American-Eurasian Journal of Toxicological Sciences 3 (1): 28-35, 2011
ISSN 2079-2050

Investigation on Silver Retention in Different Organs and Oxidative Stress Enzymes in Male Broiler Fed Diet Supplemented with Powder of Nano Silver

Farhad Ahmadi and Ardeshir Hafsy Kordestany

Islamic Azad University, Sanandaj Branch, Kurdistan, Iran

Abstract: This research was carried out to investigation of retention silver (Ag) in different organs and faeces of broiler chicks. A total of 160 one-day-old male broiler allocated in a randomized completely design (CRD) with four group (60 birds), four replicates and 10 broiler in each experimental pens. Experimental diets were included: A) Control (without nano-silver), B) control+5ppm nano-Ag/kg, C) control+10ppm nano-Ag/kg and D) control+15ppm nano-Ag/kg and nano-Ag added to basal diet of broilers throughout research (21-42 days). At the end of study, from any treatments one bird with means's treatment selected and take sampling blood from wing vein and then slaughtered. Immediately after slaughtered, Visceral organs such as: Liver, heart, gizzard, spleen, pancreas, thymus, lungs and tight and breast muscle. Also, to determine rate of Ag in bird's faeces, samples were weekly taken during study and then dried, stored until analysis. Silver concentration in different tissues by using gamma radioactivity method has measured and Ag of rate expressed as ppb (Parts per billion) into different organs and feces. Results showed no significant difference among treatment point of view time saturation of tissues with silver and seem to be quickly distributed by blood circulation. Examination of different organs showed that Ag was accumulated in different tissues of broiler. The highest concentrations Ag were found in breast and leg muscles, spleen, pancreas, faeces, thymus and heart of muscles in the ranking order. We had significantly observed increasing trend concentration of Ag in different tissues corresponding to increase level of nano-silver among control and other group ($p<0.01$).

Key words: Broiler · Oxidative stress enzyme · Silver retention · Different organs

INTRODUCTION

Nanotechnology is an enabling technology that deals with structures ranging from approximately 1-100 nm in at least one dimension [1]; Scientific Committee on Emerging and Newly Identified Health Risks [2]. The nanosize results in specific physicochemical characteristics that may differ from those of the bulk substance or particles of larger size. This effect is mainly attributed to high surface area to volume ratio, which potentially results in high reactivity. One of the substances used in nanoformulation is silver (nano-silver). It has been used since ancient times for jewellery, utensils, monetary currency, dental alloy, photography, explosives, etc. [3]. Nanoparticles are used in bioapplications as therapeutics, transfection vectors and fluorescent labels [4-7]. Despite their widespread use, there is a serious lack of information concerning the toxicity of Ag-NPs to humans and their underlying cellular reactions. Recent studies have shown that Ag-NPs accumulation in the liver could induce cytotoxicity via oxidative cell damage [8-10]. Nano-silver is also used in washing machines because of its antimicrobial activity [11]. Elementary silver or silver ions are used as active compounds in many different household water filters advertised for instance by Internet for catastrophe areas. Silver is considered toxic for humans and the recommendations of the World Health Organization (WHO) permit maximal concentrations of 0.1 mg of silver ions in drinking water disinfection [12] but EPA [13] recommends maximally 0.05 mg/l. Silver is known to accumulate from water in many organisms such as brown algae [14], shell fish [15 - 17] and fish (18,19]. Silver nitrate is absorbed through the lung and mucous membranes [12]. Silver ion is transported in blood and bound into globulins [13]. Most accumulation has been reported to occur in mammalian liver and skin [20-22]. The systemic toxicity of silver is not well documented when silver compounds are used in wound repair treatments [23]. A major part of original animal tests used for the toxicity evaluation for WHO Guidelines for drinking water

Corresponding Author: Farhad Ahmadi, Islamic Azad University, Sanandaj Branch, Kurdistan, Iran.
Tel: +98-871-638-7112-3, Fax: +98-871-638-71100, E-mail: k_a183@ymail.com.

disinfection by silver has been made between1940s and 1970s [12]. Because analysis technique and toxicology have developed and got new tools since those days, there is a need to revaluate those animal tests. Silver ions are able to pass the blood-brain barrier and cerebrospinal fluid-brain barrier [24]. For example, in rats silver is found, e.g. in the hypothalamic neurons [25] disturbing hippocampus (area of the brain) development [24]. Silver has been reported to affect lipid per oxidation in rat liver, but not in kidney and brain [24]. Cardiac necroses and decrease of blood glutathione per oxidase has been reported in silver treated swine [26]. It was reported that silver retention in active myofibrils of cats [27]. Due to its microbicidal effects in water per oral silver also affects intestinal microbes [28] and potentially provokes gastrointestinal disorders. Silver compounds are known to decrease the creatine clearance which may be due to its potential nephrotoxicity [29]. The aims of current research were:

- To measure the possible silver retention into tissues and different organs of broiler chicks,
- To find relevance between different levels nano-Ag and retention rate its in tissues and different organs and 3) to investigate bioenvironmental effect by excretion of silver due to faeces.

