Journal of Chemical Health Risks

Journal of Chemical Health Risks (2014) 4(3), 7–13

ORIGINAL ARTICLE

Toxicity Properties of Silver Nanoparticles on Lactate Dehydrogenase Activity and Histological Changes of Heart and Embryo Tissues in Pregnant Mice (NMRI)

Bagher Seyedalipour * [1], **Ameneh Arefifar**[2], **Ramezan Khanbabaee** [3], **Masumeh Oshrieh** [2]

[1]Department of Cellular and Molecular Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran

[2]Department of Developmental Biology, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Iran

[3]Department of Cellular and Molecular Biology, Islamic Azad University, Qaemshahr Branch, Qaemshahr,Iran

(Received: 29 April 2014 Accepted: 30 June 2014)

KEYWORDS

Ag NPs

Embryonic development

Lactate dehydrogenase

Histological

ABSTRACT: The rapid advancement of nanotechnology enables us to use nanoscale particles. This material in terms of physical, chemical and biological characteristics are unique compared to larger particles. Increased cell division, apoptosis, oxidative stress has been associated with toxic effects of nanosilver. The aim of this study was to evaluate histipotological changes and enzyme activity in nanoparticle silver trated pregnant NMRI mice. These experimental were performed on 35 NMRI mice used for treatment with Ag Nps. The average weight of the animals was 30 ± 3 g that divided into five groups of seven were injected intraperitoneally. After mentioned treatment, the blood sampling was done of NMRI. The collected tissues were washed with saline and fixed in Boin´s fluid and stained with hematoxylin and eosin for histopathology evaluation. After data collection, statistical analysis was done using SAS software. Histological observations showed that the silver nanoparticles had a major effect on fetal development in each experimental groups compared to the control group. No change of histological characteristics of heart tissues was observed in Ag-nps groups as compared to the control group. Different concentrations of silver nanoparticles increased levels of enzyme lactate dehydrogenase , but no significant differences were observed between control and treated groups ($P<0.05$). Toxicity of silver nanoparticles injected intraperitoneally into the experimental groups were evaluated which had unfavorable effects on embryonic development. So, further investigates are suggested to predict AgNPs toxicity.

INTRODUCTION

Rapid advancing of Nanotechnology enriches us to use particles with nano scales in dimensions of 1to 100 nanometers [1]. Nanoparticles have specific characteristics of physical, chemical and biological as compared to larger particles [2]. These physical and chemical proper-

---

* Corresponding author: b.seyedalipour@umz.ac.ir (B. Seyedalipour).

ties enable them to act as catalysts in biochemical reactions and define their biological activities. Among all different types of nanoparticles, metal nanoparticles including gold and silver are used widespread in different aspects of life such as medicinal and industry due to its specific biological properties [3]. Zinc is essential for growth, development, DNA synthesis and other cellular processes, and on the other hand, despite the widespread exploitation of ZnO nanoparticles, growing and production of nano-ZnO and its useful applications in biological systems. So far few studies on the toxic effect of ZnO nanoparticles on health and the development of generation were done.

Nanosilver, in the form of colloidal silver, has been used for more than 150 years and registered as a biocidal material [4]. One of the applications of silver nanoparticles due to its antimicrobial properties is for wound dressings and dental tools [5]. Importantly, nanosilver is a very effective fungicide [6] as well as having antiviral properties [7]. The results showed that silver nanoparticles are able to pass through the gastrointestinal tract (GIT), dermal, and lung barriers into the blood, and thereby become distributed throughout the body [8]. It is well established that the tissue distribution of nanoparticles is size dependent [9]. Using nanotechnology has different defects such as other technologies. Therefore the safety and the probability of damage should be considered. Silver nanoparticles has been reported as the materials with high toxicity as compared to other materials especially after the systemic uses, the nanoparticles is small enough to pass from smallest capillary vessel of body and biological membranes and be effective on physiology of any cells in body [10]. Researchers showed that the toxicity of these nanoparticles is dose and size dependent. The mice that received different sizes of silver nanoparticles the nanoparticles were cumulated in liver, spleen, lung, heart, testis and brain after injecting of nanoparticles into blood flow [11]. Nanoparticles can damage organs and different tissues through producing free radical and stress oxidation mechanism means attacking free radical to tissues [12]. Nanoparticles can pass from the wall of blood vessels, so it can easily insert to embryo under the development [13]. Lactate dehydrogenase is the enzymes that at high concentration can be found in liver, heart and skeletal muscle and the low concentration is in the pancreatic, kidney, stomach and red blood cells. This enzyme is intracellular enzyme and lead to reversible oxidation of lactate to pyruvate. Pathological increasing of LDH activities is observed in many diseases such as heart, liver, muscle and cancers [14]. Considering the widespread application silver nanoparticles and the value of health and human prolonging generation, necessity of precise histological study is essential in different doses of silver nanoparticles on heart and embryonic tissue.

