International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 2; Issue 4; July 2017; Page No. 01-10

Silver nanoparticles: Biomedical applications, toxicity, and safety issues

***Shashi Pratap Singh, Dr. C.S. Bhargava, Dr. Vishal Dubey, Ashish Mishra, Yatendra Singh**

Assistant Professor, Advance Institute of Biotech and Paramedical Sciences, Kanpur, Uttar Pradesh, India

Abstract
Elemental or metallic silver (Ag) is a very malleable and ductile transition metal that is white metallic luster appearance. Nano-particles are fine particles that have size nano-meter in range, that have dimension less than 100 nm (1nm-100nm). As their nano sized they have special physiochemical properties. Silver nano-particles (AgNPs) are one of most widely used nano-material that are used in personal care products, dressings as treatments for external wounds, ointments, and surgical instruments because of their effective antibacterial activity. AgNPs have broad spectrum antibacterial action that acts on both gram-negative and gram-positive bacteria both, including antibiotic-resistant strains. AgNPs (diameter 5-20 nm, average diameter ~10 nm) also exhibit the antiviral property against HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkey pox virus. AgNPs as well as nano silver-derived solution and their product have showed the potent anti-inflammatory properties. As the consumption of nano-silver products are increasing, the chances of adverse effect on human health and environment are increasing. Generally AgNPs are less toxic than silver ion, but here are several in vitro in vivo studies shown that on exposure of AgNPs leads to cytotoxicity, immunotoxicity and genotoxicity to vertebrates. AgNPs causes blood diseases and colon cancer when it has been found in the blood and colon of patients respectively. kim *et al* 2008. Reported than 28 oral exposures of AgNPs at 30 mg/kg, 300 mg/kg and 1000 mg/kg in sprague dawley rats show dose dependent distribution of AgNPs in various organs and gender specific two fold more accumulation in female kidneys in comparison to male kidney. Usually the in vivo studies, in vitro studies with AgNPs showed that shown genotoxic effects, induction of DNA strand breaks, micronuclei, and chromosomal aberrations – at low non-cytotoxic doses in different types of human and mammalian cells.

Keywords: Silver nanoparticles (AgNPs), Cytotoxicity, Immunotoxicity, Genotoxicity

Introduction

Silver

Silver name comes from the Anglo-Saxon word "*siolfur*" meaning "*silver*" (the origin of the symbol Ag comes from the Latin word "*argentum*" meaning "*silver*"). Throughout the history, silver is used by human because it has different properties like pure silver has white metallic luster. It is used as a precious commodity in currencies, ornaments, jewelry, electrical contacts and photography, among others. One of the most beneficial uses of silver is that as a potent antibacterial agent that is toxic to fungi, viruses and algae. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria as well as because of its reputation of limited toxicity to humans.

Elemental or Metallic (Ag) Silver Characteristics and Sources

Elemental or metallic silver (Ag) is a very malleable and ductile transition metal that is white metallic luster appearance (41. Brooks, 2010; Lenntech, 2010; Wikipedia, 2016), Pure silver has the highest electrical conductivity (higher than copper that is currently used in many electrical applications) and thermal conductivity and has the lowest contact resistance (1 Brooks, *et al*. 2010).

Silver is stable in pure air and water, and in the presence of ozone or hydrogen sulfide or sulfur in the air or water may result in silver tarnishing (2. Hammond, *et al*. 2000) [2]. Silver has the common oxidation state 0 and +1, but other oxidation state (+2 and +3) are also known. It has many isotopes with ^{107}Ag being the most common (3.Smith & Carson, 1977).

Applications of Elemental Silver

Silver was applied in practical medicine such as eye treatment and the treatment of skin ulcers, in the 19th century (4.Foot Defense, 2010) [4]. Silver and its compound have been extensively used in electrical conductors, electrical contacts, catalysis, photography, electronics, mirrors, drinking water filtration systems, swimming pool filtration systems, healthcare products and medical tools (4.Clement *et al*., 1994) [5].

Silver Nano-Particles (AgNPs)

Nano-particles are fine particles that have size nano-meter in range, that have dimension less than 100 nm (1nm-100nm). As their nano sized they have special physiochemical properties (6.Buzea *et al*., 2007) [6, 35]. AgNPs contain many silver atoms or ions that clustered together to form a particle 1-100 nm in size (7. Fabrication S. *et al*.) [7]. AgNPs are one of most widely used nano-material that are used in personal care products, dressings as treatments for external wounds, ointments, and surgical instruments because of their effective antibacterial activity (8. Kim T-H *et al*. 2012) [8]. The major reason for development of nano-silver containing products is that it has the strong antimicrobial properties. There are more than 1000 consumer products that contain nano-materials, roughly 25% are claimed to contain silver nano-particles. AgNPs containing consumer products are food contact materials (such as cups, bowls and cutting boards), odor-resistant textiles, electronics and household appliances, cosmetics and personal care products, medical devices, water disinfectants, room sprays, children's toys, infant products and 'health' supplements (9.Fauss, *et al*. 2008) [9]. One of the

most commonly used nano-material is AgNPs which has the biocidal activity, it is mainly used as a coating or embedding agent for medical purposes: for wound dressings, surgical instruments, implants and bone prostheses, AgNPs coatings are also used for manufacturing odor-resistant textiles, Furthermore, AgNPs is marketed as deodorants, room sprays, water cleaners, laundry detergents, in food packaging and wall paints (10. Mirta M *et al*. 2014) [10].

Properties of nano-silver

Surface effect and auantum effect these are two primary factor that difffertiate the nano-material to bulk material. These factors affect the chemical reactivity of materials as well as their mechanical, optical, electric, and magnetic properties. AgNPs also has unique optical and physical properties that are not present in bulk silver, and which are claimed to have great potential for medical applications (11.Roduner, *et al*. 2006) [11].

i) Antibacterial properties

AgNPs have broad spectrum antibacterial action that act on both gram-negative and gram-positive bacteria both, including antibiotic-resistant strains (12.Burrell *et al*. 1999.) [16] Normally antimicrobial efficacy of AgNPs depend size and concentration, high concentration leads to more effective while the smaller particle size kill bacteria at low rate. Sadeghi *et al* investigated that AgNPs have good antibacterial properties (13. Sadeghi B *et al*. 2012) [13]. In general the smaller AgNPs size show more effective antibacterial effect as comparison to larger, The average diameters under 10 nm are most effective, In smaller particle size atom are present in higher concentration on surface which leads to greater reactivity and biocidal activity (14.Vikas Gupta *et al*. 2007). Although the exact mechanism AgNPs on bacteria and bactericidal action are not proper understood but based on the other studies that showed that nano-particle penetrate the bacterial cell wall (15.Morones *et al*., 2005) [15, 17].

ii) Antifungal properties

AgNPs have effective fungicidal property that act against broad spectrum of common fungi that including genera as Aspergillus, Candida, and Saccharomyces. Although the exact mechanism of AgNPs against fungi are not understood, but mechanisms similar to that of the antibacterial actions have been proposed for fungi (16.Wright *et al*., 1999) [16].