MATERIALS AND METHODS

Experimental Design and Diets: The broilers were randomly assigned to 4 experimental diets in a completely randomized design (CRD). There were 10 birds per replicate and each treatment had 4 replicates. Feeding of the experimental animals was *ad libitum* and all animals had free access to fresh cool water and nano-silver with different levels added in basal diet throughout research. All diets were formulated to meet nutrients requirements on the recommendations of NRC [30], basal diet showed in table1. Experimental treatments were included:

A. Control (without nano-silver),
B. control+5ppm nano-Ag/kg,
C. control+10ppm nano-Ag/kg and
D. control+15ppm nano-Ag/kg

Birds Housing: Birds were housed randomly in pens with I×1.2×1 m floor space. Per bird, one running inch drinking space per bird and two running inch-feeding spaces per bird were given to each group. Round drinkers and round feeders were used for drinking and feeding the birds.

Table 1: Ingredients and composition of diets in different stage of study (1-21d)[1]

Ingredients	% (21-42)
Maize	59.38
Soyabean meal	33.61
Maize oil	5.89
Dicalcium phosphate	1.03
Limestone	0.65
Salt	0.20
DL-Methionine	0.19
pre mixture1	0.50
Total	100
Nutrient composition	
ME (kcal/kg)	3000
Crude protein (%)	20.18
Lys (%)	1.20
Met (%)	0•53
Met + Cys (%)	0.81
Ca (%)	1.00
Available P (%)	0.45

[1]Supplied the following per kilogram of diet: vitamin A, 25,000 IU; vitamin D, 5,000 IU; vitamin E, 12.5 IU; vitamin K, 2.5 IU; vitamin B1, 1.0 mg; vitamin B2, 8.0 mg; vitamin B6, 3.0 mg; vitamin B12, 15 µg; folic acid, 250 µg; nicotinic acid, 17.5 mg; calcium Pantothenate, 12.5 mg; Fe, 80 mg; Cu, 10 mg; Mn, 80 mg; Se, 0.15 mg; I, 0.35 mg.

The lighting regime was supplied continues in the shed (23L-1D). Temperature of brooding was started from 33°C and decreased every week 3°C and then 18-21°C constant until final study.

Litter: Wood shaving was used as the litter for rearing the chicks and formaldehyde and Potassium permanganate at the ratio of 2:1 was used for fumigation of the shed.

Faeces Sampling: To determine of concentration of Ag excreted by animal faeces, were sampled as randomly twice (21 and 42 d) during the experiment, then was mixed, dried and stored until analysis.

Blood Sampling: At the end of research, from any treatment one bird as randomly selected and before slaughtering, takes about 5ml as blood sample that were drawn by vein of wing. The samples were immediately frozen on dry ice and stored at -20°C for subsequent measurement assay blood of parameters by gamma-radioactivity. After slaughtering bird's sample, the heart, spleen, liver, pancreas, lungs, kidneys, brain, muscles of leg (thigh muscle), breast and gastronomies (M. gastrocnemeus) were collected and weights express

as percentage of body weight. Ag-retention in tissues were measured by using 658 kV Ag-peak and Ge-detector connected to an ORTEC Multichannel Analyzer (EG & E Ortec, Oak Ridge, USA) using a known concentration of 110m AgNo3 in aqueous solution as reference.

Stastical Analysis: Data collected were subjected to the Analysis of Variance (ANOVA) method of SAS [31]. Significant values were compared using the Duncan's multiple range tests. The model was used the following:

$$Yij = \mu + Ti + \varepsilon ij \quad (1)$$

Where:
Yij = Observation,
μ = Population mean,
Ti = Nano-Ag effect (I = 1 to 4),
εij = Experimental error

RESULTS

The results related to silver retention in different broiler tissues and oxidative stress activity has been given in tables 2 and 3 respectively. Data showed that different levels of nano-Ag had significantly effected silver deposit in different tissues in comparison to treatment (P<0.01). In addition, a trend of increasing silver retention was simultaneity observed to increase nano-Ag levels. Therefore, with attention to the result we can present silver retention had ascending titer as the following (on the basis ppb): Breast muscle > gastrocnemius muscle > muscle femur > spleen > liver > pancreas > heart > kidney > lungs > faeces > bursa fabricius. But, silver retention in different muscles was significantly higher than other tissues (P<0.01).