The aim of this study was to evaluate the amount of change in lactate dehydrogenase enzyme and the effects of silver nanoparticles on embryonic development in pregnant NMRI mice.

MATERIALS AND METHODS

In this experimental study, colloidal silver nanoparticles with an average diameter of 20 nm were used. Initially, the concentration of the nanoparticle colloids were prepared using deionized distilled water. Then, in sterile conditions, a half ml of nanosilver concentrations 50, 100, 200 and 400 mg/kg were injected into each mouse. For this purpose, 35 female NMRI mice weighing approximately 30 ± 3 g and aged approximately 8 weeks from the Pasteur Institute of Iran (Amol) purchased and for two weeks in order to prepare for the examination in room animals with humidity (70 -50), and adequate laboratory temperature (23 ± 2°C) and room enough light (12 h light, 12 h dark) were maintained. In this experiment, 35 female NMRI mice were divided into five groups including control and four experimental groups. Two days after the first injection and observation of vaginal plug after mating was considered zero time of pregnancy. On day 16 of pregnancy, the blood samples were collected and the mice were killed by cervical dislocation and embryos and heart were isolated, and then the measurement of fetal and heart weight were performed. For biochemistry analysis, blood samples were taken from the inner corners of the eyes of mice by using of capillary tube. Serum biochemical analysis was carried out to determine the serum level of total lactate dehydrogenase (Pars Azmoon Lot no, 92006). For measurement of LDH enzymes blood samples were centrifuged at 5000 rpm for 15 min. After separation of serum samples until the enzymatic measurements were frozen and kept at -20^{o}C. Then using of enzymatic kits from Biochemistry CO. (according to the procedure described in the kit purchased from the Pars Azmoon, Co., Iran) and by suggested method of International Federation of Clinical Chemistry (IFCC), and using Cobas Mira Analyzer, enzymatic assays were performed. Embryos and heart were placed in Bouin's solution for fixation. Tissue samples were embedded in paraffin and 5 micron sections were prepared and stained with hematoxylin and eosin (H and E) for histopathology evaluation. Then for observation of cellular damages, tissue samples were evaluated by a histopathologist by invert microscope. After data collection, Generalized linear model (GLM) procedure in SAS software was used for the analysis of data. The model included the treatment and initial weight of each mouse. Least square means of treatments were used for the comparison of the means.

RESULTS AND DISCUSSION

The proliferation of the nanotechnologies with the production of engineered nanoparticles presents a dilemma to regulators regarding hazard identification mostly for human health. Nanoparticles can be found in environment and is widely used in medicinal sciences. Moreover, its effect and application on cancer cure was reported, but it seems that it could negatively effect on non-cancer cells [15]. Nanoparticles may remain in body for long time, so the investigation of its toxicity is necessary [16]. IntraProtaneal injection of silver nanoparticles with concentrations of 50, 100, 200 and 400 mg/kg in pregnant NMRI mice showed that the embryo weight in experimental groups significantly decreased ($P \leq 0.05$) as compared to control group (Figure 1). Microscopic observations showed that silver nanoparticles injected into pregnant NMR mice during pregnancy had created delayed fetal development and organogenesis.

