent.

In the present study, physiological effects of nanosilver particles have been evaluated at different concentrations on serum ALT and WBC count in male Wistar rats. Hepatic damage induced by intraperitoneally injection of nanosilver particles in rats, has possibly caused severe irritation of oxidant system in these cells. In 1989, Machiedo et al showed that free radicals induced by nanoparticles can cause destruction of red blood cells. [9] Susan et al. in 2009 showed that with changes in the diameter of nanoparticles, their distribution in body tissues and their effects will become different [11].

The smaller the diameter of the nanoparticles is, the more its influence to cells and its molecular effects on the intracellular mechanisms will increase. In the present study, nanosilver particles average diameter of 4 nm and its shape is spherical [10, 12]. Due to higher contact surface and more influence on the cell membrane in higher doses, nanosilver particles lead to the influence in white blood cell mitochondria and changes in their enzyme activity. Apoptosis pathways are activated regarding the liver cells in the dose 50ppm. Sriram et al. in 2010 proved that nanosilver particles can activate Caspase mitochondrial enzymes, especially Caspase3 in lymphoid cancerous cells and cause planned death or apoptosis in them [14]. In the present study, considering the diameter and shape of used nanoparticles, anti-apoptotic pathways has been probably strengthened in white blood cells and apoptotic pathways in hepatocytes. In fact, free radicals from the nanosilver particles have attacked hepatocytes and released ALT stored in them and entering into the blood serum; whereas; the immune response of rats to an external factor has been the increase of the number of white blood cells for phagocytosis of nanosilver particles [13].

Considering the importance of white blood cells in defending the body and the important role of hepatocytes in detoxification, any changes made in their structure and number can cause very large physiological changes for human body. On the other hand, wide use of different nanosilver particles in the whole world, especially in our country, requires more accurate and comprehensive studies on the effects of these nanoparticles on blood cells. The use of laboratory rats as animal models, and various treatment methods and nanoparticles with different combinations and

diameters presents new horizons for further research to investigate applications of nanotechnology in physiology [15].

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

No conflict.

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