Results related to concentration of oxidative stress enzymes were displayed in table3. This data had showed that silver-Ag with any level had significantly effect on total of oxidative stress enzymes in comparison to control treatment (p<0.01). Also, we observed a linear incrassating enzyme concurrently with incrassating levels of nano-silver.

DISCUSSION

The researches about investigating silver deposition in live organs are continuous. Silver accumulated and deposit in different broiler tissues, especially into different body muscles. The results of research showed that the silver of deposit in the human body has been found to follow the descending order: skin, liver, adrenals, brain, thyroid, caeca, ovary and trachea [32]. Silver concentrations in different human tissues including brain tissues showed, any how, a great (more than 100-fold)

Table 2: Mean of silver retention (ppb/g of tissue) in organ and tissues of broiler chicks during study1

Different organs	Control+5ppm nano-Ag/kg	Control+10ppm nano-Ag/kg	Control+15ppmnano-Ag/kg
Spleen	5.87±0.33b	6.37±0.33b	7.57±0.33a
Heart	4.92±0.78c	5.19±0.78b	6.72±0.78a
Lungs	3.42±0.81b	3.92±0.81a	4.32±0.81a
M. gastrocnemius	9.28±0.24b	10.18±0.24a	10.88±0.24a
M. breast	10.21±0.21c	12.01±0.21b	13.51±0.21a
M. femur	9.21±0.18c	11.21±0.18b	14.21±0.18a
Liver	5.92±0.78b	6.32±0.78b	6.81±0.78a
Kidney	4.52±0.81c	5.02±0.81b	5.72±0.81a
Spleen	6.09±0.31b	7.09±0.31a	7.49±0.31a
Pancreas	5.22±0.78b	6.02±0.78a	6.62±0.78a
Bursa	2.17±0.65b	2.57±0.65b	3.17±0.65a
Faeces	3.49±0.15b	4.41±0.15a	4.89±0.15a
Blood	7.63±2.29c	9.63±2.19b	2.33±2.21a

1a-c: Means within a row with different subscripts differ significantly (p<0.01).

Table 3: Effect of nano-particle silver on erythrocytes CAT, SOD, GSH and MDA enzymes in broiler chicks (mean±SEM)[1]

Treatment	Cat(U/mgHb)	SOD(U/gHb)	GSH(U/gHb)	MDA(μmol/gHb)
A. Control (without nano-Ag)	2.45±0.58b	2278.50±424.59a	43.69±8.04b	1.92±0.15c
B. Control+5ppm nano-g/kg	2.28±0.42b	2195.31±446.80a	44.21±7.81b	2.01±0.11c
C. Control+10ppm nano-g/kg	2.94±0.19a	1925.21±346.07b	48.30±9.28a	2.61±0.09a
D. control+15ppm nano-g/kg	2.62±0.27a	1925.51±336.25b	47.73±3.87a	2.13±0.08b

[1]a-c: Means within a column with different subscripts differ significantly (p<0.05).

deep cerebella nuclei [24]. Kai *et al.* [34] reported that Ag accumulated in mice, especially into cerebellum (lower back portion of the brain) and M. soleus (bottom of a shoe) also, high concentrations of silver exist in the slow-twitch oxidative. Ahmadi, *et al.* [35] reported that different levels of nano particle silver had significantly effect on retention of Ag in breast and femur muscle and faeces ($P<0.01$). This result were agreement with the finding data Kai *et al.* [34] who observed that concentrations of silver in muscles gastrocnemius (M. gastrocnemius) were found to negatively correlate with cerebrum and positively with blood and kidneys.