iii) Antiviral properties

AgNPs (diameter 5-20 nm, average diameter ~10 nm) also exhibit the antiviral property against HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkey pox virus. Anti-HIV mechanism of silver nano-particles is based on the inhibition of the initial stages of the HIV-1 cycle. AgNPs mainly bind to glycoprotein (gp) 120, thus inhibit cluster of differentiation (CD) 4-dependent binding, fusion, and infectivity. AgNPs have very effective veridical agent to block HIV-1 cell-free and cell-associated infection (Malcolm M. X. *et al*. 2014). Silver nano-particle show the more anti HIV-1 activity (98%) as compared to Gold nano particle (6-20%). Interaction of silver nanoparticles with HIV-1 was exclusively within the range of 1-10 nm (17.Elechiguerra *et al*., 2005) [15, 17].

iv) Anti-inflammatory properties

AgNPs as well as nano silver-derived solution and their product have showed the potent anti-inflammatory properties. Recent study has suggested that the visual and histological signs of inflammation were reduced in inflammatory cells of dermis, expression of pro-inflammatory factor like cytokines transforming factor(TGF)-β, tumor necrosis factor (TNF)-α, and interleukin (IL)-8 were also reduced (18.Nadworny *et al*., 2010) [18]. Topically applied AgNPs shown potent the anti-inflammatory properties and wound healing properties (Chaloupka *et al*. 2010). In animal models AgNPs alters the expression of matrix metalloproteinase (photolytic enzymes that are important in various inflammatory and repair processes) (19. Kirsner *et al*., 2001) [19].

v) Anti-biofilm properties

AgNPs inhibit the formation of biofilms, Biofilms are complex communities of surface-attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix. (20. Percival *et al*., 2007) [20].

Application of Nano-Silver

i) Medical Applications

There are many medical application of AgNPs like including diagnosis, treatment, drug delivery, coating tools and medical devices. It is used coating medical tools and materials used in the areas of surgery, anesthesiology, cardiology and urology (21. Wijnhoven *et al*., 2009) [21].

a) Silver coated medical devices: AgNPs are used for the impregnation of medical devices such as surgical masks. The main advantage of impregnation of medical devices with silver nano-particles is that it protects both outer and inner surfaces of devices and there is continuous release of silver ions providing antimicrobial activity (14.Vikas Gupta *et al*. 2007).

b) Silver dressings: Dressings play a major part in the management of wounds (14.Vikas Gupta *et al*. 2007).

c) In orthopedics: AgNPs is also used in orthopedics in areas like as additives in bone cement, coating of implants for joint replacement and bone prostheses (22.Tolaymat *et al*., 2010) [22].

There are several uses of AgNPs in medical field like as for diagnosis, treatment, drug delivery, contraceptive devices, wound dressings, medical textiles, medical device coating, antimicrobial gel formulation for topical use, antimicrobial surface fictionalizations of plastic catheters and antimicrobial gel formulation for topical use (14Vikas Gupta *et al*. 2007).

ii) Scientific Applications

AgNPs have the remarkable physical, chemical and optical properties, that allows for their utilization in various scientific applications. AgNPs are widely used for surface enhanced raman scattering (SERS). Raman scattering by molecules could be enhanced if the analyte molecules are adsorbed on rough metal surfaces. As a consequence of the SPR and SERS, silver nano-particles are a promising tool for sensing applications, including detection of DNA sequences, laser desorption/ionization mass spectrometry of peptides, colorimetric sensors for histidine, determination of fibrinogens in human plasma, real-time probing of membrane transport in living microbial cells, enhanced IR absorption

spectroscopy, colorimetric sensors for measuring ammonia concentration, biolabeling and optical imaging of cancer, optical sensors for zeptomole, biosensors for detection of herbicides, and glucose sensors for medical diagnostics (23. Amro El-Badawy *et al*. 2010) [23]. AgNPs also used in such as immunoassays and DNA/RNA detection, because their effects include fluorophore quenching at short distances, spatial variation of the incident light field, and change in the radioactive decay rate (21.Wijnhoven *et al*., 2009) [21].

iii) Application in consumer products

There are so many diverse range of consumer product in which the AgNPs used like applications including air sanitizer sprays, socks, pillows, slippers, face masks, wet wipes, detergent, soap, shampoo, toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones, Nano-silver products targeted towards children and infants include: strollers, toys (stuffed animals), wet wipes, mats and bedding, baby bottles, nipples and pacifiers (14.Vikas Gupta *et al*. 2007). The major resion for nanosilver used is that it has strong anti-microbial properties (23.Amro El-Badawy *et al*. 2010) [23].

Toxicokinetics of Nano-silver

Absorption

Although the data available for nano-particle absorption and internal systemic exposure of

Nano-material are limited, its depend on its properties, characterization and nature of the individual particles and the local barriers present in different organs. In general for skin the uptake of nano-material is very low or absent. Oral or inhalational exposure of nano-materias for systemic availability is also low as comparison to molecules. There are several studies suggested absorption of AgNPs after oral admintration. AgNPs (60nm) on repeated oral 28 days administration have investigated and Ag content was determined by AAS technique, a dose dependent accumulation of AgNPs was observed blood, brain, kidney, liver, lung, stomach and testes (22.Tolaymat *et al*., 2010) [22]

Distribution

The major target organs for AgNPs distribution after systemic availability are the spleen, liver and kidneys, whereas there is less distribution to other organs. Furthermore, high levels of silver were sometimes noted in the testes. Sex differences also observed in different papers that is in female Ag concentration is more as compared to female. AgNPs (7.9nm) (citrate coated) after a single IV administration of 1 and 10 mg/kg b.w. in rats, the bioavailability and tissue distribution until 96 h after administration using ICP-MS for Ag detection. Tissue distribution was only determined in the lungs, liver and kidneys. In all three organs Ag could be detected up to 96 h after administration with the highest levels present in the liver (24.Park *et al*. 2011) [24].

Sung *et al*., 2009 evaluated that rats were exposed to aerosolized silver nano-particles (18-19 nm) for 90 days by inhalation (higher concentrations of 0.049, 0.133 and 0.515 mg/m3) systemic distribution with dose dependent silver could be Ag could be demonstrated in the liver, kidneys, olfactory bulb, brain and blood in addition to the lung. Concentrations were similar in males and females except in kidneys where the female kidneys accumulated two or three times more silver (25.Sung *et al*. 2009).

Metabolism

Liver is the detoxifying organ when ingestion is the entrance route to the body. HepG2 (human hepatoblastoma) cell line may be used for xenobiotic metabolism studies as it maintains many specialized functions of normal liver parenchyma cells such as synthesis and secretion of plasma proteins and cell surface receptors (1.Faedmaleki F *et al*. 2014). After inhalation Significant amounts of silver were observed in the liver. At each time point analyzed, 9-21% of the AgNPs lung content was observed in the liver (24. Park *et al*. 2011) [24].

Excretion

In kim *et al*. 2008 reported than 28 oral exposure of AgNPs at 30 mg/kg, 300 mg/kg and 1000 mg/kg in sprague dawley rats show dose dependent distribution of AgNPs in various organs and gender specific two fold more accumulation in female kidneys in comparison to male kidney (22. Tolaymat *et al*., 2010) [22].

