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Detection of Silver Nanoparticles (Nanosil®) LC_{50} in Silver Carp (*Hypophthalmichthys molitrix*) and Goldfish (*Carassius auratus*)

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Abstract: Nanometer sized silver can flow into water ecosystems, where it can exert a variety of physiological effects in aquatic animals, including fish. Nanoparticles may also have ecotoxicological effects after being discharged into aquatic ecosystems. Detection of LC_{50} is one of the common ways in evaluation of aquatic toxicants. LC_{50} is the ambient aqueous chemical activity causes 50% mortality in an exposed population. The current research aimed to examine the effect of nanometer sized silver of an Iranian commercial nanosilver (Nanosil®) on the mortality response of two freshwater fish Silver Carp (*Hypophthalmicthys molitrix*) and Goldfish (*Carassius auratus*) and define the toxicity relationship between nanoparticle and survival of these species. Fish were exposed to the commercial doses of nanometer sized silver (0, 2, 2, 20, 50, 100 and 200 ppm). LC_{50} was determined with probit analysis. As there can found LC_{50} in goldfish (83.9 ppm) was higher than silver carp (66.4 ppm). Increased mortality was concomitantly observed with AgNPs-exposed, which suggests AgNPs-induced mortality might provoke higher-level consequences.

Key words: Ag · Fish · Mortality · Nanotechnology · Toxicity Test

INTRODUCTION

Calculations of LC_{50} are based on two important assumptions. The first assumption is that the exposure time associated with the specified LC_{50} is sufficient to allow almost complete chemical equilibration between the fish and the water. The second assumption is that the specified LC_{50}, the minimum LC_{50} that kills the fish during the associated exposure interval. Fortunately, most reliable LC_{50} satisfy these two assumptions [1]. The 96-h LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms [2].

Silver nanoparticles (AgNPs) have the most commonly used in our planet, including spectrally selective coatings for solar energy absorption, chemical catalysts and especially antimicrobial sterilization, which has many applications made them one of nonmaterial's [1-3]. Widely used nanoparticles, such as silver nanoparticles, will most likely enter the ecosystem and may produce a physiological response in many animals, possibly altering their fitness and might ultimately change their densities or community populations. Open access literature regarding the toxicity of nanoparticles (NPs) is still emerging and gaps still exist in our knowledge of this area [3].

Despite the dramatic increase in the use of silver nanoparticles (AgNPs), little data is available on their potential harmful effects on the ecosystems [4]. Toxicological researches on nanoparticles are more limited, with only a few studies on the acute toxic effects of NPs on aquatic animals [4-7], so in current study conventional median lethal concentration tests were conducted on two freshwater fish silver carp (*Hypophthalmicthys molitrix*) and gold fish (*Carassius auratus*), as they may provide insights to the potential toxic effects of AgNPs on aquatic environments and introduce most toxicant material from Iranian nanotechnology company. Given the importance of *H. molitrix* and *C. auratus* in freshwater ecosystems, information concerning the ecotoxicity of widely used nanomaterials on these species could be valuable in relation to aquatic nanoecotoxicology.

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MATERIALS AND METHODS

Acute toxicity tests were conducted on silver carp (~ 45 g and 18 cm) and goldfish (~ 15 g and 7 cm) obtained in commercial fish farms, Gorgan-Iran. Only healthy fish, as indicated by their activity and external appearance, were maintained alive on board in a fiberglass tank. Samples transferred to a 400-L aerated tank equipped with aeration with 200 L of test medium. All samples were acclimated for one weeks in a 15 aerated fiberglass tanks at 18°C under a constant 12:12 L: D photoperiod [8].

In this study the effective doses of AgNPs (nano products from Iranian nanotechnology company, Nanosil®) were compared in two freshwater fishes. A water soluble form of Ag ions size <100 nm, made by Kimiafaam Company, Tehran, Iran were homogenously dispersed in deionized water by sonication. As we did not had exact effective dose of Nanosil®, during the toxicity test it was used as commercial dose. This was a liquid product that for antimicrobial activities in veterinarian purposes.

Groups of 21 fish were exposed for 96 h in fiberglass tank. Test medium was not renewed during the assay and no food was provided to the animals. Values of mortalities were recorded at time 24, 48, 72 and 96 h [9] in order to calculate the 96h-LC_{50} for silver and its confidence limits (95%) by Boudou and Ribeyre [1]. Also LC_{50} values were calculated from the data obtained in acute toxicity bioassays, by Finney's [10] method of ''probit analysis'' and with SPSS computer statistical software. In Finney's method, the LC_{50} value is derived by fitting a regression equation arithmetically and also by graphical interpolation by taking logarithms of the test chemical concentration on the X axis and the probit value of percentage mortality on the Y axis [10].

