INTERNATIONAL JOURNAL OF ADVANCE RESEARCH AND DEVELOPMENT

(Volume3, Issue2)

Available online at www.ijarnd.com

Toxicity of Silver Nanoparticles

Sonal Bhasker More[1], Pooja Belapurkar[2], Gaurav Patil[3], Mahalaxmi Mohan[4]

Student, Mahatma Gandhi Vidyamandir's Pharmacy College, Nashik, Maharashtra

Head of the Department, Mahatma Gandhi Vidyamandir's Pharmacy College, Nashik, Maharashtra

ABSTRACT

The application of nanoparticles (NPs) has increased recently because of the important antimicrobial activities of these nanomaterial's, allowing their use in several industrial sectors. Due to these applications. There is an increasing concern related to the biological impacts of the use of silver nanoparticles on a large scale and the possible risk to the environment and health. Nowadays, some recent studies have been published based on the investigation of potential inflammatory effects, diverse cellular impacts of silver nanoparticles and other important issue related to nanoparticle toxicity. These nanoparticles damage the genetic material, by crossing the cell membranes. Therefore, there is an increasing interest in the analysis of potential nanoparticle toxicity. With the increasing application of NPs in medical contexts, it is becoming necessary for a better understanding of the mechanisms of silver NPs biological interactions and their potential toxicity. Some unique properties of silver NPs, such as antibacterial, antifungal, antiviral, anti-inflammatory activity and toxicity to invertebrates and vertebrates are discussed in this article. Finally, Potential toxicology considerations of Ag NPs, both in vitro and in vivo, are also addressed.

Keywords: Silver Nanoparticles, Toxicity, Antibacterial, Mechanism etc.

1. INTRODUCTION

Silver is a naturally occurring precious metal, mostly as a mineral ore in association with other elements. It has been positioned as the 47th element in the periodic table, having an atomic weight of 107.8 and two naturale isotopes 106.90 Ag and 108.90 Ag with abundance 52 and 48%. It has been used in a wide variety of applications as it has some special properties like high electrical and thermal conductivity (1). Ancient civilizations used this precious metal in medicine, eating utensils, plates, cups, food containers, jewelry, money, clothes, building materials, and as a disinfectant for water and human infection.

Whenever silver metals and silver dressings are used in reasonable amounts has no negative effects on the human body and it has a natural antimicrobial activity (2) towards many pathogens such as bacteria (3) Viruses, fungi, yeast etc. Silver salts have been used for the treatment of mental illness, nicotine addiction, gastroenteritis and infectious diseases like syphilis and gonorrhoea (4). Use of new silver coated catheters which are because they stop the infections that were common with the old ones (5). To protect us from food poisoning, silver particles are now being put on cutting boards, table tops, surface disinfectants, and refrigerators. Silver is woven and impregnated into fabrics to kill bacteria that cause body odour.

Even in its bulk form, silver is extremely toxic to fish (6), algae, some plants, fungi (7) crustaceans and bacteria like nitrogen fixing heterophic and soil forming chemolithotrophic bacteria (8).

In the environment or in living organisms silver can be present in different forms. The most common is metallic silver, silver salts (ionic silver), silver complexes and colloidal silver. Metallic silver dissolves in acids and salts like e.g. silver nitrate contain silver in the form of hydrated silver cations.

Silver cations can be complexed with various organic ligands and even if silver cations are still present in the molecule, the overall charge of the complex can be known which are not dissociated at all in the solution or biological liquids. Colloidal silver is a colloidal state of silver-containing particles in water with 1nm to 1 micron silver or silver-containing particles. The Thomas Graham (in 1800) who was later on called as in the form of super-fine metallic form or in the form of insoluble silver compounds, finally dispersed in a solution which shows small or high turbidity. Nano silver means usually nano-sized particles of nanometre size and the term is connected to colloidal silver or identical. There are now many commercial nano-silver refers to the nanoparticulate size of the active surface area with better porosity than commercial silver (8).

Nanotechnology has been rapidly growing in recently years and has impact on diverse areas like as the economy and the environment. In this context, the number of commercial products comprising nanomaterial is now increasing. Out of all commercially available nano-sized materials, Silver nanoparticles are most used nano compounds (9) owing to its potent antimicrobial property (10). The novel properties of silver nano properties have been used in medicine, cosmetics, household and medical products as well as in textile and food products (11, 12). Silver nano particles have attracted increasing interest due to their unique physical, Chemical and biological properties including high electrical and thermal conductivity, Surface-enhanced Roman scattering chemical stability, Catalytic activity and nonlinear optical behavior (13, 14).

There is also growing evidence that is being toxic to bacteria, silver nanoparticles are also highly toxic to mammalian cells (15). Silver nano particles have been shown to damage brain cells (16), Liver cells (17). Even with prolonged exposure to colloidal silver or silver salt deposits of metallic silver under the skin cause skin diseases like argyria or argyrosis (18).

Nanosilver can be used in a liquid from such as a colloidal (Coating & Spray) or in Shampoo (liquid) & can also appear embedded in a solid polymer master batch or be suspended in a bar of soap (Solid). Nanosilver used in the textile industry, AgNPs create a long-term protective barrier against bacterial, fungal pathogens (19-21), According to the project on Emerging Nanotechnologies (22) over 1300 manufacturer identified, out of these around products utilizing Nanosilver. So nanosilver is the largest and fastest growing class of nanoparticles in consumer product applications.

The development of nanotechnology has resulted in a growing public debate on the toxicity and environmental impact of direct and indirect exposure to nanoparticles (23, 24). Actually, Nanoparticles may have higher toxicity than bulk materials (25). Nanoparticles toxicity on human health and environment is not explored (26). Concerning human health, The toxicity evaluation of AgNPs has been carried out in different cellular models such as human lung fibroblast (27). Indeed, Oxidative stress and severe lipid peroxidation have been observed in fish brain tissue when exposed to NPs (28). This review concludes that there are some publications focussing on silver nano particles toxicities. It has been observed that AgNPs lead to an increase in ROS associated with DNA damage, apoptosis, and necrosis (29, 30).

In this context, our aim of this review is to present and discuss the new publications of silver nanoparticles toxicity and highlight their importance.

2. NANO SILVER PARTICLE SYNTHESIS (NSP)

Different synthetic NSP routes lead to variable sizes, shapes, morphology, and even stability. Generally, these methods can be classified into three broad categories: physical, chemical, and biological (or green) synthesis.