Toxicity and Health Effects

Silver Toxicity

Chemical plants, industries and manufacturing sector that AgNPs compounds and its surrounding communities which comes in body by Inhalation, ingestion and dermal contact of dusts or fumes containing silver. Drake & Hazelwood (2005) [26] described that soluble silver compounds were more easily absorbed, hence, had the potential to produce adverse effects in comparison to metallic silver. Silver comes in dermal contact by several application like burns creams, use of dental amalgams and acupuncture needles, catheters, accidental punctures, and from contact with jewelry and silverware On chronic esposure silver produces permanent grey or blue grey discoloration of the skin (26. Drake *et al*. 2005) [26]. (argyria Figure1)

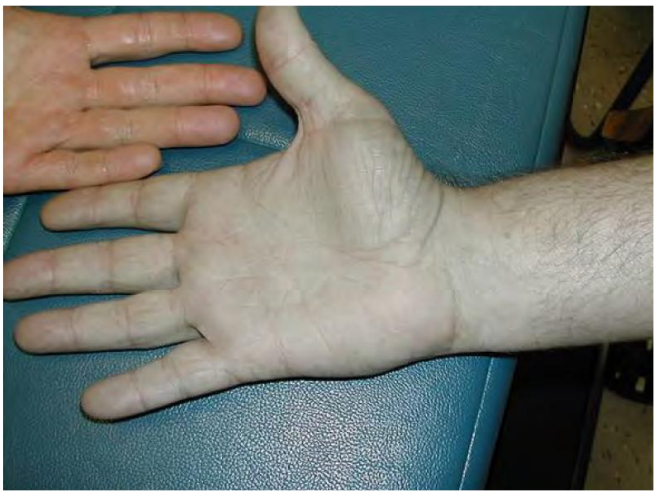

Fig 1: Systemic argyria of the skin from ingestion of colloidal silver (bottom hand) when compared to normal pigmentation (top hand) (27.Wadhera & Fung, 2010).

Pala *et al*. (2008) [28] described a case study of a craftsman, he was 71 year old, working from the age of 17, he was producing the in silver containing products like as vases,

plates, trays and frames by using cutting tools, welding and hammering silver sheets. His working bench was approximately 30-40 cm from his face, and he was working daily at least 8 hours in silver exposure environment. On ocular examination of the craftsman, it was diagnosed that craftsman eyes showed bilateral conjuctival-corneal argyrosis (Figure-2) without systemic intoxication (28.Pala *et al*. 2008) [28]. Rosenman *et al*. (1979) [29] also reported that respiratory irritation, abdominal pain and decreased night vision in workers exposed to silver nitrate and silver oxide dusts over one to ten years (29.Rosenman *et al*. 1979) [29].

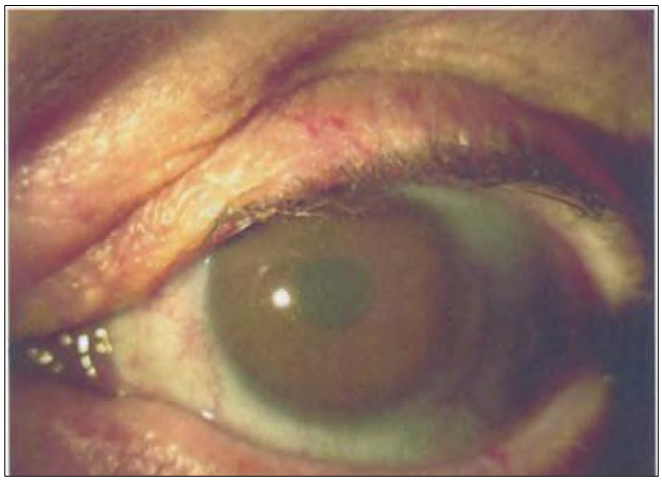

Fig 2: Conjuctival-corneal argyrosis in the craftsman occupationally exposed to silver. Reprinted from J. Occup. Health, Vol. 50, Pala, G., Fronterre, A., Scafa, F., Scelsi, M., Ceccuzzi, R., Gentile, E., Candura, S.M., Case Study: Ocular Argyrosis in a Silver Craftsman, pp521-522 (28.Pala *et al*. 2008) [28].

AgNPs Toxicity

As the consumption of nano-silver products are increasing, the chances of adverse effect on human health and environment is increasing. AgNPs has different physiochemical characteristics (such as its size, surface area, solubility, ability to aggregate, chemical composition and surface chemistry) and biological activities than characteristics of bulk silver, due to these physiochemical properties nano-silver have large surface area which leads to increased toxicity due to the activity of free silver ions released by the nano-particles.

Generally AgNPs are less toxic than silver ion, but here are several studies shown that on exposure of AgNPs leads to cytotoxicity, immunotoxicity and genotoxicity to vertebrates in vitro and in vivo (30. Lingling Huo *et al*. 2001). When AgNPs are inhaled, it lead to their migration to the olfactory bulb, where they locate in mitochondria, translocation to the circulatory system, liver, kidneys, and heart (31. Oberdörster, G. *et al*.) [31]. AgNPs causes blood diseases and colon cancer when it has been found in the blood and colon of patients respectively (32, 33.Gatti, 2004 *et al*., Gatti *et al*.) [32, 33]. The antibacterial property of AgNPs make it lethal to the bacteria as well has toxic effect on human cell, similarly lethal concentration of AgNPs for bacteria are also lethal for both keratinocytes and fibroblasts (34. Poon & Burd *et al*., 2004) [34].

On exposure of AgNPs through inhalation cause diseases include asthma, bronchitis, emphysema, lung cancer, and neurodegenerative diseases. Similarly gastrointestinal tract associated with crohn's disease and colon cancer. AgNPs that enter the circulatory system are related to occurrence of arteriosclerosis, blood clot, arrhythmia, heart diseases, it also cause autoimmune diseases such as systemic lupus erythematosus, scleroderma, and rheumatoid arthritis (35.Buzea *et al*., 2007) [6, 35]. (Figure-3). On oral admintration AgNPs were found in major body organ like as the kidneys, liver, spleen, lungs, brain, and the gastrointestinal (GI) tract. Some nano-particles passed through the GI tract and were rapidly excreted in feces and urine, indicating that they can be absorbed across the GI barrier and into the systemic circulation (36. Hagens, *et al*., 2007) [36]. AgNPs should also be regarded as a particular source of risks because of their potential toxicity to humans. On exposure of AgNPs cause the developed bluish-colour skin. Other studies have widely investigated toxicities such as oxidative damage in cellular systems (37.Danielle McShan *et al*. 2014) [37].