Another research has been reported that the material used to fill a cavity in a tooth release of silver in rats, with relatively low concentrations of silver in the cerebellum in comparison to kidney and liver Hultmant, *et al.* [36]. Nan particles have shown biological functions such as killing pathogenic bacteria and viruses (e.g. flu), but research has also shown that nanoparticles may produce adverse effects (dose related) in human cells on contact. Human neural cells, such as hippocampus cells in the CNS, are the most sensitive and delicate cells in bio organisms and are responsible for brain functions and emotions. They are vulnerable to ischemia, oxygen deficiency and external factors. One of the great concerns in science and technological development in the twenty-first century is that nanoparticles may produce potential functional and toxicity effects on human neural cells owing to their ability to pass through biological membranes [37]. Exposure to nanoparticles (such as Ag) in the body is also becoming increasingly widespread through antibacterial fabrics and coatings. However, effects from the presence (or even accumulation) of metal nanoparticles in the brain and through the BBB have not yet been fully studied. Small-sized particles have better mobility and it is expected that the transportation of nanoparticles across the Brain-border- blood (BBB) is possible either by passive diffusion or by carrier-mediated endocytosis [38]. In addition, nanoparticles may be taken up directly into the brain by trans-synaptic transport [39]. For example, Ag nanoparticles can enter via the BBB and accumulate in different regions of the brain [40] and this may be beneficial for drug delivery, but may also pose a risk to the patient [41, 42]. It has also been reported that nanoparticle exposure can induce impairments to normal neurons [43], microglia, [44] and even aggravate the process of brain pathology [45].

Beresford *et al.* [46] has mentioned accumulation of radioactive silver from the Chernobyl accident in a ewe brain, but he could investigate only one animal and the distribution of silver between cerebellum and other brain tissue was not reported. Silver concentration in human brain was found to be dependent on age and on the number of dental amalgam fillings [47] and this chronic release of silver alters monocyte metabolism [48]. The mechanisms of the silver by Saeki *et al.* [22] considered hair molting to be the principal excretion in seals and they also found silver in amniotic fluid. Hanson *et al.* [49] found that the removal of silver was slow and some silver from lactating rats was transferred to milk. The cerebellum has a major role in the adjustment of coordinated muscle activity. The silver accumulation in cerebellum and cerebrum may vary between mammals as the size ratio also varies between these organs among different mammalian species. Absorption of nano-silver into the human body may occur via inhalator, oral and dermal routes of exposure. In kinetic terms, absorption represents the process by which unchanged compounds (e.g., nano-silver) proceed from the site of administration to the central blood circulation and subsequently to the organs. The respiratory system represents a major port of entrance for nano-silver. The distribution and disposition of nano-silver in the respiratory tract depends on various factors including particle size and breathing force. In addition, due to the small diameter of the nano-silver, Brownian diffusion also determines deposition, resulting in a deep penetration of nano-silver in the lungs and diffusion to the high lung surface area presented in the alveolar region. An additional absorption route for inhaled nano-silver could be from the olfactory mucosa of the respiratory tract into the central nerve system via the olfactory nerve [50]. Indeed, inhaled 15 nm nano-silver has been demonstrated to be present in the olfactory nerve and brain of rats after inhalation [51]. This suggests that for inhaled nano-silver, the olfactory nerve represents an additional port of entry into the brain, circumventing the restricted blood-brain barrier [50, 52]. When nano-silver has passed the barriers (i.e., lung epithelia, intestinal lining and dermis) at the site of entry, the systemic circulation may be reached. Further distribution throughout the entire body may take place via the systemic circulation. In principle, the binding of plasma proteins influences the ability of a particle to traverse cell membranes and other kinetic parameters (e.g., its distribution and half-life), half life plasma-protein binding. In general for compounds in the blood (e.g., pharmaceuticals), the unbound fraction exhibits the observed effect. Therefore, when nano-silver reaches the systemic circulation, the particles can, potentially, interact with plasma proteins, coagulation factors, platelets and

red and white blood cells. An effect of plasma-proteins on the distribution, elimination and toxic potency of nano-silver particles can be expected, similar to described protein (serum albumin) effects on quantum dots [53]. The previously discussed inhalation studies with 15 nm nano-silver particles in rats revealed low, but detectable, concentrations of silver in blood and subsequent distribution to organs including liver, kidney, heart, lymph nodes and brain [51, 54]. Kim *et al.* [10], found a dose-dependent accumulation of silver content in a broad range of tissues including blood, liver, lungs, kidneys, stomach, testes and brain in a recent oral toxicity study of 60 nm silver nonmaterials in rats.