The 95% confidence limits of the LC_{50} values obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan [11]. Probit transformation adjusts mortality data to an assumed normal population distribution that results in a straight line. Probit transformation is derived from the normal equivalent deviate (NED) approach developed by Tort, who proposed measuring the probability of responses (i.e., proportion dying) on a transformed scale based in terms of percentage of population or the standard deviations from the mean of the normal curve [12].

$LC_{1,10,30,50,70,90,99}$ values were derived using simple substitution probit of 1,10,30,50,70,90 and 99 respectively for probit of mortality in the regression equations of probit of mortality vs. silver. The 95% confidence limits for LC_{50} were estimated by using the formula LC_{50} (95% CL) = $LC_{50} \pm 1.96$ [SE (LC_{50})]. The SE of LC_{50} is calculated from the formula: $SE(LC50) = 1/b\sqrt{pnw}$ Where: b=the slope of the silver/probit response (regression) line; p=the number of silver used, n = the number of animals in each group, w = the average weight of the observations [13]. At the end of acute test, Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) were determined for each endpoint measured. In addition, the maximum acceptable toxicant concentration (MATC) was estimated for the endpoint with the lowest NOEC and LOEC [14].

RESULTS

The mortality of silver doses for Nanosil® 0, 0.2, 2, 20, 50, 100 and 200 ppm after acute exposure to the Silver carp and Goldfish were examined during the exposure times at 24, 48, 72 and 96 h (Tables 1-2). Fishes exposed during the period 24-96h had significantly increased number of dead individual with increasing concentration. There were significant differences in number of dead fish between the duration 24-96 in each. There was 100% mortality at 200 ppm of Nanosil® within the 96 h after exposure for silver carp and goldfish and no mortality at 20 ppm within the 96 h exposure times for silver carp and goldfish.

Table 1: Cumulative mortality of silver carp during acute exposure to Nanosil® (n=21, effective dose)

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
Control	0	0	0	0
0.2	0	0	0	0
2	0	0	0	0
20	0	0	0	0
50	0	3	6	10
100	5	9	12	17
200	21	21	21	21

Table 2: Cumulative mortality of goldfish during acute exposure to Nanosil® (n=21, commercial dose)

	No. of mortality			
Concentration (ppm)	24h	48h	72h	96h
Control	0	0	0	0
0.2	0	0	0	0
2	0	0	0	0
20	0	0	0	0
50	0	0	3	6
100	5	5	9	13
200	18	18	21	21

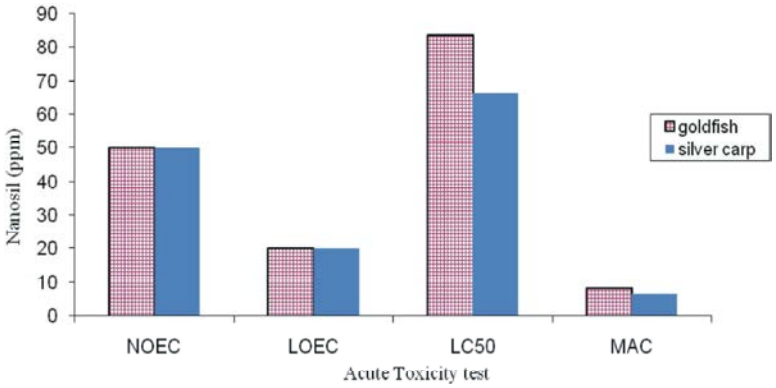

Fig. 1: Acute toxicity testing statistical endpoints of silver nanoparticle

Table 3: Lethal Concentrations (LC_{1-99}) of Nanosil® (mean ± Standard Error) depending on time (24-96h) for silver carp

	Concentration (ppm - effective dose) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	68.8±5.6	16.4±0.45	3.93±0.36	1.04±0.34
LC_{10}	88.9±5.6	54.8±0.45	41.2±0.36	30.3±0.34
LC_{30}	103.±5.6	82.6±0.45	68.2±0.36	51.6±0.34
LC_{50}	113.±5.6	101.±0.45	87.0±0.36	66.4±0.34
LC_{70}	123.±5.6	121.±0.45	105.±0.36	81.1±0.34
LC_{90}	138.±5.6	148.±0.45	132.±0.36	102.±0.34
LC_{99}	158.±5.6	187.±0.45	170.±0.36	131.±0.34

Table 4: Lethal Concentrations (LC_{1-99}) of Nanosil® (mean ± Standard Error) depending on time (24-96h) for goldfish

