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Developmental toxicity and DNA damaging properties of silver nanoparticles in the catfish (*Clarias gariepinus*)

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ABSTRACT

Although, silver nanoparticles (AgNPs) are used in many different products, little information is known about their toxicity in tropical fish embryos. Therefore, this study evaluated the developmental toxicity of waterborne silver nanoparticles in embryos of *Clarias gariepinus*. Embryos were treated with (0, 25, 50, 75 ng/L silver nanoparticles) in water up to 144 h postfertilization stage (PFS). Results revealed various morphological malformations including notochord curvature and edema. The mortality rate, malformations, and DNA fragmentation in embryos exposed to silver nanoparticles increased in a dose- and embryonic stage-dependent manner. The total antioxidant capacity and the activity of catalase in embryos exposed to 25 ng/L silver nanoparticles were decreased significantly while the total antioxidant capacity and the activity of catalase were insignificantly increased with increasing concentrations in the embryos from 24 to 144 h-PFS exposed to 50 and 75 ng/L silver nanoparticles. Lipid peroxidation values showed fluctuations with doses of silver nanoparticles. Histopathological lesions including severely distorted and wrinkled notochord were observed. The current data propose that the toxicity of silver nanoparticles in *C. gariepinus* embryos is caused by oxidative stress and genotoxicity.

1. Introduction

Nanosilver has been consolidated into consumer products including materials, contraceptives, beauty care products, youngsters' toys, medical equipment, air filters, water filters, and residential washing machines [1]. The usage of consumer products containing silver nanoparticles (AgNPs) indicates that AgNPs may be released into the aquatic environment [2]. Aquatic ecosystems are often impacted by chemical pollution, originating from consumer products; therefore, fish provide a good experimental model for monitoring toxicity in aquatic systems because they are extremely sensitive to pollutants [3–5]. AgNPs can cause defects in the spinal cord, heart, and eye [6]. Asharani et al. [7], Griffitt et al. [8], and Yeo and Kang [9] have also observed an increase in mortality and hatching delay as well as various types of malformations. Moreover, some authors have that AgNPs could cause cellular and DNA damage in addition to roles as carcinogens and oxidative stress agents [10–12].

Different types of morphological abnormalities are reported in fish exposed to environmental pollutants, such as fin erosion, skull deformation, jaw deformities, and skeletal deformities such as lordosis, scoliosis, and kyphosis; opercular deformity; fin deformity; lower lip protrusion; gill deformity; ocular disorders; scale deformity and disorientation; and neoplasia or hyperplasia [13–16].

It is well established that during toxicity studies, stressors essentially target energy metabolism which is of prime concern in fish physiology [17]. Energy metabolism is a complex that includes a range of enzymes. Modifications to these enzymes or their substrates reflect changes in energy metabolism, which can then be used as significant indicators of stress factors [18,19]. Oxidative stress has been linked with some pathological processes in fish diseases caused by pollution [20]. Environmental pollutants can cause the production of reactive oxygen species (ROS) in living organisms and accordingly may result in oxidative stress, which is a possible explanation for the toxicity of nanoparticles [21,22]. It has been reported that AgNPs cause DNA fragmentation in adult catfish [22] and that can cause defects in offspring. Furthermore, catfish have been regarded as a model organism for studies of embryology and genotoxicity because artificial spawning is easy to perform [15,22,23]. Therefore, the aim of this study is to investigate oxidative stress in embryos of the African catfish *C. gariepinus* in response to AgNPs using antioxidant enzymes as biomarkers.

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2. Materials and methods

2.1. Preparation and characterization of silver nanoparticles:-

AgNPs were purchased from Sigma-Aldrich, St. Louis, MO, USA. The physical characteristics of the particles according to the manufacturers data were: size (≤100 nm), purity (99.5%), trace metal basis, surface area (5.0 m^2/g), and density (10.49 g/cc). The preparation and description of AgNPs were reported by Sayed [22].

2.2. Gamete collection

Criteria for selection and rearing of mature *C. gariepinus* were described by De Graaf and Janssen [24]. The catfish specimens were kept in 100-L glass tanks and acclimatized for a two-week period at 27–29 °C, PH = 7.56, and dissolved oxygen 88–94% saturation. The photoperiod was a 12L:12D cycle and the catfish specimens were fed on a commercial pellet diet (3% of body weight/day).

For the collection of semen, male fish were anesthetized with 200 mg/L Ms-222 (tricaine methane sulfonate, Crescent Research Chemicals, Phoenix, AZ, USA) buffered with 800 mg/L sodium bicarbonate and the testes were removed surgically. Blood was cleaned from the testes by surgical towels. The sperm from the testes were pressed through a mesh fabric into a sterile dry Petri dish and used directly for dry fertilization. For the collection of eggs, ovulation was artificially induced. Females were injected intraperitoneally with pellets (gonadotropin-releasing hormone analogue, D-Ala6, Pro9-NEt) containing 2.5–3.0 mg of water-soluble dopamine antagonist metoclopramide (Interfish Ltd, Hungary) dissolved in 0.65% NaCl. One pellet was used per kg body weight. After injection (10–11 h), the fish were stripped and the eggs were collected in clean dry plastic containers; dry fertilization was then performed [15].