The results in table3 show that Silver has significantly effect on total of oxidative stress enzymes in comparison to control treatment ($p<0.01$). Erythrocyte MDA level was incrassating at control treatment in comparison to other groups, 5, 10 and 15 ppm ($p<0.01$). CAT, GSH and SOD activities were significantly difference in comparison to control group ($p<0.01$) and among other treatment. After absorption nano-silver from GIT and entered to blood systemic circulation, the particles can, potentially, interact with different metabolites like plasma proteins, coagulation factors, platelets and red and white blood cells [53]. Silver nanoparticles in most studies are suggested to be non-toxic. But due to their small size and variable properties they are suggested to be hazardous to the environment [55]. Studied the toxicity of different sizes of silver nano-particles on rat liver cell line (BRL 3A) (ATCC, CRL-1442 immortalized rat liver cells) was previously studied [8]. The authors found that after an exposure of 24h the mitochondrial cells displayed abnormal size, cellular shrinkage and irregular shape Braydich -Stoll et *al.* [55] reported the toxicity of silver nano-particles on C18 - 4 cells, a cell line with spermatogonial stem cell characteristics. From the study, it was concluded that the cytotoxicity of silver nano-particles to the mitochondrial activity increased with the increase in the concentration of silver nano-particles. Recently, reported that nano-particles and nano-materials such as manganese, copper and silica generate free radicals and oxidative stress [56]. The results of researches showed that silver nano-particles can damage to different organs and tissue such as liver cells [55]. In general, the liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. It is a logical assumption that ingested silver nano-particles might have impact on the liver, since the liver serves as the first check point for everything absorbed through GIT before becoming systemic. Researches showed function of mitochondria [8, 55] that exposure to silver nano-prarticles significantly decreased the mitochondria; seem to be sensitive targets of cytotoxicity of silver nano-particles. In the study with BRL 3A liver cell line, depletion of GSH level and increased ROS was found in association with mitochondrial perturbation, suggesting that oxidative stress might mediate the cytotoxicity of silver nano-particles. Recently, it has been found that Ag+ seems to perturb mitochondria through interactions with thiol groups of the mitochondrial inner membrane. As these effects of Ag+ could be completely blocked by sulfhydryl reagents, e.g. reduced glutathione (GSH), the endings clearly suggest that mitochondria are under oxidative stress when the cells are exposed to Ag+ [57]. In rat models, copper toxicity has been seen to accompany MDA generation [58]. Excessive copper accumulation in the liver has also been shown to depress superoxide dismutase (SOD) and Se-GSH peroxides activities and to result in high MDA in the serum and liver homogenates due to the lipid per oxidation induced by copper overload [59]. Reactive oxygen species formation, GSH oxidation and lipid per oxidation have also been seen to be induced by copper [60]. Researchers were concluded that the cytotoxicity of silver nano-particles to the mitochondrial activity increased with the increase in the concentration of silver nano-particles. Recently, reported that nano-particles and nano-materials such as manganese, copper and silica generate free radicals and oxidative stress [57]. The results of researches showed that silver nano-particles can damage to different organs and tissue such as liver cells [8].

In general, the liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. It is a logical assumption that ingested silver nano-particles might have impact on the liver, since the liver serves as the first check point for everything absorbed through GIT before becoming systemic. Researches showed function of mitochondria [56], that exposure to silver nano-prarticles significantly decreased the mitochondria; seem to be sensitive targets of cytotoxicity of silver nano-particles. In the study with BRL 3A liver cell line, depletion of GSH level and increased ROS was found in association with mitochondrial perturbation, suggesting that oxidative stress might mediate the cytotoxicity of silver nano-particles. Recently, it has been found that Ag+ seems to perturb mitochondria through interactions with thiol groups of the mitochondrial inner membrane. As these effects of Ag+ could be completely blocked by sulfhydryl

reagents, e.g. reduced glutathione (GSH), the endings clearly suggest that mitochondria are under oxidative stress when the cells are exposed to Ag+ [57]. In rat models, copper toxicity has been seen to accompany MDA generation [9]. Excessive copper accumulation in the liver has also been shown to depress superoxide dismutase (SOD) and Se-GSH peroxidase activities and to result in high MDA in the serum and liver homogenates due to the lipid per oxidation induced by copper overload Reactive oxygen species formation, GSH oxidation and lipid per oxidation have also been seen to be induced by copper [59]. Also, we observed that the liver concentrations of silver were relatively low in comparison to muscle tissues, although liver is rich in various thiols and phosphates. In sheep, humans and seals, the liver has been reported to be the major site organ of silver deposition [22, 46]. Because, the major portion of the incorporated silver was bound in rats in the basal membrane of liver also, silver has a high affinity for SH groups and it decreases GSH-px activity, it may cause exhaustion of glutathione in liver [24].

