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International Journal of Current Research
Vol. 9, Issue, 06, pp.52381-52384, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

SILVER NANOPARTICLES TOXICITY AND BIOASSAY STUDIES IN FRESHWATER FISH "*CYPRINUS CARPIO*"

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ARTICLE INFO

Article History:
Received 14th March, 2017
Received in revised form
03rd April, 2017
Accepted 28th May, 2017
Published online 30th June, 2017

Key words:
Silver nanoparticles,
Cyprinus carpio,
Bioassay studies,
Physical and chemical properties.

ABSTRACT

Congenital Nanoparticles are found to be reactive because of their small sizes leading to a high volume surface ratio which in turn leads to a lot of binding sites for metals and other compounds. The toxicity of nanoparticles depends on the shape, size, structure of the particle and aggregation. The aggregation, in turn depend on different types of factor in the aquatic environment such as pH, organic matter, ionic strength and composition. Bioassay studies (Acute and sublethal toxicity) were identified in fish *Cyprinus carpio* and the changes were observed. The lethal concentration LC50 for freshwater fish *Cyprinus carpio* were found to 0.1ppm. Silver nanoparticles were exposed for 24 and 96h and the values were noted as 1.0 and 0.5ppm. During the toxicant exposure various changes were observed like increase of mucus secretion, neurobehavioral changes, DNA gets damaged, the heart rate were increased, the liver cells, the stem cells and the swim bladder were affected. The growth and reproduction were stopped and the sterility were existed in fish *Cyprinus carpio* due to the exposure of Silver nanoparticles.

Citation: Dr. Thangam, Y. and Ranganayaki, P. 2017. "Silver nanoparticles toxicity and bioassay studies in freshwater fish "*Cyprinus Carpio'*"", *International Journal of Current Research*, 9, (06), 52381-52384.

INTRODUCTION

Silver nanoparticles (AgNPs) are the most commonly used in our planet, including spectrally selective coatings for solar energy absorption, chemical catalysts especially antimicrobial sterilization, has many applications made them one of nonmaterial's (Pal, *et al*., 2007). Widely used nanoparticles, such as silver nanoparticles, most likely enter the ecosystem and produce physiological response in many animals especially in fishes, possibly altering their fitness and ultimately change their densities or community populations. The toxicity of NPs derived from mammalian studies that have focused on respiratory exposure, from *in vi*tro assays using mammalian cells (Lovern *et al*., 2007). Open access literature regarding the toxicity of nanoparticles (NPs) is still emerging and gaps still exist in our knowledge of this area (Sung, 2007). Despite the dramatic increase in the use of these NPs, little data is available on their potential harmful effects on the ecosystems. Toxicological researches on nanoparticles are more limited, with only a few studies on the acute and sublethal toxic effects of Nps on aquatic animals were identified (Lovern *et al*., 2007). Acute and sublethal toxicity data help to identify the mode of toxic action of a substance and provide information on doses associated with target-organ toxicity and lethality that can be used in setting dose levels for repeated-dose studies. Several routes of discharge of Silver nanoparticles are through synthesis, production, transferring goods. Different models of routes of silver and silver nanoparticles have been modeled (Blaser *et al*., 2011). These show that silver ends up in sewage waste treatment, into sludge, some into surface waters. Few study investigated persistent singly dispersed silver nanoparticles, behaved in different freshwater systems. The experiment demonstrated that AgNP partly agglomerated in the sediment at early release point. However, some fractions were kept stable for a while. This could pose a risk for AgNP to travel to marine waters. Concentrations of dissolved silver have been measured around ng/L in aquatic environments (Luoma, 2008). Also, higher concentrations, over 0.1 mg/L, have been found in surface waters. The toxicity of nanoparticles depends on the shape, size, structure of the particle and aggregation (Choi *et al*., 2008). The aggregation, in turn, will depend on different types

