



## GROWING CONCERNS FOR A SAFE DOSE OF SILVER NANOPARTICLES

## Biological Science

<b>Prasanna Shah*</b>	Department of Physics, Acropolis Institute of Technology and Research, Indore, India *Corresponding Author
<b>Akanksha Raj</b>	Department of Zoology, University of Delhi, Delhi, India
<b>Akanksha Singh</b>	Department of Zoology, University of Delhi, Delhi, India
<b>Namita Agrawal</b>	Department of Zoology, University of Delhi, Delhi, India

## ABSTRACT

Silver nanoparticles (AgNPs) play an important role in nanoscience and nanotechnology, particularly in consumer goods and nanomedicine. Although several noble metals have been used for such purposes, an ever-increasing interest in the study of AgNPs due to its rare and unusual properties resulted in a large number of research publications, highlighting the fact that its applications are numerous and multidimensional. Many research papers have illustrated the significant benefits and simultaneously the harmful effects of the AgNPs. Such studies are necessary for the better understanding of the properties of AgNPs and its application in a tailored way which can thereby lead to their proper usage.

In the present review, we are trying to weave a thread between how usage of higher and optimum dosage can change the multi-functional bio-applications of AgNPs from toxic to beneficial. Today, the AgNP based products are considered a boon due to its antibacterial, antifungal, antiviral, anti-inflammatory, anti-angiogenic and anti-cancer activity but the need of this hour is to evaluate a safe dose before its incorporation in the consumer goods. Therefore, the present review deals with the studies related to the impact of different dosage of AgNPs reported in several research publications including ours, considering both *in vitro* and *in vivo* studies using model organisms.

## KEYWORDS

Silver nanoparticles, Nanotoxicity, Safe dose, *in vitro*, *in vivo*

## 1. INTRODUCTION

Nanotechnology deals with the study of nano-sized particles (NPs) with a size of less than 100 nm in at least one dimension. Nanotechnology covers a broad spectrum of research fields such as physics, chemistry, biology and engineering where various types of physical, chemical and biological processes are explained at nano level (Roco & Bainbridge, 2005). The challenging part in the field of nanotechnology is its proper synthesis, characterization and ultimately usage of optimum dosage in the consumer goods for which they have been synthesized.

Recently, owing to their unusual extraordinary properties, nanoparticles have gained significant attention and have been introduced into large variety of consumer products. Due to extraordinary antibacterial property, silver has become part of food and medicine since ancient times (Ahamed, AlSalhi, & Siddiqui, 2010; Tran, Nguyen, & Le, 2013). With the great advancement and better understanding of nanotechnology, silver is being specifically engineered in the scale of nanometers referred as AgNPs and used in a wide variety of consumer products such as cosmetics, food packaging, wound dressing, biomedical devices, clothing, disinfectant products, textiles and also for diagnostic and therapeutic applications.

AgNPs can access the human body through direct skin contact or ingestion and has the potential to subsequently propagate to the secondary target organs where they damage the cellular structures and DNA, causing tissue injury (Chen & Schluesener, 2008).

*In vitro* and *in vivo* reports on AgNPs discussed in the later part of this review strongly suggests that the negative impact of these nano sized particles on living organisms are of a serious concern, particularly, if used in abundance. However, dosage studies conducted using model organisms for *in vivo* impact of AgNPs clearly demonstrates that a safe limit of dosage can be determined in these materials and that safe dose can be advantageous for consumer goods. Earlier, synthesis of the AgNPs was mainly dependent on physical and chemical method but due to several limiting factors mainly toxicity, biological method became part of AgNPs synthesis that has significantly low toxic effect. However, a systematic dosage evaluation of AgNPs synthesized by biological method is still missing.