In conclusion, the results of current research were indicated that:

- Nano particle of silver can absorb systemic due to GIT, which was in agreement with finding others researchers.
- Nano-silver could arrive to different organs and tissues due to blood circulation and then retention Ag in different tissue.
- Accumulation of silver (Ag) into muscles was further in comparison other organs, therefore, this situation has negative affected in the human healthy due to consumption parts of edible carcasses broiler for example: breast and femur muscle.
- The rate of silver retention not the same in different organs, this situation may be related to activity, blood flow rate in the organs.
- Excretion silver (Ag) due to faeces, may be caused contamination of environment.
- Nanosilver had negative affected on oxidative stress enzymes concentration.
- In addition, to understand total effects of nanosilver as nonmaterials, need to further research.

REFERENCES

1. British Standards Institution (BSI). 2007. PAS136 Terminology for nanomaterials. Accessed from the website: http://www.bsiglobal.com.
2. Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR). Opinion on: The scientific aspects of the existing and proposed definitions relating to products of nanoscience and nanotechnologies. European Commission Health & Consumer Protection Directorate-General. Directorate C-Public Health and Risk Assessment, 2008, C7-Risk Assessment.
3. Cheng, D.J. Yang and Y. Zhao, 2004. Antibacterialmaterialsofsilvernanoparticles application in medical appliances and appliances for daily use. Chin. Med. Equip., 4: 26-32.
4. Kreuter, J. and S. Gelperina, 2008. Use of nano particles force rebralcancer. Tumori., 94: 271-277.
5. Su, J., Zhang, L. Liu, Y, Huang and R.P. Mason, 2008. Exploring feasibility of multicolored Cd Tequantum dots for invitro and *in vivo* fluorescent imaging. J. Nanosci. Nanotechnol., 8: 1174-1177.
6. Tan, W.B., S. Jiang and Y. Zhang, 2007. Quantum-dot based nanoparticles for targeted silencing of HER2/neugene via RNA interference. Biomaterials., 28: 1565-1571.
7. Yoon, K.Y., J. Hoon, Byeon, J.H. Park and J. Hwang, 2007. Susceptibility constants of Escherichia coli and Bacillus subtilis silver and copper nanoparticles. Sci. Total Environ., 373: 572-575.
8. Hussain, S.M., K.L. Hess, J.M. Gearhart, K.T. Geiss and J.J. Schlager, 2005. *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol *in vitro*., 19: 975-983.
9. Hsin, Y.H., C.F. Chen, S. Huang, T.S. Shih, P.S. Lai and P.J. Chueh, 2008. The effect of nanosilver is mediated by a ROS-and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T 3 cells, Toxicol. Lett., 179: 130-139.
10. Kim, S., J.E. Choi, J. Choi, K.H. Chung, K. Park, J. Yi and D.Y. Ryu, 2009. Oxidative stress dependent toxicity of silver nanoparticles in human hepatomacells. Toxicol. *In vitro*., 23: 1076-1084.
11. Vigneshwaran, N., A.A. Kathe, P.V. Varadarajan, R.P. Nachane, Y. and R.H. Balasubramanya, 2007. Functional finishing of cotton fabricsusing silvernanoparticles. J. Nanosci. Nanotechnol., 7: 1893-1897.
12. WHO, Silver, 1996. Guidelines for Drinking-water Quality; Volume 2, Geneva, New York., pp: 338-343.
13. EPA, 1980. Ambient water quality criteria for silver. Washington, DC. EPA., 5/80/071.