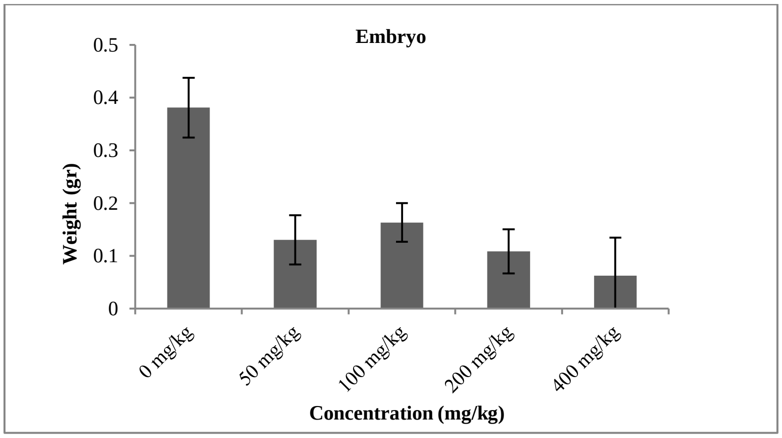

Figure 1. Comparison of 16-day fetal weight in various concentrations of silver nanoparticles with the control group in NMRI mice. Decreased embryo weight at different concentrations is significant compared to the control group. A value $p \leq 0.05$ was considered to be statistically significant and results display as Mean±SD

Macroscopic observation revealed that administration of different doses of silver nanoparticles had profound effects on embryonic development. As shown in Figure 2, embryos organs in control group compared with treated group are naturally grown and spinal are bones and also fetal weight in the experimental groups compared with the control group significantly decreased (Figure 2).

Histology comparison of treatment group with control group in pregnant mice showed that the silver nanoparticles caused that delaying in growth and development of embryo. As shown in Figure 2 in concentration of 50 mg/kg the organs were formed but a little delay was observed. In concentration of 100 mg/kg a delay in growth and malformation in organs could be observed. In 200 mg/kg the organs grows partially and embryo remained in initial stages. In concentration of 400 mg/kg there was not organogenesis and the embryo was in the initial stages that showed pass of silver nanoparticles from the blood-placenta dam and penetrate to developing embryo and effects on embryo cells.

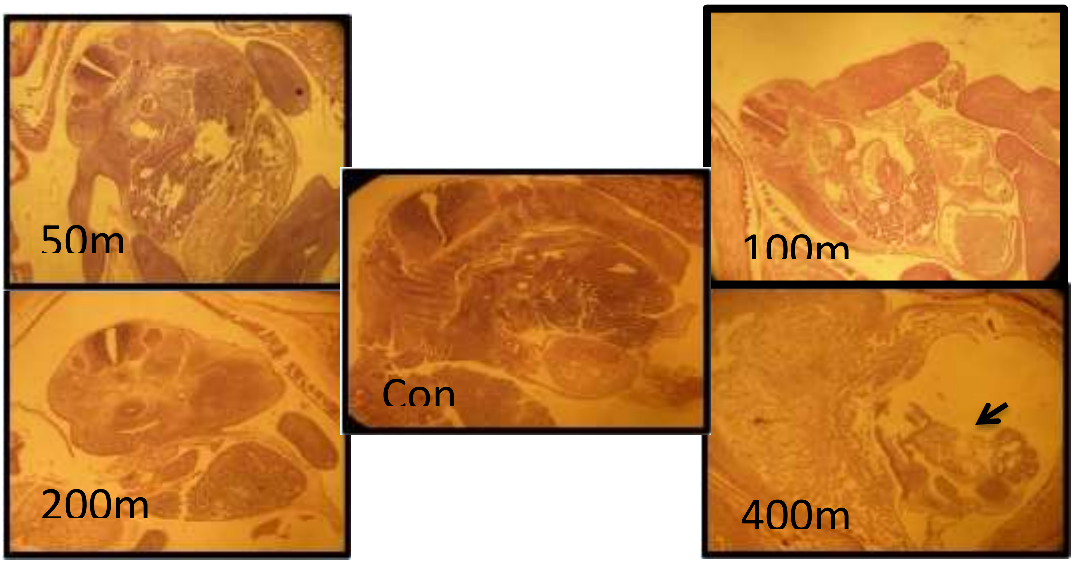

Figure 2. Transverse sections of the embryos pregnant mice (NMRI) after treatment with different doses of AgNPs. (A) Normal embryos of NMRI mice as a control (B) experimental group were treated with concentration 50, 100, 200, 400 mg/kg. The sections were stained with H & E. The magnification was 4×. Comparison of 16-day-old embryos in control group and different concentrations of silver nanoparticles in NMRI mice demonstrate growth retardation, especially in dose 200 and 400 mg/kg.