Physical Synthesis

Evaporation/condensation and laser ablation are the main physical techniques for deriving nano silver from metal samples. The evaporation/condensation technique uses a furnace tube under atmospheric pressure to produce NSPs; however, conventional furnace tubes have several drawbacks, such as high energy consumption, and require a long time to achieve thermal stability. *Jung et al* used a small ceramic heater with a local heating area, thus the evaporated vapour could cool at a suitable rate and a high concentration of nano silver could be obtained (31). Laser synthesis employs the laser ablation of metals in solution without chemical reagents, which leads to pure nano silver colloids (32).

Recently, Tien et al reported a novel arc-discharge method of producing silver suspension in pure water without any surfactants or stabilizers (33).

Chemical Synthesis

Chemical reduction is the method which is mostly used the method of nanosilver synthesis and uses silver salt, reducts, and a stabilizer or capping agent as three main components to control NSP growth. Among these, silver nitrate is a silver salt that is often used for NSPs, due to its low cost and chemical stability compared to the other available silver salts.
(33).The reductants include borohydride (34), citrate (35), ascorbate (36) and hydrogen gas (37).

Borohydride is a strong reducing agent that can result in small particles with a faster reduction rate because borohydride can also act as an NSP stabilizer and avoid aggregation of NSPs during its decomposition (37).

3. POTENTIAL APPLICATIONS OF SILVER NANOPARTICLES

Antibacterial Property of Silver nanoparticles

NSPs have a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria and antibiotic-resistant bacteria strains (38) Antimicrobial efficacy of NSPs depends on their size and concentration. Normally, a high concentration leads to a more effective antimicrobial activity, while particles of small sizes can kill bacteria at a lower concentration. Not only the from size and concentration but also shape influences the antimicrobial efficiency of NSPs. Sadeghi *et al* investigated the antimicrobial activity of different nanosilver shapes, which included silver nanoplates, silver nanorods, and silver nanoparticles, on *Staphylococcus aureus* and *E. coli*. They found that silver nanoplates had the best antimicrobial activity (39). It has also been reported that NSPs combined with various antibiotics have better antimicrobial effects than NSPs or antibiotics alone. Li et al, for example, found a greater antibacterial effect on *E. coli* when amoxicillin and silver nanoparticles were combined than when they were applied separately (40) although the antimicrobial effect of nanosilver has been widely studied, the exact mechanism of NSPs is still elusive.

Antiviral Properties

NSPs are also an antiviral agent against HIV-1, (41) hepatitis B virus, (42) respiratory syncytial virus, (43) herpes simplex virus type 1, (44) and monkey pox virus (45). It has been observed that NSPs have higher antiviral activity than silver ions, due to species difference as they dissolve to release Ag0 (atomic) and Ag+ (ionic) clusters, whereas silver salts release Ag+ only (46) Lara found that the anti-HIV mechanism of nanosilver is based on the inhibition of the initial stages of the HIV-1 cycle (47). NSPs can bind to glycoprotein (gps) 120, thus inhibit cluster of differentiation (CD) 4-dependent binding, fusion, and infectivity. They act as an effective virucidal agent to block HIV-1 cell-free and cell-associated infection. Furthermore, NSPs inhibit post-entry stages of the HIV-1 life cycle (47). Although the mechanism underlying their viral-inhibitory activity is not yet fully understood, NSPs could be considered to be a broad-spectrum agent against a variety of viral strains and are not prone to developing resistance.

Anti-inflammatory Properties

NSPs show anti-inflammatory properties in both animal models and in the clinic. In the swine model with contact dermatitis induced by topically applying 1, 2 dinitrochloro- benzene, nanosilver altered the expression of pro-inflammatory cytokines transforming growth factor-b and tumour necrosis factor-a (48). Shin and Ye found that NSPs attenuated nasal symptoms in allergic rhinitis mice and inhibited OVA-specific immunoglobulin E, IL-4, and interleukin-1, and that inflammatory cell infiltration and goblet cell hyperplasia was inhibited by nanosilver (49). In a human clinical study, wound dressing containing NSPs promoted the healing of chronic leg ulcers by not only reducing bacteria numbers in the wound bed but by decreasing inflammatory response as well. NSPs ability to reduce cytokine release and matrix metalloproteinase, (50) decrease lymphocyte and mast cell infiltration (51) and induce apoptosis in inflammatory cells (52) may explain their anti-inflammatory mechanisms.

Medical NSP Applications for Wound Dressings

Robert Burrell developed the world's first commercially available nanosilver product (Acticoat™; Smith and Nephew, London, UK) to treat various wounds and wound healing activity, including burns, chronic ulcers, toxic epidermal necrosis, and pemphigus (53). Huang et al observed that NSP-loaded wound dressings significantly reduced the healing time by an average of 3-35 days and increased bacterial clearance from infected wounds compared to silver sulfadiazine, with no adverse effects (54). However, scientists showed that nanosilver-loaded wound dressings could enhance healing in superficial burn wounds but made no difference in deep burn wounds, compared with 1% silver sulfadiazine (55). This result suggests NSPs accelerate re-epithelialization but not angiogenesis.

Recently, new dressings are being fabricated with the aim of increasing antibacterial efficacy and promotion of wound healing. Lu *et al* developed a wound dressing composed of NSPs and chitosan, and found that it significantly increased wound healing during treatment of deep partial-thickness wounds and inhibited infection, as well as diminished the risk of silver absorption, compared with 1% silver sulfadiazine dressings (56).

Cardiovascular Implants

The first cardiovascular medical device containing silver in the clinic was a prosthetic silicone heart valve coated with the silver element, which was designed to prevent bacterial infection on the silicone valve and to reduce inflammation response (57). However, metal silver may cause hypersensitivity, inhibits normal fibroblast function, and leads to paravalvular leakage in patients (58). NSPs are safe and nontoxic in medical devices, unlike metal silver. Therefore, Andara *et al* synthesized a new nanocomposite with NSPs and diamond-like carbon as a surface coating for heart valves and stents, and found that the surface of the nanocomposite showed antithrombogenic and antibacterial properties (59). In addition, they constructed antibacterial multilayer films containing NSPs, and investigated their antibacterial, mechanical, and hemodynamic properties in vitro for use in cardiovascular implant coating.

Catheters

The lot of research has been conducted to investigate NSPs as antibacterial materials for coating catheters, including central venous catheters and neurosurgical catheters. Silver line (60) and ON-Q Silver Soaker™ (61) are two commercially available medical catheters containing NSPs to prevent catheter-associated infections (62) Medical catheters are prone to bacterial infection, which can rapidly spread to the wound and its surrounding, and lead to serious complications. Because of their superior antibacterial properties and lack of observed toxicity, NSPs can decrease the incidence of bacterial infection and complications after surgery, thus they have been widely accepted for use in medical catheters.