	Concentration (ppm - commercial dose) (95 % of confidence limits)			
Point	24h	48h	72h	96h
LC_1	35.6±0.48	35.6±0.48	16.4±0.45	5.08±0.37
LC_{10}	84.6±0.48	84.6±0.48	54.8±0.45	40.5±0.37
LC_{30}	120.±0.48	120.±0.48	82.6±0.45	66.1±0.37
LC_{50}	144.±0.48	144.±0.48	101.±0.45	83.9±0.37
LC_{70}	169.±0.48	169.±0.48	121.±0.45	101.±0.37
LC_{90}	204.±0.48	204.±0.48	148.±0.45	127.±0.37
LC_{99}	253.±0.48	253.±0.48	187.±0.45	162.±0.37

Median lethal concentrations of 1%, 10%, 30%, 50%, 70%, 90% and 99% test were in Tables 3-4. Because mortality (or survival) data were collected for each exposure concentration in a toxicity test at various exposure durations (24, 48, 72, or 96 hours), data can be plotted in other ways; the straight line of best fit is then drawn through the points. These were time-mortality lines. As there can found LC_{50} of goldfish was higher than silver carp.

Toxicity Testing Statistical Endpoints are in Fig. 1. LOEC and NOEC were same for studied fishes (20 and 50 ppm respectively), however LC_{50} (the median Lethal Concentration) had significant different between species. The Maximum Acceptable Toxicant Concentration (MATC) of Nanosil® for silver carp and goldfish were 6.6 and 8.3 ppm respectively.

DISCUSSION

Aquatic toxicity tests may provide insights to the relative sensitivity of silver carp and goldfish to AgNPs, which may also provide suitable data on the impact of nanoparticles on water environment, as these species hold important positions in aquatic ecosystems. A significant increase in mortality was observed in silver carp and goldfish exposed to acute dose of Nanosil®, however this acute effect was higher in silver carp.

In determining the toxicity of a new chemical to fish, an acute toxicity test is first conducted to estimate the median lethal concentration (LC_{50}) of the chemical in water to which organisms are exposed [15]. The relationship between the degree of response of test organisms and the quantity of exposure to the chemical almost always assumes a concentration-response form [15], As in our results the *y*-axis represents percentage mortality and the *x* -axis represents concentration of silver. Both variables increased with distance from origin. The cumulative responses to silver concentrations yield the sigmoid (S-shaped) curve.

Variability in acute toxicity even in a single species and single toxicant depending on the size, age and condition of the tested species along with experimental factors. The differences in acute toxicity even may be due to changes in water quality and test species [16]. In the present study, LC_{50} values indicated that silver is more toxic to the studied fish, especially silver carp.

LC_{50} obtained in the present study compared with corresponding values that have been published in the literatures for other species of fish, show different LC_{50}

in different species and even different time, but lower value of LC_{50} for studied fish was important and confirm sensitively of aquaculture species to low silver doses. Although the LC_{50} under a defined set of environmental conditions can provide useful information, the numeric value cannot used in the field.

AgNPs have showen cytotoxicity and harmful effects on fish species in medaka (*Oryzias latipes*) fish species at early-life stages as experimental models, the developmental toxicity of silver nanoparticles was investigated following exposure to AgNPs at high concentrations (=400µg/l) [17].

In recent years there are published a few toxicological studies related to the nano silvers, for examples a study on the sized effect of AgNPs using rainbow trout (*Oncorhynchus mykiss*) has been published [18]. In the study, rainbow trout were exposed via the water to commercial silver particles of three nominal sizes: 10nm [N(10)], 35nm [N(35)] and 600-1600nm [N(Bulk)] for 10 days. When the uptake of AgNPs from the water medium into the tissues of exposed fish was measured, the uptake level was low of the tested silver particles, N (10) were found to be the most highly concentrated within gill tissues. In this research, four different sized-AgNPs were prepared and repeated-dose toxicity was evaluated after administration to rat. Furthermore, inflammatory responses were evaluated to investigate the hazardous effects of AgNPs.

In our research, the toxicities of AgNPs on silver carp and goldfish were evaluated. The results suggested that AgNPs may have toxic potential toward these species, especially on the silver carp and AgNPs-induced mortality might provoke higher-level consequences, which could comprise a contribution to the knowledge on the aquatic toxicity of AgNPs on aquatic ecosystems, for which little data are available.

However, further researches on the mechanism behind AgNPs-induced damage and mortality are needed to better explain the ecotoxicity of AgNPs in freshwater fishes. Based on the results of this study, it is suggested that small-sized Nano silvers are more active to exert toxicological or biological responses and they induce mortality responses by repeated water exposure.

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