The results of the effects of silver nanoparticles on zebrafish embryo showed that the silver nanoparticles caused disturbances in organogenesis and growth and development of zebrafish embryo. Moreover the silver nanoparticles affected the regulatory transcription mechanism to form muscle of heart [17]. The iron oxide nanoparticles have toxicity effects on growth and development of Balb/c mice [13]. The toxicity effects of nanoparticles on oval can affect the ovulation process with activating caspase routes and producing oxidative stress [18]. It can be concluded that silver nanoparticles are entering the tissues and organs in the developing embryo cause that oxidative stress through activating caspase routes and affected embryo development.

Histological study of the heart and LDH enzyme activity

The results of this study showed that different concentrations of silver nanoparticles increases the levels of enzyme lactate dehydrogenase, but no significant difference between control and treated groups was observed (Figure 3). As shown in Figure 4, no change of histopathological examination of heart muscle was observed between control and treated groups (Figure 4). In recent study the effects of silver nanoparticles with diameter of 20 nanometers was assessed in pregnant NMRI mice. The heart tissue was stained with Hematoxilin and Eosin to histopathology assessment. There was not any significant difference among doses in histopathology studies of tissue section of mice heart. Beside, different concentrations of silver nanoparticles increase the enzyme level but there was not any significant difference for enzyme activities between the control and treatment groups. This results in not in agreement with the findings of Naghsh [19] concerning toxicity effects of silver nanoparticles on hearth tissue, but is in agreement with the activity level of lactate dehydrogenase.

Cytotoxicity effects of nanoparticles (Titanium Oxide [TiO_2], zinc oxide [ZnO], magnesium oxide [MgO], silver [Ag] and gold [Au]) on Balb/c mice cells showed that cytotoxicity of all nanoparticles were dose-dependent so that the high cytotoxicitys correlated with high concentration of nanoparticles. At concentration (1000 g/L) of Ag, Au, TiO_2, ZnO and MgO nanoparticles cell death and membrane damage and LDH enzyme leakage were observed [20].

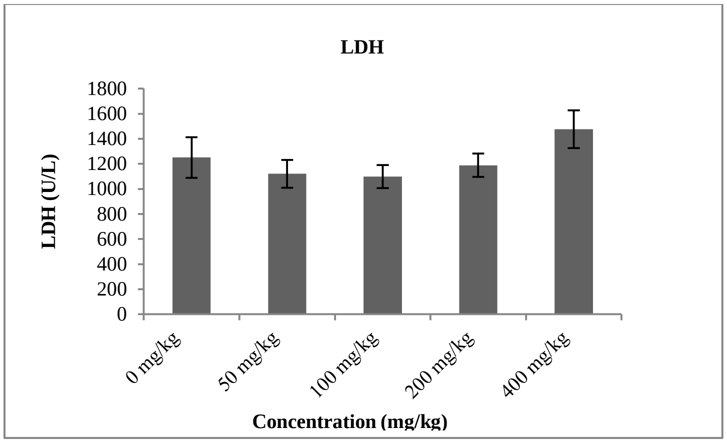

Figure 3. LDH enzyme changes in NMRI mice after 16 days of treatment with different concentrations of silver nanoparticles. Increase of LDH activity in the experimental groups was not significant compared to the control. A value $P \leq 0.05$ was considered to be statistically significant and results display as Mean±SD