Bone Cement

Alt *et al* evaluated antibacterial activity of plain poly (methyl methacrylate) bone cement loaded with different NSP concentrations in vitro, and found that bone cement-loaded 1% nanosilver completely inhibited the proliferation of *Staphylococcus epidermidis*, *methicillin-resistant S. epidermidis,* and *Methicillin-resistant S. aureus*, with no significant difference between the nanosilver bone cement and the nontoxic control group in quantitative and qualitative cytotoxicity tests (63). NSPs were also added to ultra-high-molecular-weight polyethylene for fabricating inserts for total joint replacement components, and it was found that NSPs drastically reduced the wear and tear of the polymer (64).

Dental Materials

NSPs also have applications in dental instruments and bandages. Yoshida et al showed that a resin composite incorporated with NSP-containing materials had a long-term inhibitory effect against *Streptococcus mutans* (65). Yamamoto et al also showed that a resin composite containing silver ion-implanted fillers released silver ions with antibacterial effects on oral *streptococci.* (66) In

addition, Magalhaes et al showed that incorporating NSPs in endodontic filling materials provided a significantly enhanced anti-bactericidal effect against *Streptococcus milleri*, *S. aureus*, and *Enterococcus faecalis* (67) NSPs in dental adhesives are also very effective against streptococci without affecting the adhesive mechanical properties, thus enabling their use in orthodontic treatments (68).

Biodiagnosis

NSPs can be used for bio-diagnosis, where plasmonic properties of NSPs strongly depend on size, shape, and dielectric medium that surrounds it (69) Zhou et al developed a silver nanoparticle array biosensor for clinical detection serum p53 in head and neck squamous cell carcinoma (70). NSPs are also employed to produce dual-imaging/therapy-immunotargeted nano shells to locate cancer cells and can absorb light and selectively destroy targeted cancer cells through photo thermal therapy (71).

Toxicity to Animals

- **Silver Nanoparticle toxicity on *Daphnia magna***

Silver nanoparticles size 35 nm effects on *Daphnia magna* were studied and the results showed that 100% mortality occurred for 1µg/ml during 96h and 43.33% for 0.1µg/ml treatment. In chronic exposure, *Daphnia magna* was exposed to lower concentration of silver nanoparticles showed significant toxicity at 0.001 µg/ml (72). Genotoxicity and ecotoxicity assessment of AgNPs (50nm) were studied on the fresh water crustacean *Daphnia magna*. Acute toxicity up to 2µg/ml concentration showed 100% mortality(244;LD50~1.2µg/ml) DNA stand breaks increased after exposure to AgNPs to approximately 4&8 time higher levels than the negative control at the concentrations of 1& 1.5µg/ml respectively, Increased mortality was parallelly observed with DNA damage in the silver nanoparticles. Which suggest that AgNPs induced DNA damage in the silver nanoparticles, which suggest that AgNPs induced DNA damage high provoke higher level consequences. The results of comparative study suggest that the former were slightly more toxic (73).

- **AgNPs toxicity on *zebra* fish-**

The response of zebra fish, *Daphnia* and *Pseudokirchneriella subcapitata* to 20-30 nm commercial silver nanoparticles was studied. AgNPs induced toxicity after 48hrs with an LD50 of 7.0-7.2 µg/ml in zebra fish and 0.040- 0.067 µg/ml in Daphnia. In *P.Sulcapitata* the LD50 was 0.19µg/ml. No effects on survival up to 5µg/ml were observed for soluble metal treatment, zebra fish presented on LD50 of 0.022µg/ml and for Daphnia the LD50 was found to be 0.008µg/ml. This result shows a greater toxicity of silver soluble metal compared with AgNPs (74). Effect of 5-20nm AgNPs on zebra fish was studied by Asha Rani et al (2005). The LD50 between 25 and 50 µg/ml causes alteration to cell nucleus and breaking of DNA.

- **AgNPs toxicity to Rat / mice**

Short term and high doses of AgNPs on rats /mice causes hepatotoxicity and oxidative stress (75). There are evidences that AgNPs administration to wistar rats/mice altered some biochemical parameters. Repeated use of AgNPs orally for testing may cause a mild to moderate toxicity. A dose dependent toxic responses in liver tissues are observed. Silver nanoparticles proves to be highly toxic through repeated oral gavage exposure. AgNPs cause inflammation mediator expression in liver histology. Large size particle not able to penetrate the cell membrane effectively but pressurizes the area where it abnormally accumulates (76). Small sized AgNPs are more active to exert toxicological response and they induce organ toxicity and inflammatory responses by repeated oral administration (77).

The current study showed that silver nanoparticles were predominantly localized in the liver in both sexes of mice/rats and this accumulation of nanoparticles in livers caused remarkable hepatic toxicity (78). Nanosilver particles enter into the body, especially in the gastrointestinal tract, is in colloidal form (79).

As liver organ is able to actively remove compounds from the blood and transform them into chemical forms that can be easily be excreted (80). So, silver nanoparticles might have an impact on the liver as a major organ of detoxification. Increasing serum AST and ALT levels indicated that liver tissues were damaged. These were confirmed by histological microscopy and by some other studies for instance in histological microscopy and by some other studies for instance in the histological analysis (81).

Nanosilver elevates the hepatic enzyme. So, that AST level in serum elevates. Silver nanoparticle accumulates mainly in the liver because it contains high level of thiol rich proteins such as glutathione (82, 83). Arora S, in 2009 reported AgNPs (7- 20nm) with final concentration of 10-200 µg/ml (10-200ppm) can cause oxidative stress, apoptosis and decreased cell viability in fibroblasts and liver cells which is isolated from swiss albino mice or rats & AgNPs proves to be highly toxic to primary hepatocytes (84,85). Histopathological examination of the liver revealed that various alterations denoting the hepatotoxic effects of AgNPs including hepatocellular degeneration, necrosis, and individual apoptosis, several studies confirmed that liver is the largest organ for the effect of silver nanoparticles (36-89). The AgNPs were highly toxic in rats/mice liver cell (90).

Lung and liver were the major largest tissues for prolonged AgNPs exposure and no observable adverse effect level (NOAEL) of AgNPs was determined as 100µg/ml (91). Another exposure route of AgNPs, Oral rout may be important in many consumer products such as tooth paste, reusable bottles, nursing nipples, kitchen utensils and toys (92, 93). When mice were treated with AgNPs small

sized AgNPs were accumulated in brain, lung, liver, kidney, and tests. While, when rats/mice were treated with AgNPs by injection, AgNPs trans located to the blood circulation and distributed throughout the main organs especially in the kidney, liver, spleen, brain, and lungs in the form of particles (94).

