IJTA

International Journal of Therapeutic Applications

ISSN 2320-138X

HEPATOTOXIC EFFECT OF CHARACTERIZED SILVER NANOPARTICLE

Jyoti Prakash Pani[1], S .P .Mishra[2], R.S. More[1], Sanjay Singh[2], Royana Singh[1*]

[1]Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

[2]Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

[2]Pharmaceutical division, IIT, Banaras Hindu University, Varanasi, Uttar Pradesh, India

ABSTRACT

Silver nanoparticles (NPs), is widely used in the manufacture of various types of consumer products. It is widely applied in textile industries, optics, electronics, catalysis, magnetic and medical fields, which includes therapeutic and diagnostic. It has antibacterial and antifungal property. It belongs to novel types of materials but pose potential risks to human as well as animal health. It negatively affects mice at higher dose when repeatedly induced through oral gavages. It creates toxicity in target organ liver predominantly by accumulating inside it and enhances oxidative stress in vital organs by over production of ROS in cell and depletion of antioxidant enzyme level in tissue. The effect of Ag-NPs on hepatic Catalase and glutathione (GSH) levels were variable in different treated groups compared with the control.

Key words : Magnetic stirring and cooling method, smaller size silver nanoparticle toxicity, oral gavages exposure.

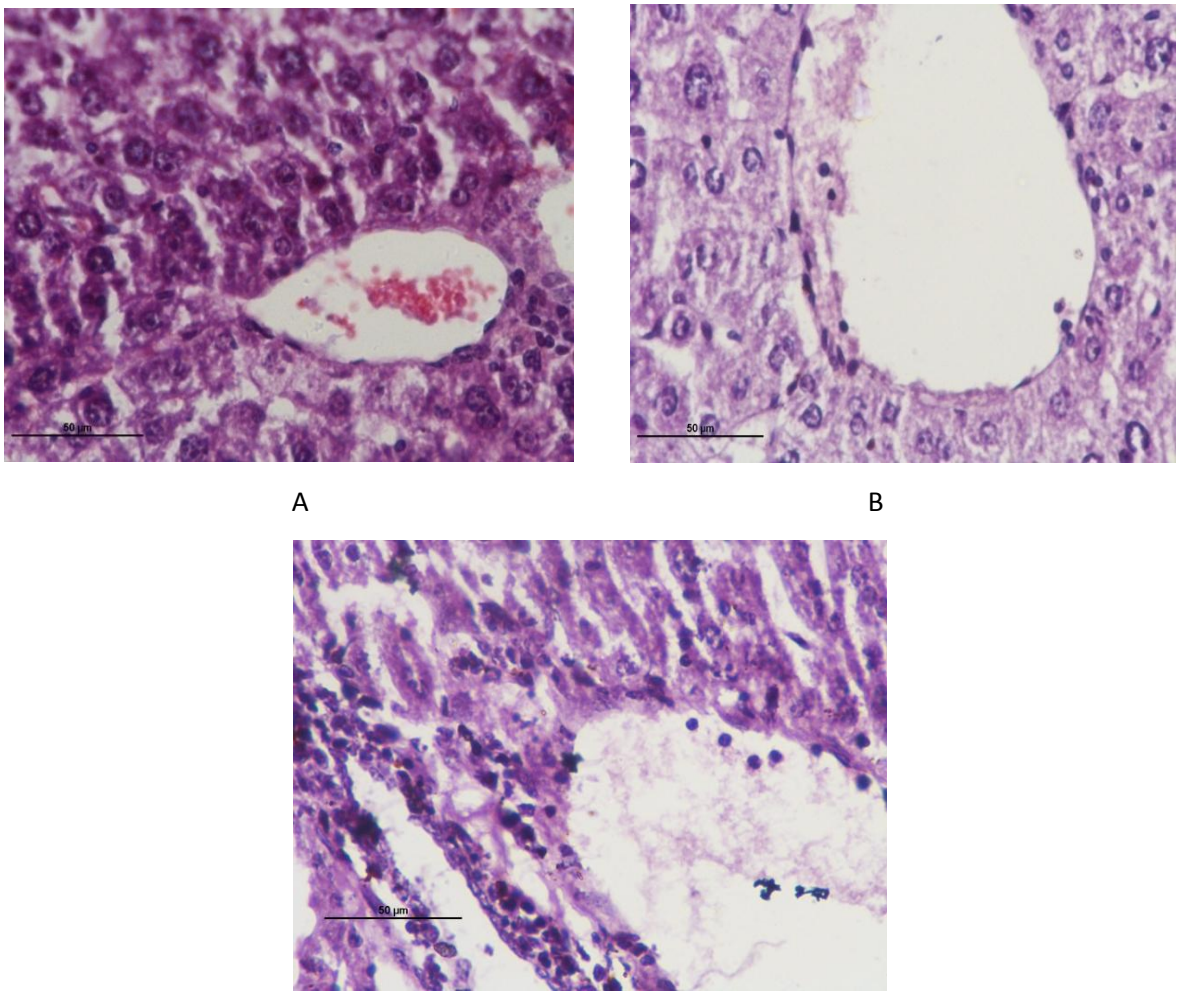
INTRODUCTION

In Nanotechnology a Nanoparticle is defined as a small object that behaves as a whole unit in terms of its transport and properties. A particle having one or more dimensions of the order of 100 nm either less or above is called nanoparticle. Why silver nanoparticle is important among the all nanoparticles? because of its applications on catalysis, optics, electronics, medical field and other areas due to their size dependant optical, electrical and magnetic properties. It is also used in biotechnology and bioengineering. Silver nanoparticles (AgNPs) are used in manufacture of nearly about 400 consumer products primarily. It also exhibits antibacterial and antifungal properties. Various types of Clothes and related products for human utilization are made by silver nanoparticle as principal constituent, such as AgNPs fabrics[1] and products of personal hygiene maintenance[2]. Some of the silver nanoparticle products are air medium conveyable, such as nasal sprays or air freshener [2] some are include wound dresser[3], some are comes under nanosilver toothpastes category, and some are designed as colloidal silver suspensions nutritional supplements[4]. Silver nanoparticle reaches directly to the target organ liver via bloodstream in all application to the body, it reach the bloodstream through inhalation[5], through the skin[6] and through the GI tract[7]. When silver nanoparticles translocate to the bloodstream passing after any exposure routes, it usually accumulates in the target organ liver. Previous studies have shown a high accumulation of silver NPs in the liver after injection[8], also previous studies