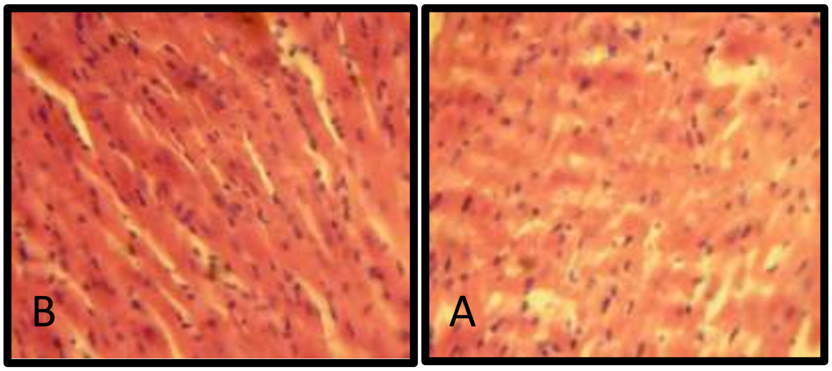

Figure 4. Comparison of heart phatological sections pregnant mice (NMRI) after treatment with different doses of Ag NPs. (A) Normal heart of NMRI mice as a control (B) Experimental group was treated with concentration 400 mg/kg. The sections were stained with H & E dye. The magnification was 40 × .There was no difference between control and treatment groups.

This difference may be due to the difference in the size of nanoparticles. Findings indicated that the effects of nanoparticles on cell can be affected by different parameters with the size of nanoparticles have higher importance. The smaller nanoparticles have more effects on cell and the structure and the shape of nanoparticles is the important parameter in cytotoxic study [21].

Although it has been reported that silver nanoparticles (Ag-NPs) have strong toxic effects to various cultured cells, the toxic effects at noncytotoxic doses are still unknown. The effect of *in vitro* toxicity of Ag-NPs at noncytotoxic doses evaluated in human hepatoma cell line and Ag-NPs exposure exhibited a significant cytotoxicity at higher doses (>1.0 mg/L) [22].

Our result revealed that the LDH activity was not changed significantly after exposure to different concentration of silver nanoparticles, which shows the safety of these particles on LDH activity.

CONCLUSION

The silver nanoparticles with a diameter of 20 nanometers cannot change the activity level of LDH and heart tissue.

This study suggests that use of Ag-NPs to evaluate the amount of change in LDH enzyme and embryonic development were dose dependent. Our findings provide crucial information that silver nanoparticles especially at high concentrations created damage and also a major influence on the development of the fetal. According to the similarities of the mechanisms within the mouse and human, there is the probability of similar variation in this field for human. The biological effects of AgNPs have exhibited, but many challenges still exist in this field. Building a proper cell model for studying the biological effects of AgNPs *in vivo* remains a challenge. For evaluating the safety of AgNPs in future should be screened some of the major factors, such as dose, time, size, shape, surface chemistry, and specific tissues. Thus, High throughput analysis is necessary to resolve problems in this field [23, 25].

ACKNOWLEDGMENTS

Hereby we wish to thank Dr. Abdollahpour for statistical analysis and Mr. Majid Ghasemi for preparation of laboratory samples and tissues. The authors declare that there is no conflict of interests. This article was extracted from the thesis of Ms. Ameneh Arefifar and the expenses of this work were discharged by himself.

REFERENCES

1. Mytych J., Wnuk M., 2013. Nanoparticle Technology as a Double-Edged Sword: Cytotoxic, Genotoxic and Epigenetic Effects on Living Cells. J Biomate Nanobiotechnol. 4: 53-63.

2. Ferreira A.J., Cemlyn-Jones J., Robalo Cordeiro C., 2013. Nanoparticles, nanotechnology and pulmonary nanotoxicology. Rev Port Pneumol. 19(1): 28-37.

3. Bhattacharya R., Mukherjee P., 2008. Biological properties of naked" metal nanoparticles. Advanced Drug Delivery Reviews. 60: 1289–1306.

4. Nowack B., Krug H. F., Height M., 2010.120 years of nanosilver history: Implications for policy makers. Environ Sci Technol. 45, 1177–1183.

5. lansdown A.B.G., 2004. A review of the use of silver in wound care: facts and fallacies. Br J Nurs. 13: 6-19.

6. Wright J.B., Lam K., Hansen D., Burrell R.E., 1999. Efficacy of topical silver against fungal burn wound pathogens. Am J Infect Control. 27: 344–350.

7. Sun R.W.Y., Chen R., Chung N.P.Y., Ho C.M., Lin C.L.S., Che C.M., 2005. Silver nanoparticles fabricated in hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. Chem Communications. 5059–5061.