Effects of Silver Nanoparticles

i) Effects of Silver on Body Weight

The growth rates were retarded in rats administered a 0.25% silver nitrate solution in the drinking water for 8½ months (this dose corresponds to 81 mg/kg of bw/day)(95). When silver was withdrawn, the body weight became normalised over the course of 10 weeks. Body weight gain decreased following the oral administration of silver acetate (9 mg of silver/kg of bw/day) (96). Regarding nano particulates silver, Shahare and Yashpal observed that oral exposure in mice for 21 days to 5 mg/kg of bw/day of 5–20 nm silver nanoparticles decreased body weight (97). In a 13 week study of the oral administration of 500 mg of 60 nm silver nanoparticles/kg of bw/day in rats, decreased body weight was observed for males only (98). It should be noted that in a range of oral investigations, no effects on body weight were reported (99-106).

ii) Effects on the Gastrointestinal Tract

High silver deposition has been demonstrated in the gastrointestinal tract in oral toxicity experiments (107,108). Three investigations reported pathological findings. Silver nanoparticles (5–20 nm) damaged the epithelial cell microvilli and intestinal glands after administration for 21 days to mice at doses of 20 mg/kg of bw (109). Moreover, Jeong *et al*. (2010) found increased numbers of goblet cells in the intestine that had released their mucu granules following the oral administration of 30 mg/kg of bw/day of 60 nm nanoparticles for 28 days. In a 13 week investigation in rats, Kim *et al*. (2010) reported abnormal pigmentation of the ileum following the administration of 125 and 500 mg/kg of bw/day of 56 nm silver nanoparticles.

iii) Effects on the Liver and Bile Ducts

Effects on several enzymes that are indicative of liver and bile duct function have been reported. Following the oral administration of ionic silver in the form of silver acetate (9 mg of silver/kg of bw/day), increased plasma alkaline phosphatase and decreased plasma urea were observed. Following the oral administration of ionic silver in the form of silver acetate (9 mg of silver/kg of bw/day) or nanoparticulate silver (9 mg of 14 nm particles/kg of bw/day) the urinary excretion of uric acid and its metabolite allantoin were increased (99). In a 28 day investigation of the oral administration of 60 nm silver nanoparticles, Kim *et al*. detected increased plasma alkaline phosphatase at doses of 300 mg/kg of bw/day in males and 1000 mg/kg of bw/day (both sexes), and cholesterol at a dose (1000 mg/kg of bw/day, only in female rats). Bile duct hyperplasia, with eosinophil infiltration of the hepatic lobules and portal tract, were also reported (108). In a 13 week investigation of the oral administration of 56 nm silver nanoparticles, rat alkaline phosphatase levels were increased at doses of 500 mg/kg of bw/day. Additionally, increase in serum cholesterol were recorded at the 125 mg/kg of bw/day dose for males sand above were recorded for females following the oral administration of 500 mg/kg of bw/day. In addition, bile duct hyperplasia was reported (99). Van der Zande *et al*. (2012) investigated the hepatotoxicity of 90 mg/kg of bw of silver nanoparticles or 9 mg/kg of bw/day of ionic silver for 28 days.

iv) Effects on Haematology and Kidney

Hadrup *et al*. (2012b) found increased haematocrit following the oral administration of 14 nm nanoparticulate silver at a dose of 9 mg/kg of bw/day for 28 days in rats. Espinosa-Cristobal et al. (2013) observed increased haematocrit, haemoglobin and blood urea nitrogen in female rat administered 14 and 36 nm silver nanoparticles at a concentration of 535 mg/L in the drinking water for 25 days (corresponding to a dose of 65 mg/kg of bw/day). Increases in these blood parameters suggest a higher need for oxygen transport in these animals, or alternatively, that silver affects the mechanisms underlying blood cell physiology. Sardari *et al.* (2012) reported histopathological findings in the spleens (decreased red pulp and increased white pulp) and kidneys (necrosis) in rats that were orally administered 1 or 2 mg/kg of bw/day of 70 nm silver nanoparticles for 30 days. Decreased red pulp potentially affects red blood cell clearance; whereas necrosis of glomerular cells, the Bowman's capsule and proximal tubular cells in the kidney potentially affect body fluid homeostasis. Hadrup *et al*. (2012b) investigated the heart and kidneys, but found no histological changes following the oral administration of 9 mg/kg of bw of ionic or 14 nm nanoparticulate silver. In addition, Kim *et al*. (2008, 2010) found no histopathological changes in the heart after up to 13 weeks of treatment with 56–60 nm silver nanoparticles at doses up to 1000 mg/kg of bw/day.

v) Effects on the Reproductive System

Changes in ovarian nuclear and cytoplasmic cell morphology, such as the disappearance of the endoplasmic reticulum and transformed mitochondrial morphology, were found in mice administered 0.03% silver nitrate in the drinking water for 1 month (corresponding to 23 mg of silver/kg of bw/day) (97). Shavlovski *et al*. (1995) investigated embryo toxicity in female rats that had been orally administered 50 mg of silver chloride/animal (corresponding to 190 mg of silver/kg of bw/day) during days 1–20 of the term. Post-implantation lethality was found to be increased, and the incidence of visceral damage in the offspring was considerably higher compared to the control group. Moreover, all of the new borns died within 24 h of birth.

vi) Effect on Central Nervous System

Whether silver crosses the blood brain barrier remains controversial, but even in absence of silver in the brain extracellular fluid, silver-induced neurotoxic effects may occur via secondary molecules that are released from the periphery. Rung by *et al*. found that 0.015% silver nitrate in the drinking water for 125 days (14 mg/kg of bw/day) induced hypoactivity in mice after a 10 day silver

withdrawal period (109). In another investigation, it was reported that an increase in liveliness was observed in rats given silver nitrate in the drinking water at 0.01% for 4 months (110).Scientist investigated the effects of the oral administration of ionic and 14 nm nanoparticulate silver for 28 days on neurotransmitters in rats (111). At doses of 2.25 mg/kg of bw/day of nanoparticulate silver and 9 mg/kg of bw/day of ionic silver (the lowest ionic silver dose investigated), alterations in noradrenaline, dopamine and 5-HT concentrations were found in the brain. Regarding human case studies (112). A case of convulsive seizures in a woman has been described following the ingestion of 200 silver containing antismoking pills per day for 40 years. In addition, Mirsattari observed myoclonic status epileptic us in man following the oral ingestion of excessive amounts of colloidal silver (113).