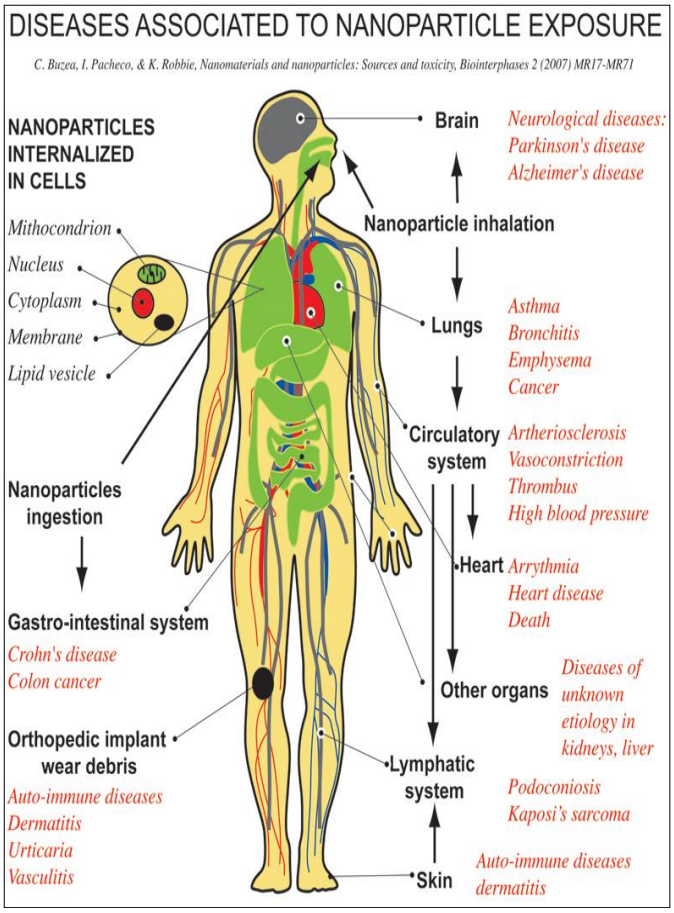

Fig 3: A schematic of the human body with pathways of exposure to nanoparticles, affected organs, and associated diseases from epidemiological, *in vivo* and *in vitro* studies. Reprinted from Biointerphases, Vol. 2 (4), 2007; Buzea [6, 35], C., Pacheco, I.I., Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity, 17-71.

Dermal Toxicity

AgNPs containing dressing, and dermal product have been used in dermal care because it have the potent anti-microbial properties and good control of wound has been achieved, but silver nano-particles and their product which contains the AgNPs leads dermal toxicity is still a topic of concern. There

are several researches researchers have demonstrated AgNPs causes the dermal cytotoxicity, (38. Chen *et al*., 2006) [43] exposed cultured keratinocytes to extracts of several types of silver containing dressings, result showed that dressing which coated with the extracts of nano-crystalline are the most cytotoxic. Keratinocyte, proliferation was significantly inhibited, and cell morphology was affected (39.El-Ansary & El-Daihan, 2009) [39]. On application of 1% of AgNPs cream (96.1% is <50 nm) in mice, leads to apoptosis of inflammatory cells but not of keratinocytes (40. Supp *et al*., 2005) [40].

AgNPs (average size 15nm) coated a topical wound dressing product is Acticoat®, one report that showed that silver poisoning after the use of Acticoat® for treatment of severe burns to the legs. The patient developed a grayish discoloration in the treated area, complained of being tired and had no appetite, on 6th day, but after 6th day, silver levels in urine and blood were found to be elevated (28 and 107 mg/kg, respectively). Acticoat® was removed and the discoloration of the face gradually faded and liver function test returned to normal values. Elevated blood silver levels were seen 7 weeks post-injury, but were negligible after 10 months. These observed adverse effects may be associated with the release of Ag^+ ions from the nanosilver dressing. Absorption of silver from Acticoat® was confirmed in 30 patients treated in another study (41.Vlachou *et al*. 2007) [41].

Respiratory Tract Toxicity

Human exposure to inhaled ambient particles, including AgNPs, may have adverse health effects nano-particles can become airborne easily due to their size and mass. When inhaled, nano-particles can go deeper into the lungs reaching more sensitive areas. After inhalation, nano-particles deposit throughout the entire respiratory tract, starting from nose and pharynx, down to the lungs (35.Buzea *et al*., 2007) [6, 35]. A study showed that when AgNPs inhaled by rats, AgNPs accumulation was seen in the lungs of the rats (1.7 mg) of which 4% was still left after seven days, but again additional toxicity parameters were not included. Inhaled AgNPs particles can be translocated to other organs such as the liver, olfactory bulb and brain, this indicate that tissue distribution on nano silver after inhalation (24.Takenaka *et al*., 2001).

Neuronal Uptake

AgNPs passage though the blood brain barrier and accumulate in the brain, leads to influence on neural cells natural physiology and development (42. Tabatabaei, *et al*. 2015) [42] silver was found in the brain of rats systemically exposed to AgNPs via inhalation, Inhaled nanoparticles are known to reach the nervous system via the olfactory nerves, and/or blood-brain barrier (35.Buzea *et al*., 2007; 83.Peters *et al*., 2006) [6, 35].

Gastrointestinal Tract Toxicity (G.I.T.)

The major route entry of nano material is gastrointestinal tract, both directly through intentional ingestion or indirectly via nanoparticle dissolution from food containers or by secondary ingestion of inhaled particles. Jeong *et al*. reported that the histological structure and properties of muco-subtances in intestinal mucosa of sprague-dawley rat aftet AgNPs administration. In experiment, he was divided the rats in four groups (10 rats in each group): control group, low-dose group (30 mg/kg), middle-dose group (300 mg/kg), and high-dose group (1,000 mg/kg). AgNPs was administered for 28 days, the treated samples showed luminal and surface particles, and the tissue contained AgNPs. AgNPs was observed in lamina propria in both the small and large intestine, in the tip of the upper villi in the ileum and in the protruding surface of the fold in the colon as a dose dependent manner. Mucus granules were found more in AgNPs treated rats as compared to control, resulting in more mucus materials in the crypt lumen and ileal lumen. Finally he invested that AgNPs induced the discharge of mucus granules along with an abnormal mucus composition in the goblet cells of the intestines (23. Amro El-Badawy *et al*. 2010) [23].

Kidney Toxicity

The toxic effect of AgNPs in in vivo mammalian system showed that AgNPs may accumulate in liver, kidney, colon, brain, and other body organs. kim *et al* 2008. reported than 28 oral exposures of AgNPs at 30 mg/kg, 300 mg/kg and 1000 mg/kg in sprague dawley rats show dose dependent distribution of AgNPs in various organs and gender specific two fold more accumulation in female kidneys in comparison to male kidney. Thus this study report suggested that 30 mg/kg b.w. as NOEAL dose of AgNPs and 300 mg/kg as LOAEL (lowest observable adverse effect level) dose. Female rats showed a higher accumulation of AgNPs in all kidney regions, including cortex, outer medulla, and inner medulla. The AgNPs were also preferentially accumulated in the basement membranes of the renal tubules in the cortex, middle and terminal parts of the inner medulla, and outer medulla (22. kim *et al* 2008).