8 Johnston H.J., Hutchison G., Christensen F.M., Peters S., Hankin S., Stone V., 2010. A review the *in vivo* and *in vitro* toxicity of silver and gold particulates: Particle attributes and biologimechanisms responsible for the observed toxicity. Critical Rev Toxicol. 4: 328–346.

9. Lankveld D.P.K., Oomen A.G., Krystek P., Neigh A., Troost-de Jong A., Noorlander C.W., van Eijkeren J.C.H., Geertsma R.E., de Jong W.H., 2010. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials. 31: 8350–8361.

10. Sharma V. K., Yngard R.A., Lin Y., 2006. Silver Nanoparticles: Green Synthesis and Their Antimicrobial Activities. J Adv Colloid Interface Sci. 145(1-2): 83-96.

11. Tang J., Xi T., 2008. Status of biological evaluation on silver nanoparticles. Sheng. Wu. Yi. Xue Gong Cheng Xue Za Zhi. 25(4): 958-61.

12. Akradi L., SohrabiHaghdoost I., Djeddi A.N., 2012. Histopathologic and apoptotic effect of nanosilver in liver of broiler chickens. Afr J Biotechnol. 11(22): 6207-6211.

13. Nori A., Parivar K., Modaresi M., Yousefi M.H., 2010. Effects of iron oxide nanoparticles on the growth and development of mice embryo. The National Conference of nano-materials and nano-technology, Najaf Abad, Islamic Azad University (Persian).

14. Negahdary M., Ajdary M., 2014. The Toxicity of Gold, Silver and Zinc Oxide Nanoparticles on LDH Enzyme in Male Mice. Ann Res Rev Biol. 4(8): 1346-1352.

15. Moss D.W., Henderson R., 1999. Clinical enzymology. In: Burtis CA, Ashwood ER. Eds. Tietz textbook of clinical chemistry 3rd ed Philadelphia: W.B Saunders Company. 617-712.

16. Warheit D.B., Webb T.R., Reed K.L., Frerichs S., Sayes C.M. 2007. Pulmonary toxicity study in rats with tree forms of ultra-fine titanium dioxide particles. Toxicol. 230(1): 90-104.

17. Kannan R.R., Jerley A.J.A., Ranjani M., Prakash V.S.J., 2001. Antimicrobial silver nanoparticle induces organ deformities in the developing Zebrafish (Danio rerio) embryos. J Biomed Sci Engin. 4: 248-254.

18. Ghorbanzadeh V., Moshtaghian J., Ebadi A.H., 2011. Influence of Nano-Silver on Graffian Follicles via Intraperitoneal Injection in Rats. Middle-East. J Sci Res. 8(1): 228-230.

19. Naghsh N., Mashayekh A., Khodadadi S., 2012. Effects of silver nanoparticle on lactate dehydrogenase activity and histological changes of heart tissue in male wistar rats. JFUMS. 2(4): 303-307 (Persian).

20. Jebali A., Kazemi B., Rafitabar H., Hekmatimoghaddam S.H., 2012. The cytotoxicity effects of different nanoparticles on Balb/c mice cells, Nanocon, Brno, Czech Republic, EU, 23 - 25. 10.2012.

21. Chen W., Liu Y., Courtney H.S., Bettenga M., Agrawal C.M., Bumgardner, J.D., 2006. *In vitro* antibacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatitei coating. Biomaterials. 27(32): 5512-7.

22. Kawata K., Osawa M., Okabe S., 2009. *In Vitro* Toxicity of Silver Nanoparticles at Noncytotoxic Doses to HepG2 Human Hepatoma Cells. Environ Sci Technol. 43(15): 6046–6051.

23. Zhang T., Wang L., Chen Q., Chen C., 2014. Cytotoxic Potential of Silver Nanoparticles. Yonsei Med J 55(2): 283–291

24. Qu Y., Li W., Zhou Y., Liu X., Zhang L., Wang L., 2011. Full assessment of fate and physiological behavior of quantum dots utilizing Caenorhabditis elegans as a model organism. Nano Letters. 11: 3174–3183.

25. Li Y.F., Chen C., 2011. Fate and toxicity of metallic and metal-containing nanoparticles for biomedical applications. Small. 7, 2965–2980.