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Original Article

Assessment of the Effect of Silver Nanoparticles on Haematological Profile and Total Protein Content in a Freshwater Fish, *Channa Punctatus* (Bloch)

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Abstract

In the present experiment effects of silver nanoparticles (AgNPs) on various heamatological parameters and total protein content of some selected tissues (*i.e.,* muscle, liver, kidney and gill) of an Indian fresh water fish, *Channa punctatus* was evaluated. Two different concentrations of biologically synthesized AgNPs (100% and 50%) were intra-muscularly injected to the experimental fish is respect of distilled water treated control at different fixation intervals (*viz*., 6 hr, 24 hr, 72 hr, 96 hr, 7 day and 15 day). Treatment of AgNPs (100% and 50%) to the experimental fishes showed a significant decrease in number of the red blood cells and increase in number of white blood cells particularly at the longer fixation intervals (7 and 15 days) in respect of distilled water treated control. Total protein content of liver and kidney showed significant increase during 15 and 30 days of exposure compared to the control. In the contrary, total protein content of gill and muscle showed a significant decrease after exposure to AgNPs in respect of the control.

Keywords: Nanoparticles; RBC; WBC; Protein; Fish.

Introduction

As a result of the wide application of nanomaterials in industry, agriculture, business, medicine and public health; nanothechnology has gained a great deal of public interest (Ju-Nam and Lead, 2008). Uses of nanomaterials are likely to result in releases into aquatic systems and may pose a risk to aquatic ecosystems (Moore, 2006). Nanoparticles are part of our daily life in form of cosmetics (Perugini et al., 2002), drug delivery system (Jin and Ye, 2007), therapeutics (Czupryna and Tsourkas, 2006) and biosensors (Prow et al., 2006). Recently, significant concerns have been expressed about the potential risk of silver nanoparticles (AgNPs), due to the current and projected high exposure (Luoma, 2008) and their likely high hazard and toxicity in the environment (Klaine et al., 2008). Indeed, a number of ecotoxicology studies have been conducted to study the effect of AgNPs in algae, bacteria, invertebrates, fish and humans in both *in vivo* and *in vitro* studies (Obserdorster, 2004; Carlson et al., 2008; Gopinath et al., 2008; Govindasamy and Abdul Rahuman, 2011). In this context in-depth studies are indeed needed to evaluate the potential risks of AgNPs in the aquatic systems as well as its inhabitants like fish. The AgNPs synthesized biologically have been widely used in the field of medicinal industries (Asz et al., 2006) but its effects on fish had been hardly evaluated. In the present study an attempt has been made to evaluate the effect of biologically synthesized AgNPs (synthesis of AgNPs has been carried out by using the extracellular filtrate of the fungal strain, *Aspergillus foetidus* MTCC8876) on the haematological parameters and total protein content of some selected tissues of an important fresh water fish *Channa punctatus*. As the fishes are directly exposed to the aquatic environment, thus the assessment of its potential risks on the haematological parameter and the total protein content of selected tissues is of immense importance in scientific research.

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Materials and Methods

Test Fish

Live specimens of *Channa punctatus* belonging to the family Channidae, weighing between 15 and 18

gm and measuring from 11 to 14 cm in length, were procured from the local fish market and properly washed in tap water and treated with 0.02% $KMnO_4$ and 0.004% formalin solution to remove external infection of fungi, algae, etc. The fishes were acclimatized in a glass aquaria (20 L capacity) containing fresh tap water (stored from deep tube well) for seven consecutive days. During acclimatization the fish were fed with TOKYO (made in Japan) twice in a day. The experiment was conducted in 15 L glass aquaria each containing 10 L tap water (pH 8.57 ±0.14, temperature 28-30^{0}C) and five number of fish were used for both the control and experimental series.

Test Chemical

Biosynthesised silver nanoparticles (AgNPs) by fungi *Aspergillus foetidus* in crude condition (100%) was collected from the Biochemistry and Biophysics Laboratory of University of Kalyani, Kalyani, West Bengal, India (Roy and Das, 2014). 50% AgNPs solution was prepared through addition of required amounts of distilled water. Both these concentrations of AgNPs were injected to the test fishes intra-muscularly for experimentation. Distilled water treated fishes were served as control.

Experimental Design

The fishes were divided into three equal groups consisting of 10 fishes in each group and injected with test chemicals @ 1ml / 100 gm body weight- (i) group-I: distilled water treated control; (ii) group-II: 50% AgNPs; and (iii) group-III: injected with 100% AgNPs at six different fixation intervals (*viz.*, 6 hr, 24 hr, 72 hr, 96 hr, 7 days and 15days). During experiment, the waste products were removed by using good quality of aquaria water filter for keeping a good environmental condition within the aquaria.