Liver Toxicity

Kim *et al*. 2010 [44, 46] study at 30 mg /kg/d, 125 mg /kg/d, and 500 mg/kg/d for 90 days, that altered alkaline phosphates and cholesterol in the male and female rat liver. He showed that AgNPs more than more than 125mg/kg lead liver damage. Bile-duct hyperplasia was also reported in this study. Thus This study suggested 30 mg/kg as a NOAEL and 125 mg/kg as LOAEL dose (44. Kim *et al*. 2010) [44, 46]. Histopathology of the liver revealed cytoplasmic vacuolization in both sexes with a clear dose dependent increase in females. Several cases of hepatic focal necrosis were seen in the high dose groups (23. Amro El-Badawy *et al*. 2010) [23].

Akram Najjaran *et al*. 2010 study showed at mice with 25, 50, 100 and 200 milligram in 28 days. The control group consumes physiologic serum instead. After 28 days to assess morphological and pathological, blood samples were obtained from ocular eye, then autopsy was done in order to evaluate enzymatic change rate of BILI -ALP -SGOT-SGPT and detect histopathology effects of nanosilver onliver tissue. In 100 and 200 milligram doses significant changes observed in hepatocytes and hepatocyte damage because of the usage of nanosilver (45. Akram Najjaran *et al* 2014) [45].

Immune System Toxicity

Takenaka *et al*. 2001 study show that AgNPs particles were detectable in the spleen (Takenaka *et al*. 2001). Eun-Jung Park *et al*. 2010 [24] study showed that nano-particles toxicity mainly depends on many factors including size, shape,

chemical composition, surface area, surface charge, and others. On oral administration of AgNPs (1mg/kg for 14 days) in mice, small-sized AgNPs (22 nm, 42 nm, and 71 nm) were distributed to the organs including brain, lung, liver, kidney, and testis while large-sized AgNPs (323 nm) were not detected in those tissues. Further in his study oral administration of AgNPs (42nm) was also investigated in mice for 28 days. By the administration of AgNPs (0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg), adverse impacts on liver and kidney were observed in a high dose-treated group (1.00 mg/kg), when determined by blood chemistry and histipathological analysis. Cytokines including IL-1, IL-6, IL-4, IL-10, IL-12, and TGF-_ were also increased in a dose-dependent manner by repeated oral administration (46.Eun-Jung Park *et al*. 2010) [24]

On application of a 1% AgNPs cream (96.1% is <50 nm) in mice) inhibited DNB-induced allergic contact dermatitis and it was also found that that the expression of two cytokines (TNF and IL 12) was suppressed (histopathological staining) and apoptosis of inflammatory cells but not keratinocytes was induced. Similar concentration-dependent anti-inflammatory effects have also been seen in guinea pigs by the same group (87.Bhol *et al*., 2004). Rat studies based on inhalation of low concentrations of 15 nm diameter AgNPs showed that soon after inhalation (30 min), nano-particles are distributed in the blood and brain, and subsequently, to organs, such as heart and kidney, while the lungs are rapidly cleared off of the nano-particles (24.Buzea *et al*., 2007; 46.Takenaka *et al*., 2001) [6, 35]

The immune system is a dynamic network of cells, tissues, and organs that work coordinated to defend the body against attacks by "foreign" invaders and protects against disease by identifying "self" and "non-self" (for example virus, fungus, bacterium) cells and tissues. It has recently been studied whether AgNPs with a narrow size distribution and protected with a monolayer of adsorbed tiopronin (Ag@tiopronin) had any functional impact on specific TLR stimulation of Interleukin-6 (IL-6) secretion in Raw 264.7 macrophages, a murine monocyte/macrophage cell line It has recently been studied whether AgNPs with a narrow size distribution and protected with a monolayer of adsorbed tiopronin (Ag@tiopronin) had any functional impact on specific TLR stimulation of Interleukin-6 (IL-6) secretion in Raw 264.7 macrophages, a murine monocyte/macrophage cell line human mesenchymal stem cells (hMSCs) and J774 A1 macrophages mediated by specific TLRs located in the cell surface or in the endocytic compartments. Moreover, the effects of AgNPs on the production of cytokines by PBMC, hMSCs, were found to strongly inhibit cytokine production, for example, of INF- γ, IL-6, IL- 8, IL-11, TNF-α and more weakly IL-5. In the case of J774 A1 macrophages the expression levels of IL-1 and IL-6 were similar to controls (48. Rebecca Klippstein *et al*.)

Rob J Vandebriel *et al*. 2014 [49] study showed that, the systemic toxicity of 20 nm AgNPs was studied in a 28-day repeated-dose toxicity study in rats. AgNPs were intravenously administered with a maximum dose of 6 mg/kg body weight (bw)/day. Several immune parameters were affected: reduced thymus weight, increased spleen weight and spleen cell number, a strongly reduced NK cell activity, and reduced IFN-γ production were observed and suggested that Intravenous AgNPs administration in a 28-day repeated-dose toxicity study induces suppression of the functional immune system (49. Rob J Vandebriel *et al*. 2014) [49].

Other blood effects

On exposure of AgNPs (60nm) by oral administration leads to induced some changes in the red blood cell compartment (increased red blood cell count, hemoglobin, and hematocrit) and on coagulation parameters (decreased active partial protrombine time) (Kim *et al*., 2008).

Reproductive System Toxicity

Gromadzka-Ostrowska J *et al*. 2012 [50] study showed that intravenous administration of AgNPs (5mg/kg, 10mg/kg single dose) on male rats and sperms were analyzed after for 1, 7 and 28 days. Decrease sperm count was reported after 1 day and 28 days. DNA damage and abnormal sperms were also reported after 7 days and 28 days after exposure (50.Gromadzka-Ostrowska J *et al*. 2012) [50]. AgNPs has toxic effects on mammalian reproductive system and can affect their fertility. As the retention of AgNPs in reproductive organs is up to two months after oral exposure (51. van der Zande M *et al*. 2012) [51]. Intravenous exposure of AgNPs (50 nm) to pregnant mice leads to accumulation in ovaries, uterus placenta, visceral yolk sac and also in embryos. (52.Austin CA *et al*. 2012) [52].

Genotoxicity, Cytotoxicity and Carcinogenicity

In a chronic subcutaneous administration of AgNPs (1.75-2.5 mg weekly), eight of the 26 (31%) rats that survived longer than 16 months developed malignant tumors. In six of the rats, the tumor arose at the site of subcutaneous injection. This was significantly higher than the historical control tumor levels that were between 1-3% (Amro El-Badawy *et al*. 2010) [23]. In vitro studies have showed that there are various toxic effects of AgNPs including cytotoxicity (Faedmaleki F *et al*. 2014), genotoxicity (53. Asha Rani PV *et al*. 2009) [53, 59], DNA damage (Faedmaleki F *et al*. 2014), apoptosis (16. Eom HJ *et al*.2010)], oxidative stress and mitochondrial damage. (Hsin, *et al*. 2008). AgNPs significantly increased cell death through an oxidative stress related mechanism in mammalian cell (54.Hsin, *et al*. 2008). In an another in vitro study showed that vitro cytotoxicity of AgNPs (15 nm diameter) in mammalian mouse C18-4 germline stem cells indicated that a AgNPs concentration of more than 5 g/ml reduced the mitochondrial function and cell viability while increasing the LDH leakage. Recently, AgNPs have been shown to cause DNA damage in mammalian cells, cytotoxicity occurs through the generation of radical oxygen species (55.Braydich-Stolle *et al*., 2005) [55].