viii) Effect of Silver Nanoparticles Administrated In vitro

Culture Cells

No genotoxicity effects were observed for different human culture cells owing to incubation with up to 10 mg/ml of capped silver nanoparticles (diameters of 6–80 nm; (114-118).However, some cases described in the literature, e.g. culture of human mesenchymal stem cells incubated with 0.1 mg/ml of albumin-capped silver nanoparticles (average size 46 nm (106) or of normal human lung fibroblast cells, or human glioblastoma cells starch incubated with capped silver nanoparticles (sizes 6–20nm (15), showed genotoxicity up to nanoparticle doses of 50 mg/ml. Albumin-capped silver nanoparticles were reported to be more genotoxic than polysaccharide-capped ones. Indeed, silver nanoparticles capped with albumin (size 70 nm) were found to be more genotoxics on a mouse peritoneal macrophage cell line (genotoxicity at around 2 mg/ml(119) compared with silver nanoparticles capped with polysaccharides(size 25 nm) on mouse embryonic stem and fibroblasts, which exhibited genotoxicity at 50 mg/ml (120). In addition, from the reviewed literature, there is a tendency for human culture cells treated with capped silver nanoparticles to be less sensitive than mouse culture cells, independent of the kind of capped silver nanoparticles, with some exceptions. This tendency should be investigated in further studies.

Table- Toxicity of Ag-NPs on Various Organisms

Cell lines/ Organisms	Particle Size	Surface Stability	Dose	Exposure time	Major outcomes	Ref.
In vitro Mouse	15nm	None	EC50: 8.75µg/ml	48h	Concentration dependant toxicity Reduced mitochondrial function drastically and increased membrane leakage	121
Rat liver-derived cell line (BRL 3A)	15 and 100nm	None	5-50µg/ml	24h	Depletion of reduced glutathione (GSH) level. Reduced mitochondrial potential and increase in ROS levels.	122
Rat alveolar macrophages	15,30 and 55 nm	None	10-75µg/ml	24h	A Size dependant toxicity mechanism of toxicity was found to be largely mediated through oxidative stress.	123
Mouse fibroblasts and liver cells	7-10nm	None	>1mg/l	24h	Cell proliferation and chemotaxis were decreased while IL-8 release was increased.	124
Rat liver mitochondria	40-80nm	None	2 and 5µg/mg protein	25min.	Cause impairment of mitochondrial function uncoupling effect on the oxidative phosphorylation..	125

4. CONCLUSION

Silver nanoparticles are one of the most attractive nanomaterials for commercialization application. They have been widely used as antibacterial, antiviral, anti-inflammatory and biomedical products. In this review, we have given a comprehensive data of AgNPs toxicity. We first gave an overview of NSPs synthesis, then reviewed applications and possible toxicity to *Daphnia magna*, Zebra fish, Rat, and mice. It has been reported that ingestion of colloidal silver has also been linked with effect on gastrointestinal tract, liver and bile duct, cardiovascular system, reproductive and nervous system etc. Having a surface area larger and smaller size of nanoparticles can make potential interactions with membrane surfaces and they can easily translocate and become distributed throughout the human body. Taking into account their physicochemical and biological properties. It is likely that nanoparticles will introduce new pathologies. Finally silver has the problem of argyria and argyrosis in human and in the environment.

5. ACKNOWLEDGMENTS

The authors are grateful to Principal Dr. S.R.Tambe, guide Dr. M. Mohan and my colleagues for their continuos support and encouragement.