Study of Haematological Profile and Total Protein Content

The blood from the caudal vein of control and treated fish was collected for heamatological investigation. RBC and WBC were examined following the procedures of Wintrobe (1957) and Sood (1996). For the study of total protein content in four selected tissues (*viz.,* liver, kidney, gill, muscle), the fishes were removed after 6 hr, 24 hr, 48 hr, 72 hr, 7 days and 15 days from each of the treated and control specimens separately and homogenized in 0.1% NaCl solution. After centrifugation at 4500g for 15 minutes, the supernatants were collected. Aliquotes containing known amount of protein for each tissue (5 to 10 micro gram) was used for estimating the quantity of protein as per the method of Bradford (Bradford, 1976). A standard curve was constructed from different known concentration of Bovine Serum Albumin (BSA) against their OD values. The amount of unknown protein (µg / gm) was calculated in the routine manner against the standard curve.

Statistical Analysis

The experiments were conducted in triplicates. Data of haematological and total protein contents have been presented as mean ± standard error (S.E.). Values of RBC and WBC of fish blood and total protein of tissues were compared statistically with control by using student's t test (2- tailed) with the help of SPSS 17. The level of significance was established at $p<0.05$.

Results

Total RBC Count

The erythrocyte count of healthy controls showed a mean value of 2.86×10^6 mm^{-3}. The fishes that were treated with AgNPs showed an alteration in mean value of RBC for both the treatment series. For 50% treated series the lowest RBC count was found at 7 days of fixation interval and the highest was at 6 hr, but the significant decrease of RBC count was found for 72 hr, 7 day and 15 day of fixation intervals ($p<0.05$) (Table-1). For 100% AgNPs treated series highest RBC count was found at 24 hr and the lowest at 96 hr interval, of which the data at 48 hr, 96 hr, 7 day and 15 day found significant ($p<0.05$) (Table-1).

Total WBC Count

The results of the total count of WBC revealed that the blood of the control fish showed a mean value of 57.84×10^3 mm^{-3}. The fishes treated with AgNPs reflect an alteration in mean value of WBC for both the treated series. For 50% treated series the lowest WBC count was found at 72 hr fixation interval and the highest was at 15 days, but the significance differences were found for 15 day of fixation interval ($p<0.05$) (Table-2). For 100% AgNPs treated series the result was significant for 7 day and 15 day of fixation intervals ($p<0.05$) (Table-2).

Total Protein Content of Selected Tissues

Total protein content of four selected tissues (*viz.*, liver, kidney, gill and muscle) of *C. punctatus* was

recorded against control for both the treatment series at different fixation intervals (Table 3 to 6). Highest value of total protein content in liver was 18.73 µg/mg for 50% and 26.88 µg/mg for 100% of AgNPs, of which the value of 48 hr and 96 hr (for 50%) and 24 hr and 15 days (for 100%) showed significant differences in respect of the controls ($p<0.05$) (Table 3). Highest value of total protein content in kidney was 12.46 µg/mg for 50% and 19.85µg/mg for 100% of AgNPs, of which the values at 6 hr (for 50%) and 24 hr and 15 days (for 100%) showed significant differences ($p<0.05$) (Table 4). The lowest value of total protein content in gill was 2.86 µg/mg for 50% and 1.76 µg/mg for 100% AgNPs, of which the result at 48 hr (for 50%) and 24 hr and 7 day (for 100%) showed significant differences ($p<0.05$) (Table 5). The lowest value of total protein content in muscle was 6.38 µg/mg for 50% and 5.43 µg/mg for 100% AgNPs, of which the result at 48 hr and 72 hr (for 50%) and 6 hr, 48 hr, 72 hr, 96 hr, 96 hr, 7days (for 100%) showed significant differences in respect of control ($p<0.05$) (Table 6).

Table 1: Effect of AgNPs on total RBC ($\times 10^6$ mm^{-3}) of *C. punctatus.* Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				2.86±0.06			
50% AgNPs	2.73± 0.002	2.06±0.001	2.26±0.001	1.49±0.005*	2.22±0.007	1.41±0.011*	1.85±0.004*
100% AgNPs	2.36±0.003	3.43±0.005	1.82±0.011*	2.63±0.009	1.14±0.008*	1.72±0.059*	1.88±0.007*

Table 2: Effect of AgNPs on t otal WBC ($\times 10^3$ mm^{-3}) of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				57.84±0.17			
50% AgNPs	58.73± 0.018	55.06±0.014	59.26±0.012	51.49±0.052	57.22±0.017	57.41±0.011	61.85±0.014*
100% AgNPs	52.86±0.032	53.43±0.053	59.82±0.11	52.63±0.019	56 .14±0.008	62.72±0.059*	64.88±0.047*