proved retention of silver particles in the liver following direct ingestion[7]. AgNPs proved to be highly toxic not only to bacteria and fungi but also to a number of animal species and cultured cells[9, 10]. AgNPs toxicity has often been associated with Ag ion release and induction of oxidative stress[10]. Studies showed an equivalent toxicity of same concentration soluble silver compared to AgNPs [11], and particles with very low solubility have elicited high toxicity in exposed species and cells[9]. The literature shows examples for both inflammatory[12] and anti-inflammatory[13] effects of nano silver, as well as conflicting results with regards to the ability of AgNPs to cause oxidative stress[12, 11]. The silver nanoparticles show

Corresponding author:

Email: singhroyana@rediffmail.com

inflammatory gene expression and releases pro-inflammatory mediators in tissue. Results of this study will definitely contribute to choose suitable endpoints for *in vitro* risk assessment of silver nanoparticles.



A B C

Fig.1 **(A-C) Photomicrograph of the mice liver A. Control showing the central vein and the hepatocytes. (40x H&E)B .The 5 mg/kg b.w. treated mice live showing disarrangement of the hepatocytes with marked inflammatory cell infiltration and congestion. (40x H&E) C.10 mg treated mice showing marked dilatation of the sinusoids, necrosis of the hepatocytes and inflammatory cell (40x H&E)**

AIM

The present study is aimed to compare the hepatotoxicty induced by small and large size Silver nanoparticle.

MATERIAL

Silver nanoparticle colloidal solution synthesized & prepared by magnetic stirring & cooling method.[14] 30 ml of 0.002M Sodium borohydride ($NaBH_4$) laboratory prepared fresh solution added to an Erlenmeyer flask. A 1 inch magnetic stir bar was placed in the flask in an ice bath surrounding the Erlenmeyer flask on a magnetic stir plate. 2 ml of 0.001M silver nitrate ($AgNO_3$) is added into the stirring $NaBH_4$ solution at approximately 1 drop per second from a sterile bottle with infusion set attached. Stirring stopped as soon as all of the $AgNO_3$ is added. Small portion of the solution is transferred to a test tube. One drop of 0.3% polyvinyl pyrollidone (PVP) added to the solution present in the test tube and a drop of NaCl to prevent aggregation and to make effective hanging of the particles in solution core. The presence of a colloidal suspension can be detected by the

reflection of laser beam from the silver particles. The preparation mixture ranged from 1200 to 1500 nm size silver nanoparticles were added respectively after suspended in normal saline. PVP (0.33%) and $NaBH_4$ (0.002 M) were added for proper stability and to prevent aggregation in core of the solution. For confirmation of the size both the test AgNPs colloidal solution send to pharmaceutical division for dynamic light scattering analysis with the help of Delsa Nano ™ Beckmann Coulter machine. $AgNO_3$ transparent crystal beads in sealed packet form of respective sizes and other chemicals were purchased from Sigma Aldrich Company USA.