6. REFERENCES

[1] Margaret IP, Lui SL, Poon VKM, Lung I, Burd A: Antimicrobial activities of silver dressings: an in vitro comparison. J Med.Microbial.55:59-63, 2006.
[2] Hill Wr, Pillsburry DM: Argyria: The Pharmacology of silver. MD: Williams & Wilkins company, Baltimore 1939.
[3] Marshall JP II, Schneider RP: Systemic argyria secondary to topical silver nitrate. Arch. Dermatol, 113:1077-1079, 1977.
[4] Samuel U, Guggenbiechler JP: Prevention of catheter –related infections: the potential of a new nano-silver impregnated catheter. Int.J.Antimicob. Agents 23S1:S75-S78, 2004.
[5] Braydich-Stolle L, Hussain S, S schlager JJ, Hofmann MC: In vitro cytotoxicity of nano particles in mammalian germline stem cells.Toxicol.Sci.88:412-419, 2005.
[6] Eisler R: A review of silver hazards to plants and animals. In Conf.Transport, Fate, and Effects of Silver in the Environment, Madison, Wisconsin 1996, pp.143-144.
[7] Albright I.J, Wilson EM: Sub-lethal effect of several metallic salt-organic compounds combinations upon heterotrophic microflora of a natural water, Water Res.8:101-105, 1974.
[8] Gaiser BK, Biswas A, Rosenkranz P, Jepson MA, Lead JR, Stone V, Tyler CR, Fernandes TF. 2011. Effects of silver and cerium dioxide micro- and nano-sized particles on Daphniamagna. J. Environ. Monit. 13: 1227–1235.
[9] Ahmed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y. 2008. DNA damage response to the different surface chemistry of silver nanoparticles in mammalian cells. Toxicol. Appl. Pharmacol. 233:404–410.
[10] Durán N, Marcato PD, De Conti R, Alves OL, Costa FTM, Brocchi M. 2010. The potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanism of actionteria, their toxicity and possible mechanisms of action. J. Braz. Chem. Soc. 21: 949–959.
[11] Hood E. 2004. Nanotechnology, diving into the unknown. Environ. Health Perspect. 112: A747–A749.
[12] Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Van De Meent D, Dekkers S, De Jong WH, van Zijverden M, Sips AJAM, Geertsma RE.2009. Nano silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 3: 109–138.
[13] Sharma V K, Yngard R A, and Lin Y 2009 *Adv. Colloid Sur. Interface* 145 83.
[14] Krutyakov Y A, Kudrynskiy A A, Olenin A Y and Lisichkin G V 2008 *Russ. Chem. Rev.* **77** 233
[15] Hussain SM,Javorina MK,Schrand AM,DuhartHM,Ali SF,Schlager JJ: The interaction of managanese nanoparticles with PC-12 cells induces dopamine depletion.Toxicol.Sci.92:456-463, 2006.
[16] Hussain SM,Javorina MK,Schrand AM,Duhart HM,Ali SF,Schlager JJ: In vivo toxicity of nanoparticles in BRL 3A rat liver cells.Toxicol. in vitro19:975-983,2005.
[17] Chen X, schluesener HJ, Nanosilver :A Nanoproducts in medical application. Toxicol.Lett. 176:1-12, 2007.
[18] Hogstrand C,Wood CM.The toxicity of silver to marine fish. In Anden AW,Bober TW (eds.):Proc.4th Int. conf. Transport, Fate and Effect of silver in the Environment,Madison,Wiscnsin 1996.pp.109-112.
[19] Garc´ıa-Barrasa J, L´opez-de-luzuriaga J M and Monge M 2011 *Cent. Eur. J. Chem.* **9** 17.
[20] Fabrega J, Luoma S N, Tyler C R, Galloway T S and Lead J R 2011 *Environ. Internat.* **37** 517
[21] Dallas P, Sharma V K and Zboril R 2011 *Adv. Colloid Interface Sci.* **166** 119
[22] PEN, http://www nanotechnology project.org
[23] Brayner R 2008. The toxicological impact of nanoparticles, *Nanotoday* 3: 48 -55.
[24] Panda KK, Achary VMM, Krishnaveni R, Padhi BK, Sarangi SN, Sahu SN, Panda BB. 2011. In vitro biosynthesis and genotoxicity bioassay of silver Nano particles using plants. *Toxicol*. In Vitro 25: 1097–1105.
[25] Donaldson K, Stone V, MacNee W. 1999. The toxicology of ultrafine particles. In Particulate Matter Properties and Effects Upon Health, Maynard LA, Howards CA (eds). Bios Scientific: Oxford; 115–127.
[26] Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science 311: 622–627.
[27] AshaRani PV, Wu YL, Gong Z, Valiyaveettil S. 2008. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19: 255;102.
[28] Oberdörster E. 2004. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile Largemouth Bass. Environ. Health Perspect. 112: 1058–1062.
[29] Arora S, Jain J, Rajwade JM, Paknikar KM. 2008. Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol. Lett. 79: 93–100.
[30] Nordberg G, Gerhardsson LS: Silver, In Seiler HG, Sigel H, Sigel A(eds): Handbook on toxicity of Inorganic Compounds. Marcell Dekker, New York 1988, pp.619-624.
[31] Jung JH, Oh HC, Noh HS, Ji JH, Kim SS. Metal nanoparticle generation using a small ceramic heater with a local heating area. *J Aerosol Sci*. 2006;37(12):1662–1670.
[32] Tsuji T, Iryo K, Watanabe N, Tsuji M. Preparation of silver nanoparticles by laser ablation in solution: influence of laser wavelength on particle size. *Appl Surf Sci*. 2002;202(1–2):80–85.
[33] Tien DC, Liao CY, Huang JC, et al. Novel technique for preparing a nano-silver water suspension by the arc-discharge method. *Reviews on Advanced Materials Science*. 2008;18:750–756.
[34] Evanoff DD Jr, Chumanov G. Synthesis and optical properties of silver nanoparticles and arrays. *Chemphyschem*. 2005;6(7):1221–1231.
[35] Pyatenko A, Yamaguchi M, Suzuki M. Synthesis of spherical silver nanoparticles with controllable sizes in aqueous solutions. *J Phys Chem C*. 2007;111(22):7910–7917.
[36] Blanco-Andujar C, Tung LD, Thanh NTK. Synthesis of nanoparticles for biomedical applications. *Annual Reports Section "A" (Inorganic Chemistry)*. 2010;106:553–568.
[37] Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine*. 2007;3(1):95–101.

[38] Sadeghi B, Garmaroudi FS, Hashemi M, et al. Comparison of the anti-bacterial activity on the nanosilver shapes: nanoparticles, nanorods and nanoplates. *Adv Powder Technol*. 2012;23(1):22–26.
[39] Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects of β-lactam antibiotic combined with silver nanoparticles. *Nanotechnology*. 2005;16(9):1912–1917.
[40] Blanco-Andujar C, Tung LD, Thanh NTK. Synthesis of nanoparticles for biomedical applications. *Annual Reports Section "A" (Inorganic Chemistry)*. 2010;106:553–568.
[41] Sun RW, Chen R, Chung NP, Ho CM, Lin CL, Che CM. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem Commun (Camb)*. 2005;(40): 5059–5061.
[42] Lu L, Sun RW, Chen R, et al. Silver nanoparticles inhibit hepatitis B virus replication. *Antivir Ther*. 2008;13(2):253–262.
[43] Taylor PL, Omotoso O, Wiskel JB, Mitlin D, Burrell RE. Impact of heat on nanocrystalline silver dressings. Part II: physical properties. *Biomaterials*. 2005;26(35):7230–7240.
[44] Baram-Pinto D, Shukla S, Perkas N, Gedanken A, Sarid R. Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate. *Bioconjug Chem*. 2009;20(8):1497–1502.
[45] Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. *Nanoscale Res Lett*. 2008;3(4):129–133.
[46] Taylor PL, Ussher AL, Burrell RE. Impact of heat on nanocrystalline silver dressings. Part I: chemical and biological properties. *Biomaterials*. 2005;26(35):7221–7229.
[47] Lara HH, Ayala -Nuñez NV, Ixtepan-Turrent L, Rodriguez-Padilla C. Mode of antiviral action of silver nanoparticles against HIV-1. *J Nanobiotechnology*. 2010;8:1.
[48] Nadworny PL, Wang J, Tredget EE, Burrell RE. Anti-inflammatory activity of nanocrystalline silver in a porcine contact dermatitis model. *Nanomedicine*. 2008;4(3):241–251.
[49] Shin SH, Ye MK. The effect of nano-silver on allergic rhinitis model in mice. *Clin Exp Otorhinolaryngol*. 2012;5(4):222–227.
[50] Castillo PM, Herrera JL, Fernandez-Montesinos R, et al. Tiopronin monolayer-protected silver nanoparticles modulate IL-6 secretion mediated by Toll-like receptor ligands. *Nanomedicine (Lond)*. 2008;3(5): 627–635.
[51] Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotechnol*. 2010;28(11):580–588.
[52] Huang Y, Li X, Liao Z, et al. A randomized comparative trial between Acticoat and SD-Ag in the treatment of residual burn wounds, including safety analysis. *Burns*. 2007;33(2):161–166.
[53] Chen J, Han CM, Lin XW, Tang ZJ, Su SJ. [Effect of silver nanoparticle dressing on second degree burn wound]. *Zhonghua Wai Ke Za Zhi*. 2006;44(1):50–52. Chinese.
[54] Lu S, Gao W, Gu HY. Construction, application and biosafety of silver nanocrystalline chitosan wound dressing. *Burns*. 2008;34(5): 623–628.
[55] Grunkemeier GL, Jin RY, Starr A. Prosthetic heart valves: Objective Performance Criteria versus randomized clinical trial. *Ann Thorac Surg*. 2006;82(3):776–780.
[56] Jamieson WR, Fradet GJ, Abel JG, et al. Seven-year results with the St Jude Medical Silzone mechanical prosthesis. *J Thorac Cardiovasc Surg*. 2009;137(5):1109–1115. e2.
[57] Andara M, Agarwal A, Scholvin D, et al. Hemocompatibility of diamondlike carbon–metal composite thin films. *Diam Relat Mater*. 2006;15(11–12):1941–1948.
[58] Ghanbari H, Viatge H, Kidane AG, Burriesci G, Tavakoli M, Seifalian AM. Polymeric heart valves: new materials, emerging hopes. *Trends Biotechnol*. 2009;27(6):359–367.
[59] Fu J, Ji J, Fan D, Shen J. Construction of antibacterial multilayer films containing nanosilver via layer-by-layer assembly of heparin and chitosan-silver ions complex. *J Biomed Mater Res A*. 2006;79(3): 665–674.
[60] Lackner P, Beer R, Broessner G, et al. Efficacy of silver nanoparticles-impregnated external ventricular drain catheters in patients with acute occlusive hydrocephalus. *Neurocrit Care*. 2008;8(3):360–365.
[61] The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, Med Line, CAS, Sci Search®, Current Contents®/Clinical Medicine, Journal Citation Reports/Science Edition, EM Base, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.
[62] http://www.dovepress.com/international-journal-of-nanomedicine-journal
[63] Alt V, Bechert T, Steinrücke P, et al. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. *Biomaterials*. 2004;25(18):4383–4391.
[64] Morley KS, Webb PB, Tokareva NV, et al. Synthesis and characterisation of advanced UHMWPE/silver nano composites for biomedical applications. *Eur Polym J*. 2007;43(2):307–314.
[65] Yoshida K, Tanagawa M, Matsumoto S, Yamada T, Atsuta M. Antibacterial activity of resin composites with silver-containing materials. *Eur J Oral Sci*. 1999;107(4):290–296.
[66] Yamamoto K, Ohashi S, Aono M, Kokubo T, Yamada I, Yamauchi J. Antibacterial activity of silver ions implanted in SiO2 filler on oral streptococci. *Dent Mater*. 1996;12(4):227–229.
[67] Magalhães APR, Santos LB, Lopes LG, et al. Nanosilver application in dental cements. *ISRN Nanotechnology*. 2012;2012:1–6.
[68] Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. *Dent Mater*. 2009;25(2):206–213.
[69] Haes AJ, Van Duyne RP. A nanoscale optical blosensor: sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles. *J Am Chem Soc*. 2002; 124(35):10596–10604.