## 2. CONTRIBUTIONS OF NANOPARTICLES

With the advent of nanotechnology, matter can be manipulated precisely that develops into new materials in the range of nanometres with unique physio-chemical properties. Current research is leading to the development of more sophisticated nanoparticle based consumer

goods and biomedical tools. Among these nanoparticles for example, AgNPs, Titanium dioxide (TiO<sub>2</sub>), Quantum dots (QDs) are widely anticipated to eventually find application in a number of commercial consumer goods, clothing, food industry, paints, cosmetics, electronics, coating application and medical products (Cheng et al., 2004; Cohen et al., 2007).

Silver due to its unique anti-microbial property has been effectively utilized for the treatment of infectious diseases like syphilis, gonorrhoea (Gulbranson, Hud, & Hansen, 2000; Drake & Hazelwood, 2005). Moreover, silver is woven and impregnated into fabrics to kill bacteria. Another widely used nanoparticles in the medical field, nano-based targeted drug delivery system has become a promising tool to provide improved pharmacotherapy by increasing the half-life of drugs (Swai et al., 2009). Various conventional imaging techniques for cancer detection such as X-ray, magnetic resonance imaging (MRI), computed tomography (CT), endoscopy, ultrasound etc have low sensitivity to differentiate between the benign and malignant lesions (Choi, Kwak, & Park, 2010). Nanotechnology represents an advantageous alternative over these currently available methods. The larger surface area to volume ratio enable nanoparticles to be tagged with moieties that is capable of specifically recognizing and binding to the molecules indicative of cancer. Certain nanoparticle probes such as quantum dots (QDs), polymer dots (PDs), gold nanoparticles (AuNPs) display various unique properties to detect cancer before the disease becomes symptomatic.

Gold nanoparticles (AuNPs) are also widely used in many consumer products and for medical purpose. The extraordinary photothermal cell damage property of AuNPs is a promising direction in both tumor therapy as well as the therapy of infectious diseases (Kennedy et al, 2011). The photothermal therapy of AuNPs is based on the heat released in the surrounding media by the excited electrons in the conduction band (Jain, Huang, El-Sayed & El-Sayed, 2008).

## 3. CONCERN FOR SAFE DOSE OF AgNPs

Surprisingly, despite significant advancement in the field of nanotechnology, very few studies have carried systematic assessment of AgNPs exposure as a function of dosage. There are primarily three main exposure routes through which nanoparticles may enter the human body viz. dermal absorption, inhalation and ingestion.

Incorporation of nanomaterials into consumer and biomedical products without a proper dosage study is a cause for concern as nanomaterials are often associated with toxicity.

### 3.1. *In vitro* STUDIES

Majority of *in vitro* nanotoxicity studies employ two different assays: functional and viability assays. Functional assays assess the effect of nanoparticles on various cellular processes whereas; viability assays deal with the cellular death caused by nanoparticles.

Most of the *in vitro* reports have shown that nanoparticles apparently get internalized by cells and make their way to various cellular compartments (Love, Maurer-Jones, Thompson, Lin, & Haynes, 2012). Published reports have also suggested that exposure to AgNPs decreased viability, disrupted plasma membrane integrity, caused mitochondrial dysfunction, induced DNA damage and thus apoptosis in human hepatoma cell lines, human fibroblast cells, mouse germline cells, human leukemia cells and rat adrenal cells (Avalos, Haza, Mateo, & Morales, 2014).

It was interesting to find that toxicity induced by AgNPs also depend upon its fabrication route. A report suggests that coated (citrate, PVP) AgNPs induce less toxicity as compared to the uncoated in the epithelial cell lines (Nguyen et al., 2013). Basically, coating of AgNPs exerts protective effects by chelating the Ag<sup>+</sup> ions and reducing its bioavailability (Yang et al., 2012).

Further, studies on rat liver derived cell lines documented a significant increase in ROS production and decrease in GSH levels at 25 and 50 µg/ml suggesting oxidative stress as the underlying mechanism of AgNPs induced toxicity (Hussain et al., 2005). Few studies also point out that toxicity of AgNPs depends on variables like particle diameter, particle size and dosage. AgNPs have been reported to interfere with various cellular components due to its unique physicochemical properties and produce variable results (Monteiro-Riviere, Inman, & Zhang, 2009). *In-vitro* models cannot establish the fate of nanoparticles inside the human body including its bioavailability, accumulation, metabolism and clearance. Hence, testing of these nanoparticles in *in-vivo* models is imperative.