Table 3: Effect of AgNPs on the total protein content (µg/mg) of liver of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				13.93± 0.007			
50% AgNPs	18.73± 0.018	15.06±0.014	9.26± 0.012*	11.49±0.052	7.22±0.017*	17.41±0.011	15.85±0.014
100% AgNPs	12.86±0.032	33.43±0.053*	19.82±0.11	12.63±0.019	11.14±0.008	12.72±0.059	26.88±0.047*

Table 4: Effect of AgNPs on the total protein content (µg/mg) of kidney of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				6.31±0.028			
50% AgNPs	12.46±0.017*	2.69±0.009	2.53±0.009	2.3±0.006	3.13±0.002	5.78±0.012	2.78±0.004
100% AgNPs	4.04±0.008	19.85±0.001*	10.37±0.164	3.06±0.01	9.93±0.026	.72±0.008	19.85±0.001*

Table 5: Effect of AgNPs on the total protein content (µg/mg) of gill of *C. punctatus*. Data presented as mean ± SE, n=5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				7.19±0.004			
50% AgNPs	9.49±0.042	3.6±0.021	2.86±0.002*	3.29±0.011	8.96±0.027	8.22±0.026	10.75±0.034
100% AgNPs	7.75±0.008	14.04±0.013*	7.43±0.079	6.22±0.036	6.29±0.025	1.76±0.012*	7.77±0.003

Table 6: Effect of AgNPs on the total protein content (µg/mg) of muscle of *C. punctatus*. Data presented as mean ± SE, n= 5. Asterisks (*) indicate the values that are significantly different ($p < 0.05$)

Treatment	Time						
	6 hour	24 hour	48 hour	72 hour	96 hour	7 day	15 day
Control				15.81±0.015			
50% AgNPs	15.57±0.015	10.17±0.003	6.38±0.003*	7.08±0.016*	9.47±0.011	20.8±0.026	14.53±0.044
100% AgNPs	5.43±0.011*	17.85±0.006	7.61±0.151*	4.25±0.043*	6.92±0.021*	7.10±0.027*	17.9±0.041

Discussion

The fate of nanoparticles in the aquatic environment, their interactions with biotic and abiotic components, and their potential to cause harm are still poorly understood, and these uncertainties are driving concerns on the risks they may pose to human and environmental health (Tessa et al., 2010). In the present experiment it was found that the total count of RBC in the experimental fishes for both the treatment series was lower than the control for all the fixation intervals (Table 1). Previously, Adakole (2012) showed that when *C. gariepinus* was exposed to metal finishing company effluents, RBC was initially increased and finally decreased after chronic exposure. Chandanshive et al. (2012) reported that decrease in RBC of fish *Labeo rohita* after exposure to mixture of heavy metals. In the present study, suppression of total RBC count as a result of AgNPs treatment may be due to destructive action of AgNPs on erythrocytes and the viability of the cells may be affected corroborated with the previous findings of Karuppasamy (2000). It was reported that multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero, 1973). On the other hand, hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al., 1987). Thus, the present results of reduction in total RBC count in *C. punctatus* treated with AgNPs may be due to the deposition of nanoparticles within the cells.

Total count of WBC was found to be increased in the longer intervals (7 days and 15 days) for both the concentration of AgNPs in respect of control (table-2). Previously, progressive increased levels of total WBC count have been reported in *C. punctatus* exposed to lead (Hymavathi and Rao, 2000), *Clarias batrachus* exposed to mercuric chloride (Joshi et al., 2002) and *Clarias gariepinus* to metal finishing company effluents (Adakole, 2012). The increase in total WBC count in the present study was as a result of direct stimulation for its defense from diseases due to the presence of nanoparticles (Singh et al., 2008), or may be attributed to alteration in blood parameters and direct effects of AgNPs (Sinha et al., 2000).

Alteration of total protein content as a result of AgNPs injection to the experimental fish was found for all the tissues examined (*i.e.*, liver, kidney, gill and muscle) (Tables 3 to 6). The exact reason of this fluctuation of total protein content at different fixation intervals is not clearly understood. However, it can be said that the increase of total protein content may be due to increased protein synthesis in the particular tissue as an effect of AgNPs. The decreased levels of total protein in fish exposed to AgNPs suggest that the protein might be used as an alternative source of energy, due to high energy demand that induced by nanosiver intoxication (Hori et al., 2006).

Thus, the present results confirmed that stress due to AgNPs does create hematological disturbances, total protein alteration, affecting the immune system and making the fish vulnerable to diseases.

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