14. Sjoblom, K.L. and J. Ojala, 1981. Initial experience with aquatic environmental monitoring programmes around Finnish nuclear power plants. IAEA-SM-248/110., pp: 515-532.
15. Smith, D.R., M.D. Stephenson and A.R. Flegal, 1986. Trace metals in mussels transplanted to San Francisco Bay. Environ. Toxicol. Chem., 5: 129-138.
16. George, S.G., B.J.S. Pirie, A. Calabrese and D.A. Nelson, 1986. Biochemical and ultrastructural observations of long-term silver accumulation in the mussel, Mytilus edulis. Mar. Environ. Res., 18: 255-265.
17. Topcuoğlu, S., E. Birol and M.Y. Unlu, 1987. Factors affecting the accumulation and elimination of silver (110mAg) in marine isopods. Mar. Environ. Res., 21: 189-198.
18. Grosell, M. and C.M. Wood, 2001. Branchial versus intestinal silver toxicity and uptake in the marine teleost Paraphrys vetulus. J. Comp. Physiol. (B)., 171: 585-594.
19. Webb, N.A. and C.M. Wood, 2002. Bioaccumulation and distribution of silver in four marine teleost and two marine elasmobranches: influence of exposure duration, concentration and salinity. Aquatic Toxicol., 49: 111-129.
20. Furchner, J.E., C.R. Richmond and G.A. Drake, 1968. Comparative metabolism of radionuclides in mammals. IV. Retention of silver-110m in the mouse, rat, monkey and dog. Health Phys., 55: 398-401.
21. Beresford, N.A., 1989. The transfer of Ag -110m to sheep tissues. Sci. Total Environ., 85: 81-90.
22. Saeki, K., M. Nakjimi, T. Loughlin, D.C. Calkins, N. Baba, M. Kiyota and R. Tatsukawa, 2001. Accumulation of silver in the liver of three species of pinnipeds. Environ. Pollut., 112: 19-25.
23. Lansdown, A.B.G. Silver, 2002. Toxicity in mammals and how its products aid wound repair. J. Wound Care., 11: 173-177.
24. Rungby, J., 1990. An experimental study on silver in the nervous system and on aspects of its general cellular toxicity. Danish Med. Bull., 37: 442-449.
25. Stoltenberg, M., S. Juhl, E.H. Poulsen and E. Ernst, 1994. Autometallographic detection of silver in hypothalamic neurones of rats exposed to silver nitrate. J. Appl. Toxicol., 14: 275-280.
26. Van Vleet, J.F. and V.J. Ferrans, 1992. Etiologic factors and pathologic alterations in selenium-vitamin E deficiency and excess in animals and humans. Biol. Trace Elem. Res., 33: 1-21.
27. Toop, J., R.E. Burke, Dum, R.P. M.J. O'Donovan and C.B. Smith, 1982. 2-Deoxyglucose autoradiography of single motor units: labelling of individual acutely active muscle fibers. Neuro Sci. Methods, 5: 283-289.
28. Kalachniuk, H.I., M. Marounek, L.H. Kalachniuk and O.H. Savka, 1994. Rumen bacterial metabolism as affected by extracellular redox potential (Metabolizm bakterii rubtsia za dii zovnishn'oklitynnoho redoks-potentsialu). Ukr. Biokhim. Zh., 66: 30-40.
29. Rosenman, K.D., N. Seixas and I. Jacobs, 1987. Potential nephrotoxic effects of exposure to silver. Br. J. Ind. Med., 44: 267-272.
30. NRC. 1994. Nutrient Requirements of Poultry. 9th ed. National Academy of Science, Washington, DC.
31. SAS Institute. 1996. User's Guide SAS Institute Inc., Cary, NC.
32. Humphreys, S.D.M. and P.A. Routledge, 1998. The toxicology of silver nitrate. Adverse Drug React. Toxicol. Rev., 17: 115-143.
33. Drasch, G., H.J. Gath, E. Heissler, I. Schupp and G. Roider, 1995. Silver concentrations in human tissues. Their dependence on dental amalgam and other factors. J. Trace. Elem. Med. Biol., 9: 82-87.
34. Kai, H.O. Pelkonen, Helvi, Heinonen-Tanski, O.P. Osmo and Hänninen, 2003. Accumulation of silver from drinking water into cerebellum and musculus soleus in Mice. Toxicol., 186: 151-157.
35. Ahmadi, F. and F. Rahimi, 2011. The Effect of Different Levels of Nano Silver on Performance and retention of Silver in Edible Tissues of Broilers. World Appl. Sci. J., 12: 01-04.
36. Hultman, P., U. Lindh and P. Hörsted-Blinslev, 1998. Activation of immune system and systemic immune-complex deposits in Brown Norway rats with dental amalgam restorations. J. Dent. Res., 77: 1415-1425.
37. Brooking, J., S.S. Davis and L. Fungus, 2001. Transport of nanoparticles across the rat nasal mucosa. J. Drug Targeting., 9: 267-279.
38. Hoet, P.H., I. Bruske-Hohlfeld and O.V. Salata, 2004. Nanoparticles known and unknown health risks.
39. Oberdörster, E., 2004. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environ. Health Perspect., 112: 1058-1062.
40. Rungby, J. and G. Danscher, 1984. Hypoactivity in silver treated mice. Acta Pharmacol. Toxicol. Copenh., 55: 398-401.
41. Sarin, H., 2008. Effective transvascular delivery of nanoparticles across the blood-brain tumor barrier into malignant glioma cells. J. Transl. Med., 6: 80.