chloroform inhalation anesthesia. The liver from each mice and group was dissected in a clean and sterile condition. The liver was cut in two halves. One was fixed in 10% formalin while the other was subjected to Anti oxidant enzyme Catalase test and Reduced Glutathione. Six micron thin serial section of the liver tissue was cut by rotator microtome and evaluated after H & E staining.

RESULT- DLS ANALYSIS OF RESPECTIVE TEST SOLUTION

The DLS analysis of both test mixture indicated average diameter of the silver particle ranged between 1200 to 1500. With an average of 1300 nm. The Poly Dispersity Index of both size silver nanoparticle colloidal solutions was measured 0.461 and 0.568 with a zeta potential analysis of -17.52mV. UV-Vis spectrophotometric analysis of the both test solution also yielded more or less the same result but maximum absorption of UV light (near about 0.35 absorbance rate) was observed for 80 nm silver particle size with maximum excitation whereas lowest absorption (>0.06 absorbance rate) was observed for 1500 nm silver particle size with minimum excitation.

Table 1: Showing the Catalase (u. mg. / prot.) activity and Reduced Glutathione values (u. mg. / prot.) in the mice liver following AgNPs as oral gavage

Parameter	Control(FT)	5 mg / kg b.w. (FT)	10 mg / kg b.w. (FT)
No. of mice	10	10	10
Catalase (unit mg/protein	25.943±35.96	25.944±16.86	25.945±15.85
Reduced Glutathione (unit mg/protein)	0.572±0.81	0.444±0.71	0.476±0.26

* Values are presented as means **±** s. d. / FT- Fresh Tissue

METHOD

Adult male and non pregnant female Swiss albino mice weighing approx. 25 gm-35gm were randomly selected from the animal house Department of Anatomy, IMS, BHU from separate aseptic breeding colonies,. The study was approved by central animal ethical committee IMS, BHU (No. Dean/2014/CAEC/614/Dt.30.05.14). The mice were kept at avg. (22-26^{0}c) with humidity 55±5% and were provided Hindustan lever diet pellets and tap water add libitum. They were run 12 hr light n dark cycle at a ratio of 1:1 inside plastic polycarbonate cage with light transferable network of stainless steel roofing.

The strong and healthy male and female mice which were heterozygous were divided into three groups (1-Control group (Sham control treated with anionic double distilled water), 2. 1200 to 1500 nm size range silver nanoparticle treated group at the dose of 5mg/ kg b.w., 3. 1200 to 1500 nm size range silver nanoparticle treated group at the dose of 10mg/ kg b.w.)

AgNPs was administered through repeated oral gavages for 28 days continuously. AT 29th day the mice were sacrificed by over dose of deep ether/

Freshly dissected liver were presented with alteration and depletion in Catalase activity in the form of optical density in 90 second gap interval from 90 to 720 seconds of UV-Vis spectrophotometric analysis in treated group. Liver tissue homogenized with 10 times normal saline (1:10) were exposed to Reduced Glutathione test. The results of optical density values obtained were imposed into Reduced Glutathione standard curve.

HISTOLOGY

6 micrometer serial sections of liver of mice were cut by rotator microtome and stained by

Haematoxylin and eosin staining. The photomicrographs exhibited severe toxic features, clumps of hepatocyte necrosis in form of multiple patches, discrete infiltration of lymphocytes which also includes cell blebbing, multiple calcified focus & discrete red cell masses in multiple areas.

Catalase enzyme elevation in treated liver issue

Catalase depletion in the fresh liver tissue was observed after doing Catalase activity test. The Catalase activity showed no difference when the control were compared with the I treated groups, p value <1.2.

Depletion of reduced glutathione activity as oxidative stress focus

Oxidative stress effect in the liver tissue has been observed in the form of depletion of Reduced Glutathione activity in treated group mice when compared with control group (p value < 0.05).