[70] Zhou W, Ma Y, Yang H, Ding Y, Luo X. A label-free biosensor based on silver nanoparticles array for clinical detection of serum p53 in head and neck squamous cell carcinoma. *Int J Nanomedicine*. 2011; 6:381–386.
[71] Loo C, Lowery A, Halas N, West J, Drezek R. Immunotargeted nano she International Journal of Nanomedicine 2014:9.
[72] Foldbjerg R, Dang DA, Autrup H. 2011. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch. Toxicol. 85: 743–750.
[73] Park BSY, Choi J. 2010. Geno- and ecotoxicity evaluation of silver nanoparticles in freshwater crustacean Daphnia magna. Environ. Eng. Res. 15: 23–27.
[74] Griffitt RJ, Luo J, Gao J, Bonzongo J-C, Barber DS. 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ. Toxicol. Chem. 27: 1972–1978.
[75] Anita K. Patlolla1, Diahanna Hackett and Paul B. Tchounwou , SILVER NANOPARTICLE INDUCED OXIDATIVE STRESS-DEPENDENT TOXICITY IN SPRAGUE-DAWLEY RATS NIH Public Access *Mol Cell Biochem*. 2015 January; 399(0): 257–268.
[76] Jyoti Prakash Pani, S .P .Mishra, R.S. More, Sanjay Singh, Royana Singh, HEPATOTOXIC EFFECT OF CHARACTERIZED SILVER NANOPARTICLE, International Journal of Therapeutic Applications, Volume 29, 2015, 13-18.
[77] Eun-Jung Parka, Eunjoo Baeb, Jongheop Yib, Younghun Kim c, Kyunghee Choid, Sang Hee Leed, Junheon Yoond, Byung Chun Leed, Kwangsik Parka Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles ,Environmental Toxicology and Pharmacology 30 (2010) 162–168.
[78] Lee TY, Liu MS, Huang LJ, Lue SI, Lin LC et al (2013) Bioenergetic failure correlates with autophagy and apoptosis in rat liver following silver nanoparticle intraperitoneal administration. Particle Fib Toxicol 10(40):1–13
[79] Chang AL, Khosravi V, Egbert B (2006) A case of argyria after colloidal silver ingestion. J Cutan Pathol 33:809–811.
[80] Cheraghi J, Hosseini E, Hoshmandfar R (2013) In vivo effect of silver nanoparticles on serum ALT, AST and ALP activity in male and female mice. Adv Environ Biol 7(1):116–122.
[81] Gatti AM, Montanari S, Monari E, Gambarelli A, Capitani F, and Parisini B (2004) Detection of micro- and nano-sized biocompatible particles in the blood. J Mater Sci Mater Med 15:469–472.
[82] 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.
[83] 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.
[84] 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.
[85] 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.
[86] 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.
[87] 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).
[88] 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.
[89] 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.
[90] 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.
[91] Sung, J.H., Ji, J.H., Park, J.D., Yoon, J.U., Kim, D.S., Jeon, K.S., Song, M.Y., Jeong, J., Han,B.S., Han, J.H., Chung, Y.H., Chang, H.K., Lee, J.H., Cho, M.H., Kelman, B.J., Yu, I.J.,2009. Subchronic inhalation toxicity of silver nanoparticles. Toxicol. Sci. 108 (2), 452–461.
[92] Chen, X., Schluesener, H.J., 2008. Nanosilver: a nanoproduct in medical application. Toxicol. Lett. 176 (1), 1–12.
[93] Edwards-Jones, V., 2009. The benefits of silver in hygiene, personal care and healthcare. Lett. Appl. Microbiol. 49 (2), 147–152.
[94] Tang, J., Xiong, L., Wang, S., Wang, J., Liu, L., Li, J., Yuan, F., Xi, T., 2009. Distribution, translocation and accumulation of silver nanoparticles in rats. J. Nanosci. Nanotechnol, 9 (8), 4924–4932.
[95] Matuk, Y., Ghosh, M., McCulloch, C., 1981. Distribution of silver in the eyes and plasma proteins of the albino rat. Can. J. Ophthalmol. 16 (3), 145–150.
[96] Hadrup, N., Loeschner, K., Bergstrom, A., et al., 2012b. Subacute oral toxicity Investigation of nanoparticulate and ionic silver in rats. Arch. Toxicol. 86 (4), 543–551.
[97] Shahare, B., Yashpal, M., 2013. Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice. Toxicol. Mech. Methods 23,161–167. http://dx.doi.org/10.3109/15376516.2013.764950 (Copyright (C) 2013 American Chemical Society (ACS). All Rights Reserved).
[98] Kim, Y.S., Song, M.Y., Park, J.D., et al., 2010. Subchronic oral toxicity of silver nanoparticles. Part. Fibre Toxicol. 7, 20. http://dx.doi.org/10.1186/1743-8977-7-20.
[99] Cha, K., Hong, H.W., Choi, Y.G., et al., 2008. Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnol. Lett. 30 (11), 1893–1899. http://dx.doi.org/10.1007/s10529-008- 9786-2.