2.3. Experimental setup and sampling

The fertilized eggs were incubated in dechlorinated tap water (pH = 7.56, dissolved oxygen 88–94% saturation, temperature 27–29C°, and photoperiod 12L:12D). Exposure started 2 h post-fertilization (2 h-PFS). Fertilized eggs were divided into four groups: one control and three groups exposed to AgNPs once with 25, 50 and 75 ng/L according to Govindasamy and Abdul Rahuman [25] and the OECD [26]. Exposure took place in a 30 × 30 × 30 cm glass tank (10 L water level), three replicates for each group (300 samples). Sampling was at intervals of 24, 48, 72, 96, 120, and 144 h-PFS after AgNP exposure. Ten embryos were collected and anesthetized with 200 mg/L Ms-222 buffered with 800 mg/L sodium bicarbonate at each sampling point and fixed at −80 °C until Measurements of enzyme activities and other samples fixed for 24 h in Bouin's solution for gross and minor external malformation and histopathology. The hatching process started at 22 h-PFS. Mortality was recorded as in the Karber method [27]. Morphological malformations were observed and reported according to Mahmoud et al. [15].

2.4. Measurements of enzyme activities

Measurements of lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were according to a modified protocol [19]. The catalase level was estimated according to a method described by Aebi [28]. Total antioxidant capacity was determined according to Abu Khalil et al. [29] using a commercial kit (Antioxidant Assay Kit, Cat. No. MAK187; Sigma-Aldrich, Cairo, Egypt).

2.5. Lipid peroxidation

Lipid peroxidation (LPO) in the embryos was determined by a procedure previously described [30]. The absorbance of each aliquot was measured at 535 nm. The rate of lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substance formed per hour per milligram of protein using a molar extinction coefficient of 1.56 M^{-1} cm^{-1} [31].

2.6. DNA fragmentation measurement

DNA fragmentation was determined by a procedure using a spectrophotometer (Micro Lab 200 Vital Scientific, Dieren, The Netherlands) at 575 or 600 nm against a reagent blank [32]. The percentage of fragmented DNA was estimated by the following formula: % of fragmented DNA = fragmented DNA/(fragmented + intact DNA) x 100.

2.7. Comet assay

The comet assay was performed according to a modified protocol based on Jarvis et al. [33] and Mekkawy et al. [19].

2.8. Histopathology

Fixed specimens were dehydrated and subsequently embedded in paraffin. Transverse serial sections 5–7 μm thick were cut and stained with hematoxylin and eosin. Sections were studied using an Omax advanced trinocular biological microscope with a 14MP USB Digital Camera (CS-M837ZFLR-C140U).

2.9. Determination of Ag bioaccumulation:-

Embryos (144 h-PFS) and water samples were used for the determination of Ag ions. Embryos (0.5 g of each) and water media (500 μ1) were added to 1.5 mL of concentrated HNO_3 and then boiled for 45 min at 100 °C to break down the embryonic tissue and dissolve all the silver content. The mixture was cooled, diluted with 500 mL of

Table 1
Effect of different concentrations of silver nanoparticles on the percentage of mortality and malformation rate during early developmental stages of the African catfish *Clarias gariepinus*, N = 3/treatment.

Parameter	Group	24-PFS[#]	48-PFS	72-PFS	96-PFS	120-PFS	144-PFS
Mortality rate%	Control	5.66 ± 0.88[**]	1.66 ± 0.33	1.33 ± 0.33	0.67 ± 0.33	1 ± 0.00	0.33 ± 0.33
	25 ng/L	5.33 ± 0.88	2.66 ± 0.67	1.33 ± 0.33	1.33 ± 0.33	3.67 ± 0.88	2.67 ± 0.67
	50 ng/L	6 ± 0.58	3 ± 0.58	3.67 ± 0.88	1 ± 0.58	5 ± 0.58[*]	3.67 ± 0.88[*]
	75 ng/L	8.66 ± 1.02	5 ± 0.58[*]	5 ± 0.58[*]	3 ± 0.58[b]	4 ± 0.58[*]	2.67 ± 0.67
Malformation rate%	Control	1.33 ± 0.33	0.67 ± 0.33	0.33 ± 0.33	1 ± 0.58	3.66 ± 0.88	2.33 ± 0.88
	25 ng/L	2.33 ± 0.33	2.33 ± 0.88	3 ± 0.58	2.33 ± 0.88	3.66 ± 0.88	2.66 ± 0.88
	50 ng/L	9.33 ± 0.88[*]	4 ± 0.58[*]	4 ± 0.58[*]	4 ± 0.58	5.66 ± 0.88	3.66 ± 2.02
	75 ng/L	14 ± 1.16[b]	8.33 ± 0.88[*]	2.33 ± 0.88	3.33 ± 1.20	3.33 ± 0.67	2.66 ± 0.88

[#]PFS = postfertilization stage. [*]Statistically significant difference (ANOVA, $p < 0.05$). [**]The numbers as mean ± SE.