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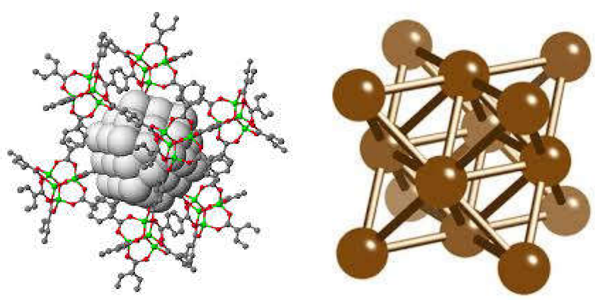
of factor in the aquatic environment such as pH, organic matter, ionic strength and composition. Nanoparticles are found to be reactive because of their small sizes leading to a high volume surface ratio which in turn leads to a lot of binding sites for metals and other compounds (Moore, 2006). This allows them to quite easily enter membrane and accumulate inside cell and cause damages (Choi *et al*., 2008). Silver nanoparticles have been reported to have an effect on reproducibility, DNA and development. Study on 5 dpf *zebrafish* embryos showed effects on survival, embryonic growth and pigmentation at high concentration of AgNP and effects on swim bladder and larval swimming at low concentration of AgNP. AgNP changes the neurobehavioral endpoints and that of changes are differently from Ag^+ (Powers *et al*., 2011). Due to their physicochemical properties AgNP act as catalyst and produce reactive oxygen species, ROS, (Choi *et al*., 2008, Moore 2006). Many of these studies have revealed silver nanoparticles (Ag NPs) to have noticeable toxicity against several cell lines as well as a number of aquatic organisms, but the mechanistic basis of these toxic effects is now an area of active research. In particular, the bioavailability of silver ions (Ag^+) from Ag NPs, considered by many as a major factor in Ag-mediated toxicity. Such studies underscore the need to understand the transport, uptake and degradation of Ag NPs under physiological conditions, to accurately assess the relative benefits and risks of using Ag NPs in commercial products.

Traditional methods in toxicology research and assessments have focused mostly on chemical agents and were not originally designed to encompass nanoparticles, so determining the toxicological effects of Ag NPs raises several challenges. For instance, Ag NPs and Ag-oxides have strong optical extinctions at visible wavelengths and can interfere with colorimetric assays such as the MTT assay, which is used to measure cell viability based on mitochondrial activity. Ecologists have warned that widespread use of such a powerful antimicrobial could have serious negative consequences for bacteria in natural systems if nano- anti microbials are released in waste streams. There is also growing evidence that as well as being toxic to bacteria, silver nanoparticles are also highly toxic to mammalian cells (Braydich-Stolle *et al*., 2005, Gopinath *et al*. 2008). Silver nanoparticles have been shown to damage brain cells , liver cells and stem cells (Braydich-Stolle *et al*. 2005). Even with prolonged exposure to colloidal silver or silver salt deposits of metallic silver under the skin cause skin diseases like argyria or argyrosis (Chen *et al*. 2007). Metal nanoparticles of silver have been demonstrated to be acutely toxic to fish with an LC_{50} value of 1–1.5 mg L^{-1} Griffitt *et al*., 2007. In contrast, aluminium, cobalt, nickel, and titanium dioxide nanoparticles were found to be less toxic to adult fish *Cyprinus carpio* with a 48-hour median lethal concentration (LC_{50}) >10 mg L^{-1} Griffitt *et al*., 2008 . Metal nanoparticles possess unique properties due to their size, shape, surface structure, aggregation characteristics, and chemical composition (Oberdorster *et al*., 2005, Handy *et al*., 2007) that differ from their respective soluble metal. Water chemistry such as salinity and pH influence the toxicity of nanoparticle is sparsely investigated (Blinova *et al*., 2010) but possibly affects the size and shape of the particles (Powers *et al*., 2006). Consequently, it is important to characterize the nanoparticles in the actual environment in order to know how they chemically interact as this likely alters their toxicity. The toxic action of silver nanoparticles was relatively rapid with signs of stress appearing within 30 min of exposure. At higher silver nanoparticle concentrations (>72 μg L^{-1}), toxicity stress signs emerged, with increased respiratory rate. The surface respiration took place, where they ultimately lost equilibrium and sank to the bottom. Few fishes displayed jerky movements and circular swimming just before they lost equilibrium. No signs of aggressive behaviour or changes in body colour were observed in any of the fishes. After 24 hours of exposure, there were no longer visible differences in behaviour between the control and the lowest exposure groups. Fish mucus (thin white branched threads), likely secreted from the gills, were observed exposed to 1.0ppm silver nanoparticles. Mucus secretion was not observed in the control and in the lower concentration exposure. In fish researches, few ecotoxicological studies on aquatic organisms have been performed, so in current study conventional median lethal concentration tests were conducted on the Common carp, as they may provide insights to the potential toxic effects of AgNPs on aquatic environments and introduce most toxicants material. Given the importance of *Cyprinus carpio* in freshwater ecosystems, information concerning the ecotoxicity of widely used nanomaterials on these species could be valuable in relation to aquatic nanoecotoxicology. To compare the toxicity on AgNPs to that of silver ions, the toxicity of silver ions was also examined in *Cyprinus carpio* using the same toxic points as used in the AgNPs toxicity assay. Silver particles especially for the nanomaterials concerned, in spite of increase in the use of nanomaterials and their ubiquitous distribution in aquatic environments, little knowledge is available on their potential toxicity on aquatic animals. Considering the potential of *Cyprinus carpio* as a bioindicator species and the importance of the toxicity of nanoparticles in ecotoxicity monitoring, the measurement of the mortality response in these species after exposure to nanoparticles could provide useful data for aquatic monitoring.