DISCUSSION

According to Knetsch ML et al In 2011, Takenaka S et al. in 2001 and Lee Y et al. in 2013 Silver nanoparticle accumulates mainly in liver because it contains high levels of thiol-rich proteins such as glutathione. [15, 16, 17] Our study also reports the same thing that is depletion in Reduced Glutathione activity is a major parameter for oxidative stress. Arora S, Jain J, Rajwade J, Paknikar K. et al in 2009 reported AgNPs (7-20 nm) with final concentrations of 10-200 µg/ml (10-200 ppm) can cause oxidative stress, apoptosis, and decreased cell viability in fibroblasts and liver cells isolated from Swiss albino mice.[18] Our study also reports AgNPs of mean 1300.15nm average size range in concentration of 5mg/kg b.w. and 10mg/kg b.w can cause the same and histological hazards. AgNPs proves to be highly toxic to primary hepatocyte (Gaiser et al., 2012; Piao et al., 2011).[9, 19] The consequences of AgNPs toxicity is oxidative stress (Carlson et al., 2008; Piao et al., 2011).[20, 19] Catalase activity in our study exhibited variable result these findings shows similarity to those of Piao et al. (2011), [19] which showed a strong decrease in reduced GSH in human Chang hepatocyte treated with AgNPs. This differentiation could be due to the type of AgNPs used. Using AgNPs at a toxic dose, as evidenced by the images with rounded off, detaching cells at the concentrations used, whereas this study only used sub lethal particle concentrations.

Histology showed cell rounded off with detaching activity in sub lethal Silver nanoparticle dose. Our study also reports inflammatory mediator expression of liver cytology and infiltration of neutrophils into the space where clump of necrotic hepatocytes are seen. Histopathological examination of liver revealed that various alterations denoting the hepatotoxic effect of silver nanoparticles including hepatocellular degeneration, necrosis and individual apoptosis were the most recognized hepatic changes that were dose dependent. Several studies confirmed that liver is the target organ for the effect of silver nanoparticles (Ji et al., 2007; Gopinath et al., 2008; Kim et al., 2008b; Sung et al., 2009), also (Hussain et al., 2005) reported that silver nanoparticles were highly toxic in rat's liver cells.[21, 22, 23, 24, 25] Silver nanoparticles reduced the activity of mitochondria which results in reduction of available energy for cells. Moreover, (Sardari et al., 2012) discussed the mechanism of silver nanoparticles induced hepatotoxicity by repeated oral administration and they found that nanoparticles are removed from the liver by macrophages due to phagocytosis process and the repetition of this process produced higher oxygen radicals.[26] The results of the current study are consistent with previous reports by (Sung et al., 2009; Kim et al., 2010).[24, 26]

This study suggests that repeated use of AgNPs orally for testing may cause a mild to moderate toxicity, as indicated by time, size and dose dependent toxic responses in liver tissues. Silver nanoparticle proves to be highly toxic through repeated oral gavages exposure.Silver nanoparticle cause inflammatory mediator expression in liver histology. DLS analysis says the size of the study used silver nanoparticle vary between 71-1300.5 nm range. Large size particle not able to penetrate the cell membrane effectively but pressurizes the area where it abnormally accumulates. Rather than causing intracytotoxicity it causes teratogenic effects at tissue level by hampering nutrition.

ACKNOWLEDGEMENTS

Author sincerely acknowledges all coauthors and UGC for all sorts of financial support.

REFERENCES

1) Kulthong K, Srisung S, Boonpavanitchakul K, Kangwansupamonkon W and Maniratanachote, R, Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat, Part Fibre Toxicol, **7**(2010)**:** 8.

2) Chao JB, Liu JF, Yu SJ, Feng YD, Tan ZQ, Liu R and Yin YG, Speciation analysis of silver nanoparticles and silver ions in antibacterial products and environmental waters via cloud point extraction-based separation, Anal Chem, 83 (2011), 6875–6882.

3) Silver S, Phung le T, Silver G, Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol, 33 (2006), 627–634.

4) Nowack B, Krug HF, Height M, 120 years of nano silver history, Implications for policy makers, Environ Sci Technol, Advance access published January 10, (2011) , Doi: 10.1021/es103316q.

5) Mühlfeld C, Gehr P, Rothen-Rutishauser B, Translocation and cellular entering mechanisms of nanoparticles in the respiratory tract, Swiss MedWkly, 138 (2008), 387–391.

6) Korani M, Rezayat SM, Gilani K, Arbabi Bidgoli S, Adeli S, Acute and sub-chronic dermal toxicity of nanosilver in guinea pig, Int J Nanomedicine, 6 (2011), 855–862.

