WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 10, 558-566. Research Article ISSN 2277– 7105

SYNTHESIS OF SILVER NANOPARTICLES IN GLUCOSE LEVEL TO FRESHWATER FISH "*CYPRINUS CARPIO*"

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Article Received on
02 July 2017,
Revised on 23 July 2017,
Accepted on 12 August 2017
DOI: 10.20959/wjpr201710-9190

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ABSTRACT

Background: AgNPs are commonly used in the aquatic organism and these nanoparticles are released into the aquatic environment through fishes. Aquatic organisms in biochemical profiles and other under stress serve as important bioindicators in the monitoring of aquatic environment. Glucose is an important source of energy in fish, especially in brain which obtains most of its energy from this carbohydrate. **Objective:** The present investigation aimed to assess the toxicity of silver nanoparticles in plasma glucose of an Indian major carp *Cyprinus carpio* in order to understand the mode of action, stress response and using of these parameters as suitable biomarkers for silver and silver nanoparticles toxicity. **Material &Methods:** Plasma glucose was estimated by O-Toluidine method (Cooper and Mc Daniel, 1970). **Results:** Silver nanoparticles in glucose level exhibited a significant decrease throughout the study period. **Conclusion:** Carbohydrate metabolism were disturbed when animals are subjected to toxic stress and immediate energy demand of the body provided by glucose. When there is a physiological demand for energy, glucose may be oxidized completely to Co_2, water and energy is released.

KEYWORDS: Silver nanoparticles, Cyprinus carpio, glucose level.

INTRODUCTION

Nanotoxicology is a study of impact of nanomaterials on living organisms and environment. It also deals with the quantitative evaluation of severity and frequency of nanotoxic effects in relation to the exposure of the organisms. Metal nanoparticles enter the environment through aquatic and terrestrial and have been used in various fields such as consumer products, industrial applications, health care technology and biochemical activities (Masciangioli and Zhang 2003; Nohynek et al. 2007). Benn and Westerhoff, 2008 stated that the AgNPs are commonly used in the aquatic organism and these nanoparticles are released into the aquatic environment through fishes. The toxicological evidence of AgNPs is still lacking and the safety measurements for these nanoparticles have to be framed. On a world wide scale the emission of nanoparticles have equipped on long term ecotoxicological effects (Handy and Shaw, 2007). Agrahari *et al*. (2007) reported that analysis of biochemical parameters could help to identify target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism. Biochemical parameters were often used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances. Aquatic bodies are traditional recipients in industrial waste containing heavy metals, salts, chemicals and pesticides which when released in higher concentrations cause deleterious effect on organisms. Persistent presence of pollutants like salts, heavy metals in aquatic ecosystem has reportedly caused metabolic stress in organisms even to extent of mortality in some cases (Shaffi *et al*., 2001). For a wide range of environmental pollutants, the aquatic ecosystem is the final sink, where non-target aquatic organism can be affected. (Osman *et al*., 2010).

Silver are formed under various conditions so called clusters which can eventually aggregate forming silver nanoparticles. Silver can be formed by the action of laser during ablation of metallic silver or silver salts. Charged silver clusters like Ag^{2+}, Ag^{3+} are formed by laser desorption/ionization of Ag and silver nanoparticles, Ag salts and Ag_n clusters with *n* upto 50-100µg/L were determined (Staudt *et al*. 2000). Study of silver and silver nanoparticles with two isotopes was observed. Due to the two isotopes of silver, in mass spectra characteristic isotopic envelops are developed. Evidently, there are three combinations of the silver isotopes yielding three peaks in mass spectrum. Silver cluster Ag_8 can be stabilized in erionite channels. Erionite is a kind of zeolite which shows cavities with diameter 0.63nm and length 1.5nm, such elongated cavities are connected by small windows with a diameter