Chemical structure

Silver nanoparticles



Physical properties of Silver nanoparticles

Chemical symbol	:	AgNP
Molecular weight	:	107.87
True Density	:	10490 kg/cm^3
Morphology	:	Spherical
Melting point	:	961.78^0c
Boiling point	:	2162^0c
Average particle size	:	<100 nm

Chemical properties

The recent years of development have also increased the use of silver nanoparticles. AgNPs is commonly used as coating for many products such as medical devices, food storage containers, handrails. AgNPs is also spun down in fabrics and some cases as powder for use in shoes. The optical and physical properties of AgNPs make it very useful in medical applications. Silver nanoparticles are increasingly being incorporated in a variety of products, including clothing, food packaging, washing machines, children's toys and medical equipment, mainly due to its antibacterial properties.

MATERIALS AND METHODS

Bioassay studies were formulated and static method were followed (APHA, 2005).

RESULTS

Silver nanoparticles were exposed to freshwater fish *Cyprinus carpio*. The toxicity of toxicant were observed for 24h and 96h. For 24h the value is 1.0ppm and for 96h 0.5ppm. The sublethal toxicity of silver nanoparticles in fish *Cyprinus carpio* is 0.1ppm ie, 1/10 of 24h value.

nitrate process a different toxicity than silver ions released from silver nanoparticles.

On the contrary, the strong optical properties of Ag NPs be useful in the context of biosensing and biological imaging and offer excellent opportunities to study NP uptake and biodistribution, *in vivo* as well as *in vitro* (Seok-Jeong oh *et al*., 2014, Gad *et al*., 2014). Ag NPs support localized surface plasmons that give rise to resonant light scattering and other optical properties (Yi Zhang *et al*., 2014), and can support a variety of bioanalytical sensing and imaging modalities. Recent applications of Ag NPs include the detection of biomarkers in Alzheimer's disease (Chao-Hung *et al*.,2014), the targeted imaging of cancer cells (Liya Guo *et al*.,2013) and the identification of pathogens by surface-enhanced Raman scattering (Wook-Joon Yu *et al*.,2013). The plasmon-enhanced optical activities of Ag NPs enable them to be tracked in real time without the need for additional labels, as well as a handle for evaluating their eventual degradation. Toxic effects were observed during *Cyprinus carpio* development. Mortality, heart rate and hatching rate were all impacted by Ag NPs.