7) Schleh C, Semmler-Behnke M, Lipka J, Wenk A, Hirn S, Schäfflr M, Schmid G, Simon U and Kreyling WG, Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration, Nanotoxicology.6 (2012) , 36–46.

8) Hirn S, Semmler-Behnke M, Schleh C, Wenk A, Lipka J, Schäfflr M, Takenaka S, Möller W, Schmid G, Simon U et al, Particle size-dependent and surface charge-dependent bio-distribution of gold nanoparticles after intravenous administration, Eur J Pharm Biopharm, **77** (2011), 407–416.

9) Gaiser BK, Fernandes TF, Jepson MA, Lead JR., Tyler CR, Baalousha M, Biswas A, Britton, GJ, Cole PA, Johnston BD et al, Interspecies comparisons on the uptake and toxicity of silver and cerium dioxide nanoparticles, Environ Toxicol Chem, 31 (2012), 144–154.

10) Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V, A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev, Toxicol 40 (2010), 328–346.

11) Powers CM, Badireddy AR, Ryde IT, Seidler FJ, Slotkin TA, Silver nanoparticles compromise neurodevelopment in PC12 cells: Critical contributions of silver ion, particle size, coating, and composition, Environ. Health Perspect, 119(2011), 37–44.

12) Nishanth RP, Jyotsna RG, Schlager JJ, Hussain SM, Reddanna P, Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: Role of ROS-NFκB signaling pathway. Nanotoxicology 5(2011),502–516.

13) Wong KK, Cheung SO, Huang L, Niu J, Tao C, Ho CM, Che CM, Tam PK, Further evidence of the anti-inflammatory effects of silver nanoparticles, Chem Med Chem, 4 (2009) 1129–1135.

14) Solomon SD, Bahadory M, Jeyarajasingam AV, Rutkowsky SA, Boritz C, Mulfinger L, Journal of Chemical Education, 84 (2007), 322-325.

15) Knetsch ML, Koole LH. New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers . 2011; 3(1): 340-366.

16) Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U et al, Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats, Environ Health Perspect, 109 (2001), 109(Suppl 4), 547–551.

17) Lee Y, Kim P, Yoon J, Lee B, Choi K and Kil K-H et al, Serum kinetics, distribution and excretion of silver in rabbits following 28 days after a single intravenous injection of silver nanoparticles, Nanotoxicology, 7 (2013), 6, 1120-1130.

18) Arora S, Jain J, Rajwade J and Paknikar K, Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells, Toxicol Appl Pharmacol, 236 (2009) 3, 310-318.

19) Piao MJ, Kang KA, Lee IK, Kim HS, Kim S, Choi JY, Choi J and Hyun JW, Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis, Toxicol Lett, 201(2011),92–100.

20) Carlson C, Hussain SM., Schrand AM, Braydich-Stolle LK, Hess KL, Jones RL and Schlager JJ, Unique cellular interaction of silver nanoparticles: Size-dependent generation of reactive oxygen species, J Phys Chem B,112 (2008),13608–13619.

21) Ji JH, Jung JH, Kim SS, Yoon JU, Park JD, Choi BS, et al, Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley-rats, Inhal Toxicol,19 (2007) 19(10),857–871.

22) Gopinath P, Gogoi SK, Chattopadhyay A and Gosh SS, Implications of silver nanoparticle induced cell apoptosis for invitrogene therapy, J Nanobiotechnol,19 (2008) (Art. no. 075104).

23) Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, Park JD, et al, Twenty-eight-day oral toxicity, genotoxicity, and gender-related issue distribution of silver nanoparticles in Sprague-Dawley rats, Inhal Toxicol,20 (2008), 575–83.

24) Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, et al, Sub chronic inhalation toxicity of silver nanoparticles, Toxicol Sci 108 (2009)2,452–461.

25) Hussain SM, Hess KL, Gearhart JM, Geiss KT and Schlager JJ, In vitro toxicity of nanoparticles in BRL 3A rat liver cells, Toxicol In Vitro, 2005, 19, 975–983.

26) Sardari RRR, Zarchi SR, Talebi A, Nasri S, Imani S, Khoradmehr A, et al, Toxicological effects of silver nanoparticles in rats, Afr J Microbiol Res, 6(2012), 5587–5593.

27) Kim YS, Song MY, Park JD, Song KS, Ryu HR , Chung YH, et al, Sub chronic oral toxicity of silver nanoparticles, Part Fibre Toxicol, 7(2010) , 20.