0.25 nm. The form of silver affects the uptake and bioavailability. The form depends on the biochemical and physiochemical condition of the environment and the bioavailability of silver is dependent on its speciation. Silver binds strongly with reduced silver, chloride, thiosulfate and organic material (Ratte, 1999). Aquatic organisms in biochemical profiles and other under stress serve as important bioindicators in the monitoring of aquatic environment. Biochemical parameters in fish are sensitive for detecting potential adverse effect and relatively early events of pollutant damage (Almeida *et al*., 2002). Biochemical methods offer promise in these areas viz, detection of states of stress, suggestion of modes of action and tentatively as tools to explain the metabolic basis for conventional fishery like growth. Vutukuru, (2003), biochemical and physiological methods of diagnosis constitute a promising approach to the problems of detecting the effects of toxic chemicals at the earliest possible stage. Glucose is an important source of energy in fish, especially in brain which obtains most of its energy from this carbohydrate (Soengas and Aldegunde, 2002). In fishes the carbohydrate and lipids play a major role as energy precursors for fishes under stress condition changes in each of these blood components have been employed as useful general indicators of stress in teleost (Radhakrishnaiah *et al*., 1992; Yildizand Benli, 2004). Fish have been described as glucose intolerant, as hyperglycemia after a glucose load can last for several hours, even more than a day. However these effects seen to be related to the fish species and, more importantly to feeding habits (Moon, 2001). Many investigators have reported increase in blood glucose under the stressful conditions of handling forced exercise thermal shock, plasma glucose has been widely monitored to study stress in fish (Teles *et al*., 2003, Pane *et al*., 2005; Gagnon *et al*., 2006).

Decrease of glucose due to silver and silver nanoparticles exposure in *Atlantic cod* (Siikavuopio and Sae, 2006). Decrease in plasma glucose level was reported by (Seok-Jeong, *et al*., 2014) in silver nanoparticles in fresh water fish *Cyprinus carpio*, Chowdhury *et al*. (2004) in *Oncoryhncus mykiss* exposed to cadmium, Pane *et al*.,(2005) in *Oncorhynchus mykiss* exposed to nickel and Gagnon *et al*., (2006) in *Oncorhynchus mykiss* and *Cyprinus carpio* exposed to copper. Decrease in plasma glucose level was reported by Bhattcharya *et al*. (1987) in *Channa punctatus* exposed to industrial pollutants, Das *et al*. (2004a) in *Catla catla* exposed to nitrite and Velisek *et al*., (2007) in rainbow trout *Oncorhynchus mykiss* due to deltamethrin. Sometimes no significant changes in plasma glucose may be observed, because under stress the fish is rapidly consuming energetic substrates. Similar degrees was recorded by Chowdhury *et al*. (2004) in *Oncorhynchus mykiss* exposed to nickel, Gagnon *et*

al. (2006) in *Oncorhynchus mykiss* exposed to copper, *Cyprinus carpio* species exposed to silver and silver nanoparticles, Seok-Jeong, *et al*., 2014. The present investigation aimed to assess the toxicity of silver and silver nanoparticles in plasma glucose of an Indian major carp *Cyprinus carpio* in order to understand the mode of action, stress response and using of these parameters as suitable biomarkers for silver and silver nanoparticles toxicity.

MATERIALS AND METHODS

ESTIMATION OF PLASMA GLUCOSE

Plasma glucose was estimated by O-Toluidine method (Cooper and Mc Daniel, 1970).

RESULTS

Table 1 represents the data on changes in the biochemical profiles of fish *Cyprinus carpio* exposed to concentration of silver nanoparticles in glucose level to sublethal toxicity. Silver nanoparticles in glucose level exhibited a significant decrease throughout the study period. The significant decrease in glucose level was directly proportional to the exposure period showing a percent decrease of at the end of -16.66, -12.17, -9.17, -7.54, -5.82 at the end of 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th}, days for glucose level. There were significant ($P< 0.05$) variation among the treatments.