### 3.2 *In vivo* STUDIES

AgNPs are known to penetrate in many organs of the human body including liver, brain, adipose tissue and lungs (Kim et al., 2008). *In vivo* models that includes mainly Mouse, Rat, zebra fish (*Danio rerio*), *Caenorhabditis elegans* and *Drosophila* serves ideal models to provide a better insight into understanding the safe vs toxic dosage, impact of nanoparticle size, duration of exposure, transgenerational effect etc.

Due to experimental limitations and ethical issues, the *in vivo* studies cannot be conducted in mammalian systems which results in lack of in-depth knowledge in nanotoxicity.

However, to get a better insight, using model organisms can be the best alternative to monitor dosage effect of AgNPs. In a recent report, zebra fish embryo treated with AgNPs displayed developmental delay, tail malformation and edema. The embryos were significantly smaller in size as compared to untreated control with smaller yolk sacs (Muth-Köhne et al., 2013). These results clearly suggest adverse effect of AgNPs in zebra fish.

Internalization of AgNPs and associated toxicity has been studied in *Caenorhabditis elegans* (Meyer et al., 2010). Uptake of AgNPs was observed in the pharynx, gut, unclaid eggs with developing embryo suggesting that AgNPs were ingested and internalized into the cells of *C. elegans*. The localization of AgNPs in the egg cell indicates trans-generational transfer of AgNPs to the offspring. Moreover, AgNPs exposure was found to cause growth inhibition in developing *C. elegans* (Meyer et al., 2010). Interaction of AgNPs with the biological surface of *C. elegans* has been reported to cause severe epidemic edema and burst; further the survival and fertility was found to get negatively influenced by high in a dose-dependent manner (Kim, Nam, & An, 2012).

From the past few years, *Drosophila* has been well acknowledged and widely used for genetic and toxicological investigations of various studies including nanoparticles (Ong, Yung, Cai, Bay, & Baeg, 2015). Several studies conducted in *Drosophila* demonstrate that AgNPs intake in *Drosophila* larvae can induce remarkable pigmentation defects, reduction in body size, loss of body weight and poor locomotor ability of adult flies (Key et al., 2011; Armstrong et al., 2013). Depigmentation in the cuticle of adult flies is caused by the

consumption of excess AgNPs and loss of melanin pigments in their body color (Armstrong et al., 2013). It is noteworthy that in humans, exposure to silver has been found to be associated with specific clinical symptoms, such as argyria, which causes an irreversible gray coloration of the skin (Payne et al., 1992).

It has also been reported that higher doses of AgNPs and its administration early during development is detrimental to the reproductive health, survival and metabolism of *Drosophila* that follows in generations to come without feeding them to AgNPs (Raj et al. 2017 a, b). All the above *in vivo* observations indicate AgNPs being toxic, if ingested at a sub-lethal or lethal dose. However, lower concentrations are quite safe and comparable to un-fed controls therefore, for the best outcome of AgNPs in consumer goods, optimum dose should be used.

### 4. CONCLUSION: OVERCOMING THE LIMITATIONS BY DOSAGE ASSESSMENT

A vast range of engineered nanoparticles are synthesized for the betterment of human life as these nanoparticles, mainly AgNPs play very important role in our day to day life. The extraordinary properties of AgNPs can only be exploited if their toxic effects on human health can be taken care of by using optimum dose. In most of the consumer products, the amount of AgNPs incorporated is not specified. Research carried out in model organisms clearly indicates the adverse effects of these nanoparticles if used in excess. Such studies warrant the need for a proper, nontoxic dosage of nanoparticles. Therefore, a systematic evaluation of AgNPs dosage is paramount to overcome the limitations. It is essential that an optimum dose must be used in the consumer products to get the desired effect from these miraculous nanoparticles.

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