There are several In vivo experiments in mice and rats have suggested that on administration of AgNPs by inhalation AgNPs are able to reach several organs, including the lungs and the liver, and to cross the blood–brain barrier. On inhalation and intraperitoneal injection short-term in vivo exposure several different sizes and doses of AgNPs then it was reported that to induce oxidative stress and inflammation (Park *et al*. 2011) [24] DNA damage detected by the single cell gel electrophoresis (comet) assay was observed in rats at intravenous doses higher than 20 mg/kg (56.Tiwari *et al*., 2011) [56]. Usually the in vivo studies, in vitro studies with AgNPs showed that shown genotoxic effects – induction of DNA strand breaks, micronuclei, and chromosomal

aberrations – at low non-cytotoxic doses in different types of human and mammalian cells (57. Penny Nymark*et al*. 2013) [57] and it was also reported that after exposure to 0.5–15 g/ml AgNPs for 24 h, a dose-dependent increase in bulky DNA adducts was reported in human lung adenocarcinoma cell (58..Foldbjerg *et al*., 2011) [58] A low non-cytotoxic dose (0.5 g/ml) of 7–10 nm AgNPs increased cell viability in human hepatoma cells, possibly due to hormesis, i.e. stimulatory effects of low levels of potentially toxic agents (57.Penny Nymark *et al*. 2013) [57].

Mechanism of toxicity

Several mechanisms of toxicity have been proposed for AgNPs, but AgNPs toxicity is mainly related to its to its transformation in biological and environmental media, its surface oxidation, silver ion release and interaction with biological macromolecules (Danielle McShan *et al*. 2014) [37]. AshaRani *et al*. reorted the mechanism of toxicity of AgNPs and antiproliferative activity of nanosilver (Fig.4) AshaRani *et al*. have suggested that the disruption of the mitochondrial respiratory chain by AgNPs increases ROS production and interruption of ATP synthesis, thus leading to DNA damage. AgNPs particles can interact with membrane proteins and activate signalling pathways, leading to inhibition of cell proliferation The AgNPs particles can also enter the cell through diffusion or endocytosis to cause mitochondrial dysfunction, generation of reactive oxygen species (ROS), leading to damage to proteins and nucleic acids inside the cell, and finally inhibition of cell proliferation (59.Asha Rani *et al*. 2009) [53, 59]

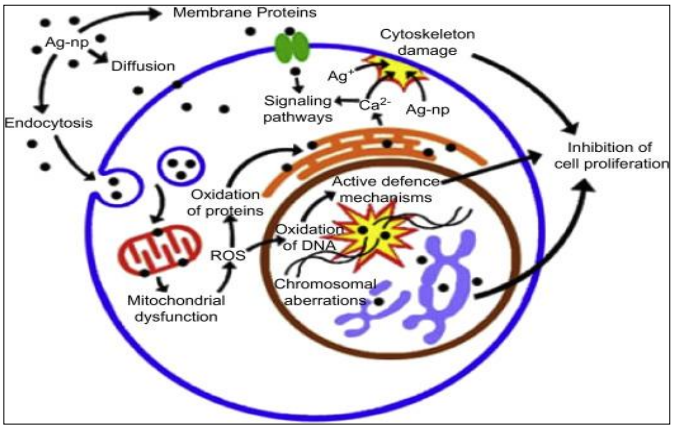

Fig 4: Proposed mechanism of AgNPs toxicity. Note. From "Anti-proliferative activity of silver nanoparticles," by P. AshaRani, M.P. Hande, and S. Valiyaveettil, 2009, BMC Cell Biol, 10, p.65. (59.Asha Rani *et al*. 2009) [53, 59]

Hsin *et al*. 2008 have studied the toxicity mechanism of AgNPs in NIH3T3 fibroblasts. They have found that treatment with nanosilver induces the release of cytochrome c into the cytosol and translocation of Bax to the mitochondria, indicating that AgNPs acts through ROS and C-Jun N-terminal kinase to induce apoptosis via the mitochondrial pathway. Interaction of AgNPs with DNA also leads to cell cycle arrest at the G2/M phase (19.Hsin *et al*. 2008)

Ag-NPs can enter into the cell by diffusion or endocytosis. Once inside the cytoplasm, they can interfere with energy production in mitochondria and promote the generation of reactive oxygen species (ROS). ROS and Ag+ ions released from Ag-NPs may cross the nuclear membrane and cause DNA damage. DNA damage can be either repaired or lead to irreversible chromosome damage or cell death (apoptosis) The mechanism of lethality of both ionic silver and AgNPs involves membrane-disruption, at bactericidal, although the exact mechanism of interaction with bacteria is still debated. Silver-ion release from AgNPs is considered to play an important role. In addition, the presence of AgNPs in bacterial membranes may lead to severe membrane disruption. Oxidative damage may also play a role in the bactericidal activity of AgNPs. (60.Costerto *et al*. 1974, Maillard and Hartemann 1995) [60].

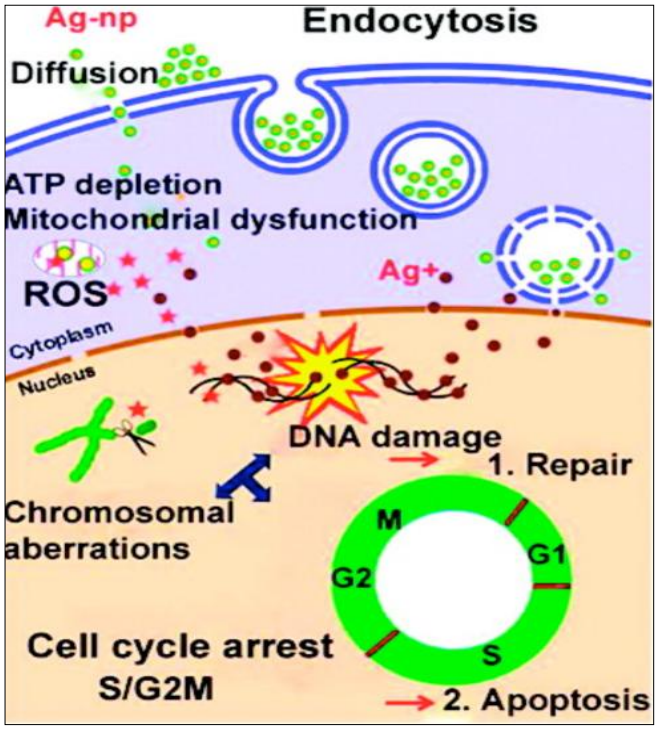

Fig 5: Hypothetical mechanisms of silver AgNPs cytotoxicity.