Table 1: Changes in the glucose content of *Cyprinus carpio* exposed to sublethal concentration of silver nanoparticles for 35 days.

S.No	EXPOSURE PERIOD	CONTROL	EXPERIMENT	CHANGE %
1	7	120 ± 0.099b	100 ± 1.375ab	-16.66
2	14	115.00 ± .123c	101 ± 1.097a	-12.17
3	21	109 ± 0.134d	99 ± 0.176a	-9.17
4	28	106 ± 0.130c	98 ± 0.339b	-7.54
5	35	103 ± 0.183e	97 ± 0.279ab	-5.82

Values are mean ± S.E. of five individual observations. (-) Denotes percent decrease over control. ** Significant at 5% level. Means in a column bearing same letter(s) are significantly different according to DMRT ($P>0.05$).

DISCUSSION

The biochemical profile changes the metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of substances Adedeji (2010). Alteration of blood chemistry may

be indicative of unsuitable environmental conditions (temperature, pH, oxygen concentration) or the presence of stressing factors, such as toxic chemicals (Barcellos *et al*., 2004; Firat and Kargin, 2010). Biochemical and physiological methods of diagnosis constitute a promising approach to the problems of detecting the effect of toxic chemicals at possible stages (Larsson *et al*., 1985). According to the use of biochemical methods constitute the area viz., detection of stages of stress, suggestion of modes of action and tentatively as tools to explain the metabolic basis for conventional fishery statistics like growth. Since pollution may induce certain biochemical changes in fishes before the drastic cellular and systematic dysfunction manifest, appropriate biochemical parameters could be used effectively as sensitive indicators. Glucose is one of the most sensitive indices of an organisms stress. Earlier reports on the changes in the blood glucose in response to stress are contradictory showing both a rise and a fall. Its high concentrations in blood indicate that the fish is under stress and intensively using energy reserves (Firat and Kargin, 2010). Glucose in serum is a major metabolite of carbohydrate metabolism (Artacho *et al*., 2007; Zhou *et al*., 2009) and blood glucose level have long been used as indicators of stress in fish (Osman *et al*., 2010).

Decrease of glucose due to silver and silver nanoparticles exposure in fresh water fish *Cyprinus carpio*, may be due to the decrease of metabolite in liver (Seok-Jeong, *et al*., 2014). Decrease in plasma glucose level was reported in silver nanoparticles in the metabolic profile altered in two fishes the *Pacu* and *Tambacu* due to short term silver exposure (Moraes *et al*., 2006). In Pacu, the glucose decreased in liver and muscle while the glycogen increased in liver and decreased in muscle. In *Tambacu*, glucose levels were higher in the liver and plasma. The *Tambacu's* liver provided glucose in a more evident way than *Pacu* under silver effect. And in silver nanoparticles glucose level decreased in fish *Cyprinus carpio* in the present study. Ruparelia *et al*., (1997) observed a significant increase in glucose level in *Sarotherodon mossambica* exposed to cadmium induced hyperglycemia has been reported. The engineered nanomaterials comprise of numerous different biochemical and physical forms and some of these materials including carbon nanotubes, carbon spheres called fullerenes (Zhu et al. 2006) and nanoparticles made from metals (Griffitt et al. 2007), metal oxides (Federici et al. 2007) or composites made of several metals (King-Heiden et al. 2009) have adverse effects on fish (Smith et al. 2007). To study the aquatic toxicity of fish in glucose level, fish species has been widely used as an indicator of pollutant and they strongly respond to stress conditions. Carbohydrate metabolism is disturbed when animals are subjected to toxic stress and immediate energy demand of the body provided by glucose.

When there is a physiological demand for energy, glucose may be oxidized completely to Co_2, water and energy is released. In the presents study silver nanoparticles exposed to fish *Cyprinus carpio*, decrease in glucose level, might be due to hypoglycemia or it might be due to stress condition of the intake of metal silver nanoparticles into the body of fish.

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