[100] Espinosa-Cristobal, L.F., Martinez-Castanon, G.A., Loyola-Rodriguez, J.P., et al., 2013.Toxicity, distribution, and accumulation of silver nanoparticles in Wistar rats. J. Nanopart. Res. 15 (6). http://dx.doi.org/10.1007/S11051-013-1702-6 (doi: Unsp 1702).
[101] Hadek, R., 1966. Preliminary report on the cellular effect of intravital silver in the mouse ovary. J. Ultrastruct. Res. 15 (1), 66–73.
[102] Jeong, G.N., Jo, U.B., Ryu, H.Y., Kim, Y.S., Song, K.S., Yu, I.J., 2010. Histo chemical study of intestinal mucins after administration of silver nanoparticles in Sprague–Dawley rats. Arch. Toxicol. 84, 63–69 (Copyright (C) 2013 U.S. National Library of Medicine).
[103] Kim, Y.S., Kim, J.S., Cho, H.S., et al., 2008. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague–Dawley rats. Inhalation Toxicol. 20, 75–583.http://dx.doi.org/10.1080/08958370701874663 (Copyright (C) 2013 American Chemical Society (ACS). All Rights Reserved)
[104] Rugby, J., 1986. Experimental argyrosis: ultrastructural localization of silver in rat eye. Exp. Mol. Pathol. 45, 22–30 (Copyright (C) 2013 U.S. National Library of Medicine).
[105] Sardari, R.R.R., Zarchi, S.R., Talebi, A., et al., 2012. Toxicological effects of silver nanoparticles in rats. Afr. J. Microbiol. Res. 6 (27), 5587–5593. http://dx.doi.org/10.5897/Ajmr11.1070.
[106] van der Zande, M., Vandebriel, R.J., Van, D.E., et al., 2012. Distribution, elimination,and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS Nano 6, 7427- 7442.
[107] Loeschner, K., Hadrup, N., Qvortrup, K., et al., 2011. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part. Fibre Toxicol. 8 (1), 18.
[108] Hadrup, N., Loeschner, K., Bergstrom, A., et al., 2012b. Subacute oral toxicity investigation of nanoparticulate and ionic silver in rats. Arch. Toxicol. 86 (4), 543–551.
[109] Shavlovski, M.M., Chebotar, N.A., Konopistseva, L.A., et al., 1995. Embryotoxicity of silver ions is diminished by ceruloplasmin-further evidence for its role in the transport of copper. BioMetals 8, 122–128. http://dx.doi.org/10.1007/bf00142011 (Copyright (C) 2013 American Chemical Society (ACS). All Rights Reserved).
[110] Rungby, J., Danscher, G., 1984. Hypoactivity in silver exposed mice. Acta Pharmacol. Toxicol. 55, 398–401 (Copyright (C) 2013 American Chemical Society (ACS). All Rights Reserved).
[111] Rungby, J., Danscher, G., 1983. Localization of exogenous silver in brain and spinal cord of silver exposed rats. Acta Neuropathol. 60, 92–98 (Copyright (C) 2013 U.S. National Library of Medicine).
[112] Hadrup, N., Loeschner, K., Mortensen, A., et al., 2012c. The similar neurotoxic effects of nanoparticulate and ionic silver in vivo and in vitro. Neurotoxicology 33 (3),416-423. http://dx.doi.org/10.1016/j.neuro.2012.04.008.
[113] Mirsattari, S.M., Hammond, R.R., Sharpe, M.D., Leung, F.Y., Young, G.B., 2004. Myoclonic status epilepticus following repeated oral ingestion of colloidal silver. Neurology 62 (8), 1408–1410.
[114] Ohbo, Y., Fukuzako, H., Takeuchi, K., Takigawa, M., 1996. Argyria and convulsive seizures caused by ingestion of silver in a patient with schizophrenia.
[115] Singh S, D'Britto V, Prabhune AA, Ramana CV, Dhawan A, Prasad BLV. 2010. Cytotoxic and genotoxic assessment of glycolipid reduced and -capped gold and silver nanoparticlesw. New J. Chem.34: 294–301
[116] Lu W, Senapati D, Wang S, Tovmachenko O, Singh AK, Yu H, Ray HPC. 2010. Effect of surface coating on the toxicity of silver nanomaterial on human skin keratinocytes. Chem. Phys. Lett. 487: 92–96
[117] Park AEJ, Yi J, Kim Y, Choi K, Park K. 2010. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol. In Vitr 24: 872–878.
[118] Ahmed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y. 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol. Appl. Pharmacol. 233:404–410
[119] Braydich-stolle L, Hussain S, Schlager J J and Hofmann M 2005 *Toxicol. Sci.* 88 412.
[120] Hussain S M, Hess K L, Gearhart J M, Geiss K T and Schlager J J 2005,*Toxicol. In Vitro* 19 975.
[121] Shin S-H, Ye M-K, Kim H-S and Kang H-S 2007 *Int. Immunopharm.* **7:**1813.
[122] Kawata K, Osawa M and Okabe S 2009 *Environ. Sci. Technol.* 43 6046.
[123] Park E, Yi J, Kim Y, Choi K and Park K 2010 *Toxicol. In Vitro* 24 872