Table 1. Calculation of log concentration / probit regression line for 24h experiment in fish *Cyprinus carpio* were exposed to different concentration of Silver nanoparticles

S.No	Conc in PPM CH	No of Fish Used	% Dead	Log Conc	Emprical Probit	Expected Probit	Wt probit	Wt Co-efficient	Wt					
				X		Y	Y		W	WX	Wy	WX^2	Wy^2	WXy
1	0.2	10	35	1.544	4.3	4.3	4.1	0.5	5	7.72	20.5	11.919	84.05	31.652
2	0.6	10	46	1.662	4.39	4.45	4.25	0.575	5.15	8.559	21.887	14.225	93.021	36.377
3	1.0	10	50	1.698	5	4.85	4.5	0.6	6	10.188	27	17.299	121.5	45.846
4	1.4	10	54	1.732	5	5	4	0.61	6.1	10.565	24.4	18.298	97.6	42.260
5	1.8	10	60	1.778	5.1	5.1	5	0.615	6.15	10.934	30.75	19.441	153.75	54.673

Table 2. Calculation of log concentration / probit regression line for 96h experiment in fish *Cyprinus carpio* were exposed to different concentration of Silver nanoparticles

S.No	Conc in PPM AgNp	No of Fish Used	% Dead	Log Conc	Emprical Probit	Expected Probit	Wt probit	Wt Co-efficient	Wt					
				X		Y	Y		W	WX	Wy	WX^2	Wy^2	WXy
1	0.1	10	40	1.602	4	3.75	3.5	0.412	4.12	6.600	14.42	10.573	50.47	23.100
2	0.4	10	45	1.653	4.52	4.3	4	0.524	5.24	8.661	20.96	14.317	83.84	34.646
3	0.5	10	50	1.695	4.95	4.7	4.54	0.619	6.19	10.492	28.152	17.784	128.035	47.717
4	0.8	10	54	1.732	5	4.9	4.7	0.603	6.23	10.790	29.281	18.688	137.620	50.714
5	1.2	10	58	1.763	5.2	5	4.99	0.627	6.27	11.054	31.318	19.488	156.436	55.214

DISCUSSION

Nanosilver is a commercial name for pure de-ionized water with superfine silver in suspension. Nanoparticles size is from 5 to 50 nm. Most of the silver is in the form of metallic silver nano-particles. The remaining silver is in ionic form. Because of the small size of the particles, the total surface area of the silver exposed in solution is minimized, resulting in the highest possible effect per unit of silver (Alt *et al*., 2004). As a result , a very small concentration of silver in Nanosilver provides greater effectiveness inside the body than silver solutions in the colloidal form of many times greater concentration. The aim of this paper is to give an overview and analyze the various toxic effects of silver and Nano silver on aquatic animal and human health and the environment. In contrast, Navarro *et al*., 2008, estimated that 1% of the silver in a carbonate-coated nanosilver suspension was free silver ions. This underlines the importance of estimating the dissolution of metal nanoparticles. In fathead minnow embryo, the 96-hour LC_{50} of nanosilver was lower than the 15 µg L^{-1} silver nitrate LC_{50} value, even though the amount of dissolved silver from nanosilver was 18–95 µg L^{-1}. Laban *et al*., 2010 therefore presented the idea that silver ions dissociated from silver

Each of these end points was affected in a dose-dependent manner (5–100 µg/l Ag NPs). Changes in morphology, edema and an overall slowing of development were also detected. In this study, uniform distribution of the nano particles within the fish embryos was also observed. Most of the information available on the mechanisms of toxicity and associated effects of Ag NPs comes from *in vitro* studies, with only limited information from *in vivo* studies. Three main mechanisms of toxicity of AgNPS have been proposed: oxidative stress, DNA damage and cytokine induction. Results from *in vivo* studies have shown that exposure of Ag NPs results in effects in different major organs.

Conclusion

Aquatic toxicity tests may provide insights to the relative sensitivity of *Cyprinus carpio* to AgNPs, which may also provide suitable data on the impact of nanoparticles in water environment, as these species hold important positions in aquatic ecosystems. A significant increase in mortality was observed in *C. carpio* exposed to 0.5 and 1.0 mg/L of nanocid® and nanosil® AgNPs. The results from Bioassay tests provides information for comparison of toxicity and dose-response among members of chemical classes. The fraction of nanosilver that is chemically available is worth considering. The nanosilver toxicity caused by chemical interactions, the toxic portion of nanoparticles originate either from silver ions dissolved from the particle or from the exposed silver atoms on the particle surface.

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