42. Muthu, M.S. and S. Singh, 2009. Targeted nanomedicines. Effective treatment modalities for cancer, AIDS and brain disorders. Nanomedicine (Lond.), 4: 105-118.
43. Tang, M., 2008. Unmodified Cd Se quantum dots induce elevation of cytoplasmic calcium levels and impairment of functional properties of sodium channels in rat primary cultured hippocampal neurons. Environ. Health Perspect, 116: 915-922.
44. Au, C., L. Mutkus, A. Dobson, J. Riffle and J. Lalli, 2007. Aschner, M. Effects of nanoparticles on the adhesion and cell viability on astrocytes. Biol. Trace Elem. Res., 120: 248-256.
45. Sharma, H.S. and A. Sharma, 2007. Nanoparticles aggravate heat stress induced cognitive deficits, blood-brain barrier disruption, edema formation and brain pathology. Prog. Brain Res., 162: 245-273.
46. Beresford, N.A., R.W. Mayes, N.M. Crout, Howard and B.J. Kanyar, 1994. Dynamic behaviour of 110mAg in sheep tissues. Health Phys., 66: 420-426.
47. Webb, N.A. and C.M. Wood, 2002. Bioaccumulation and distribution of silver in four marine teleost and two marine elasmobranches: influence of exposure duration, concentration and salinity. Aquatic Toxicol. 49: 111-129.
48. Wataha, J.C., P.E. Lockwood, A. Schedle, M. Noda and S. Bouillaguet, 2002. Ag, Cu, Hg Ni ions alter the metabolism of human monocytes during extended low-dose exposures. J. Oral Rehabil., 29: 133-139.
49. Hanson, S.R., S.A. Donley and M.C. Linder, 2001. Transport of silver in virgin and lactating rats and relation to copper. J. Trace Elem. Med. Biol., 15: 243-253.
50. Oberdörster, G., Z. Sharp, V. Atudorei, A. Elder, R. Gelein, W. Kreyling and C. Cox, 2004. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol., 16: 437- 445.
51. Takenaka S., E. Karg, C. Roth, H. Schulz, A. Ziesenis, U. Heinzmann, P. Schramel and J. Heyder, 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect., 109(Suppl. 4): 547-551.
52. Oberdörster, G., E. Oberdörster and J. Oberdörster, 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect., 113: 823-839.
53. Lovric, J., H.S. Bazzi, Y. Cuie, G.R. Fortin, F.M. Winnik and D. Maysinger, 2005. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. J. Mol. Med., 83: 377-385.
54. Ji, J.H., J.H. Jung, S.S. Kim, J.U. Yoon, J.D. Park, B.S. Choi, Y.H. Chung, I.H. Kwon, J. Jeong, B.S. Han, J. H. Shin, J.H. Sung, K.S. Song and I.J. Yu, 2007. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol., 19: 857-871.
55. Braydich-Stolle, L., S. Hussain, J.J. Schlager and M.C. Hofmann, 2005. *In vitro* cytotoxicity of nanoparticles in mammalian germ line stem cells. Toxicological Sci., 88: 412-419.
56. Hussain, S.M., A.K. Javorina, A.M. Schrand, H.M. Duhart, S.F. Ali and J.J. Schlager, 2006. The interaction of manganese nano-particles with PC-12 cells induces dopamine depletion. Toxicol. Sci., 92: 456-63.
57. Almofti, M.R., T. Ichikawa, K. Yamashita, H. Terada and Y. Shinohara, 2003. Silver ion induces a cyclosporine a-insensitive permeability transition in rat liver mitochondria and release of apoptogenic cytochrome. C. J. Biochem., 134: 43-49.
58. Sokol, R.J., M.W. Devereaux and M.G. Traber, 1989. Copper toxicity and lipid peroxidation in isolated rat hepatocytes: effect of vitamin E. Pediatr Res., 25: 55-62.
59. Zhang, S.S., M.M. Noordin and S.O. Rahman, 2000. Effects of copper overload on hepatic lipid peroxidation and antioxidant defense in rats. Vet Hum Topical., 2000, 42, 261-264.
60. Pourahmad, J. and P.J. öBrien, 2000. A comparison of hepatocyte cytotoxic mechanisms for Cu^{2+} and Cd^{2+}. Toxicol., 143: 263-273.