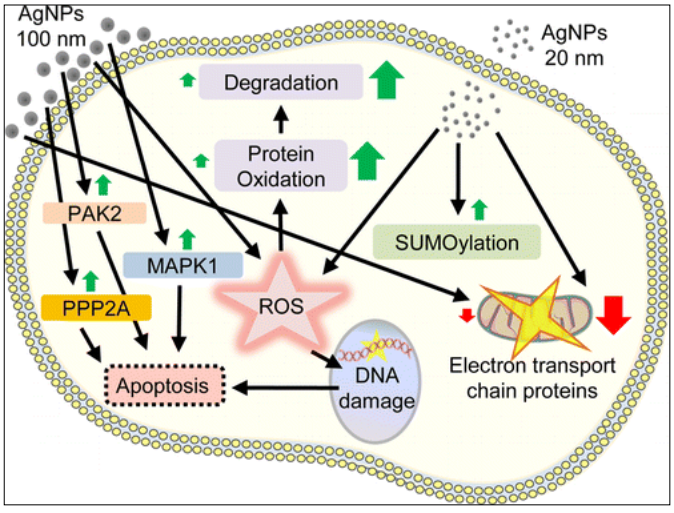

Fig 6: Mechanism to generate ROS and effect apoptosis Environmental toxicity

Environmental Toxicity

AgNPs undergoes several transformations when it is released into the environment such as silver sulphide is available in

waste water treatment plants and also in many freshwater bodies through which the processes is there to determine the bioavailability and toxicity of silver in the environment. A large fraction of the silver released to fresh water bodies to suspended particulates matter which later undergo transformations, accumulation, or resuspension which depend on physical, chemical, and biological conditions. Nowadays silver is increasingly used in a wide range of applications. For example, in sanitization of drinking water, Water disinfection, surface disinfection (like Silver-nanoparticle-embeded antimicrobial paints) cooling towers, recreational waters, textiles, plastics, aqua filters, sunscreens and other cosmetics, food and dietary supplements, antimicrobial surfaces and medical applications (61.Silver *et al*.,2006; 102.Rodriguez *et al*. 2007) [61].

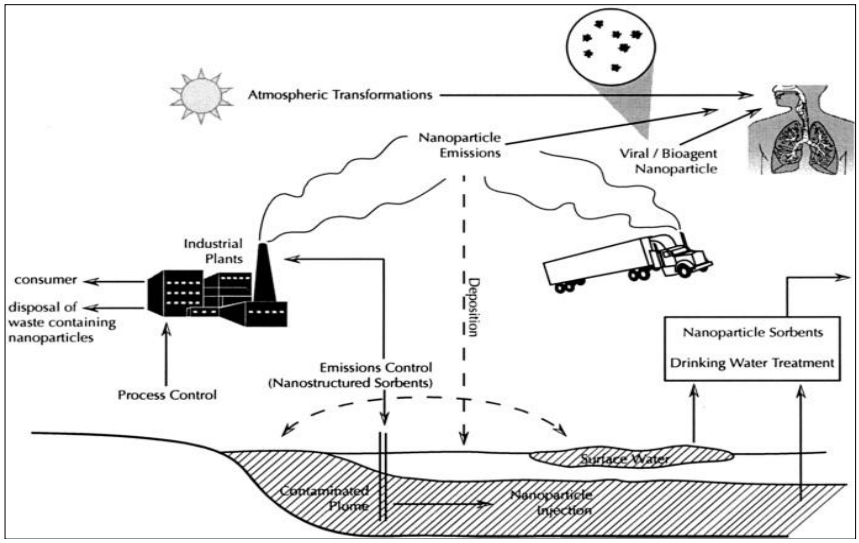

Fig 7: Environmental toxicity

References

1. Silver. http://minerals.usgs.gov/minerals/pubs/commodity/silver/silvemyb04.pdf. Date accessed. 2016.
2. Hammond CR. The Elements, in Handbook of Chemistry and Physics 81st edition. CRC press. 2000. ISBN 0849304814
3. Smith I, Carson B. Trace metals in the environment. Silver. Ann Arbor, MI, Ann Arbor Science Publishers. 1977; 2:469.
4. Foot Defense. Silver: a study of this precious metal and its use in foot care. 2010. http://www.acor.com/Downloads/ebSilverFoot.pdf. Date accessed Nov-2016.
5. Clement JL, Jarrett PS. Antibacterial Silver Metal Based Drugs, 1, 5-6. Appl. Environ. Microbiol. 1994; 73:1712-20.
6. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity, Biointerphases. 2007; 2(4):MR17-MR71.
7. Fabrication S. characterization of chitosan/ nanosilver film and its potential antibacterial application. J Biomater Sci Polym Ed. 2009; 20(14):2129-2144.
8. Kim T-H, Kim M, Park H-S, Shin US, Gong M-S, Kim H-W. Size-dependent cellular toxicity of silver nanoparticles. J Biomed Mater Res Part A. 2012; 100A:1033-1043.
9. Fauss E. The Silver Nanotechnology Commercial Inventory. University of Virginia. 2008. http://www.nanoproject.org.
10. Mirta M, Gerd L, Ivan P, Maja ZA, Slaven D, Walter G *et al*. Cellular uptake and toxicity effects of silver nanoparticles in mammalian kidney cells. 2014. (wileyonlinelibrary.com) DOI 10.1002/jat.3081.
11. Roduner E. Size matters: why nanomaterials are different, Chem. Soc. Rev. 2006; 35:583-592.
12. Burrell RE. The in vitro and in vivo antimicrobial potency of a new silver coated dressing for wound care. 1997. 51OK K955453, Washington DC.
13. 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.
14. Vikas Gupta1, Ajay Kumar. Nanosilver Products - A Review. Chemical Science Review and Letters. 2014; 3(11):717-727.
15. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT *et al*. The bactericidal effect of silver nanoparticles, Nanotechnology. 2005; 16:2346-2353.
16. Wright JB, Lam K, Hansen D, Burrell RE. Efficacy of topical silver against fungal burn wound pathogens. Am. J. Infect. Control. 1999; 27:344-350.
17. Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH *et al*. Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnol. 2005; 29:3-6.
18. Nadworny PL, Wang J, Tredget EE, Burrell RE. Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. Journal of Inflammation. 2010; 7:13.
19. Kirsner RS, Orsted H, Wright JB. Matrix metalloproteinases in normal and impaired wound healing: a potential role for nanocrystalline silver, Wounds. 2001; 13(3 Suppl):4C-12C.

20. Percival SL, Bowler PG, Dolman J. Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model. Int. Wound. J. 2007; 4:186-191.
21. Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW *et al.* Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, Nanotoxicology. 2009; 3(2):109-138.
22. Tolaymat T, El Badawy A, Genaidy A, Scheckel K, Luxton T, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. Sci. Tot. Environ. 2010; (408)5:999-1006.
23. Amro El-Badawy, David Feldhake, Raghuraman Venkatapathy *et al*. State of the Science Literature Review: Everything Nanosilver and More. 2010, 1-355 EPA/600/R-10/084,www.epa.gov.
24. Park J, Lim DH, Lim HJ, Kwon T, Choi JS, Jeong S *et al*. Size Dependent Macrophage Responses and Toxicological Effects of Ag Nanoparticles. Chem. Commun. 2011; 47, 4382-4384.
25. Sung JH, Ji JH, Song KS, Lee JH, Choi KH, Lee SH *et al.* Acute inhalation toxicity of silver nanoparticles. Toxicol Ind Health. 2011; 27:149-154.
26. Drake PL, Hazelwood KJ. Exposure-related health effects of silver and silver compounds: A review. Ann. Occup. Hyg. 2005; 49:575-585.
27. Wadhera A, Fung M. http://dermatology.cdlib.org/111/case reports/argyria/c11 jpg. Accessed. 2016.
28. Pala G, Fronterre A, Scafa F, Scelsi M, Ceccuzzi R, Gentile E. Case Study: Ocular Argyrosis in a Silver Craftsman, J. Occup. Health. 2008; 50:521-524.
29. Rosenman KD, Moss A, Argyria KS. Clinical implications of exposure to silver nitrate and silver oxide. J. Occup. Med. 1979; 21:430-435.
30. Lingling Huo, Rui Chen, Lin Zhao, Xiaofei Shi, Ru Bai, Dingxin Long *et al*. Silver nanoparticles activate endoplasmic reticulum stress signalling pathway in cell and mouse models: The role in toxicity evaluation. Biomaterials. 2015; 61:307-315
31. Oberdörster G. Pulmonary effects of inhaled ultrafine particles, Int. Arch. Occup. Environ. Health. 2001; 74(1):1-8.
32. Gatti AM. Biocompatibility of micro- and nano-particles in the colon. Part II, Biomaterials. 2004; 25:385-392.
33. Gatti AM, Montanari S, Monari E, Gambarelli A, Capitani F, Parisini B. Detection of micro- and nano-sized biocompatible particles in the blood. J. Mater. Sci.: Mater. Med. 2004; 15(4):469-472.
34. Poon VK, Burd A. *In vitro* cytotoxity of silver: Implication for clinical wound care. Burns. 2004; 30:140-147.
35. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity, Biointerphases. 2007; 2(4):17-71.
36. Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips AJAM. What do we (need to) know about the kinetic properties of nanoparticles in the body?, Regulatory Toxicology and Pharmacology. 2007; 49:217-229.
37. Danielle McShan, Paresh C Ray *et al*. Molecular toxicity mechanism of nanosilver. journal of food and drug analysis. 2014; 22:116-1 27.
38. Chen KL, Elimelech M. Aggregation and Deposition Kinetics of Fullerene ©Nanoparticles. Langmuir. 2006; 22:10994-11001.
39. El-Ansary A, Al-Daihan S. On the toxicity of therapeutically used nanoparticles: An overview, J. Toxicol. 2009, 9. doi:10.1155/2009/754810.
40. Supp AP, Neely AN, Supp DM, Warden GD, Boyce ST. Evaluation of cytotoxicity and antimicrobial activity of Acticoat® burn dressing for management of microbial contamination in cultured skin substitutes grafted to athymic mice, Journal of Burn Care & Rehabilitation. 2005; 26(3):238-246.
41. Vlachou E, Chipp E, Shale E, Wilson YT, Papini R, Moiemen NS. The safety of nanocrystalline silver dressings on burns: A study of systemic silver absorption. Burns. 2007; 33:979-985.
42. Tabatabaei *et al*. Prenatal Exposure to Silver Nanoparticles Causes Depression Like Responses in Mice. Indian Journal of Pharmaceutical Sciences Indian J Pharm Sci. 2015; 77(6):681-686.
43. Peters A, Veronesi B, Calderón-Garcidueñas L, Gehr P, Chen LC, Geiser M *et al*. Translocation and potential neurological effects of fine and ultrafine particles a critical update, Part. Fibre Toxicol. 2006; 3:13.
44. Kim YS, Song MY, Park JD *et al*. Subchronic oral toxicity of silver nanoparticles. Particle and fibre toxicology. 2010; 7(20):111.
45. Akram Najjaran1, Nastaran Asghari Moghaddam *et al*. Toxicity effects of nanosilver on liver enzymes, liver and lung tissues. International journal of Biomedical Engineering and Science (IJBES). 2014; 1(1):11-15.
46. Eun-Jung Park, Eunjoo Bae, Jongheop Yi, Younghun Kim *et al*. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environmental Toxicology and Pharmacology. 2010; 30:162-168.
47. Bhol KC, Alroy J, Schechter PJ. Anti-inflammatory effect of topical nanocrystalline silver cream on allergic contact dermatitis in a guinea pig model. Clin. Exp. Dermatol. 2004; 29:282-287.
48. Rebecca Klippstein, Rafael Fernandez-Montesinos *et al*. Silver nanoparticles interactions with the immune system: implications for health and disease. Silver Nano-particle. 309-322.
49. Rob Vandebriel J, Elisa CM Tonk, Liset J de la Fonteyne-Blankestijn *et al*. Immunotoxicity of silver nanoparticles in an intravenous 28-day repeated-dose toxicity study in rats. Particle and Fibre Toxicology. 2014, 11-21.
50. Gromadzka-Ostrowska J, Dziendzikowska K, Lankoff A, Dobrzynska M, Instanes C, Brunborg G *et al*. Silver nanoparticles effects on epididymal sperm in rats. Toxicology letters. 2012; 214:251-258.
51. van der Zande M, Vandebriel RJ, Van Doren E, Kramer E, Herrera Rivera Z, Serrano-Rojero CS *et al*. Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. ACS nano. 2012; 6:7427-42.

52. Austin CA, Umbreit TH, Brown KM, Barber DS, Dair BJ, Francke-Carroll S *et al*. Distribution of silver nanoparticles in pregnant mice and developing embryos. Nanotoxicology. 2012; 6:912-22.
53. Asha Rani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS nano. 2009; 3:279-90.
54. Rinna A, Magdolenova Z, Hudecova A, Kruszewski M, Refsnes M, Dusinska M. Effect of silver nanoparticles on mitogen-activated protein kinases activation: role of reactive oxygen species and implication in DNA damage. Mutagenesis. 2015; 30:59-66.
55. Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol. Sci. 2005; 88:412-419.
56. Tiwari D, Jin T, Behari J. Dose-dependent in vivo toxicity assessment of silver nanoparticle in Wistar rats. Toxicol. Mech. Methods. 2011; 21:13-24.
57. Penny Nymarka, Julia Catalána d, Satu Suhonena, Hilkka Järventaus *et al*. Genotoxicity of polyvinylpyrrolidone-coated silver nanoparticles in BEAS 2B cells. Toxicology. 2013; 313:38-48.
58. Foldbjerg R, Dang D, Autrup H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch. Toxicol. 2011; 85:743-750.
59. Asha Rani P, Hande MP, Valiyaveettil S. Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 2009; 10:65.
60. Costerton JW, Ingram JM, Cheng KJ. Structure and function of the cell-envelope of Gram-negative bacteria. Bacteriol. Rev. 1974; 38:87.
61. Silver S, Phung lT, Silver G. Silver as biocides in burn and wound dressings and bacterial esistance to silver compounds. J Ind Microbiol Biotechnol. 2006; 33(7):627-34