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Oxidative Stress Bio-markers in *Labeo rohita* Fingerlings Exposed to Silica Oxide Nanoparticles

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ABSTRACT

Background: The present study was conducted to study the Oxidative stress bio-markers in *Labeo rohita* fingerlings exposed to Silica oxide nanoparticles.

Methods: A total number of 180 fishes were randomly divided into 4 groups with 15 fishes each *viz.* E_0 (control), E_1 (SiO_2NPs @ 2 mg/L), E_2 (SiO_2NPs @ 4 mg/L) and E_3 (SiO_2NPs @ 6 mg/L) and each group was set in triplicates. After acclimatization period of 14 days fishes were exposed to SiO_2NPs in groups E_0, E_1, E_2 and E_3 and oxidative stress was estimated every 15 days up to experimental period of 90 days.

Result: Findings showed a significant decline in Catalase activity and Superoxide dismutase and an increase in Lipid peroxidation at the end of the experimental period in the fingerlings exposed to Silica oxide nanoparticles.

Key words: Bio-markers, *Labeo rohita*, Oxidative stress, Silica nanoparticles.

INTRODUCTION

Nanoparticles are defined as particles with all external dimensions within the nanoscale (1-100 nm) (ISO, 2023). They are increasingly being synthesised due to their wide range of industrial applications (Dhawan *et al.,* 2010; Yokel and Mac Phai, 2011; Radad *et al.,* 2012). Nanoparticles are of great interest in a variety of fields due to their unique properties such as small size (1-100 nm in diameter), higher surface area to volume quantitative relationshipand completely different electronic, magnetic, opticaland mechanical properties. However, because these particles have a larger surface area to volume quantitative relation, they are more chemically reactive, resulting in an accumulation of reactive oxygen species (ROS).

Nanosilica, or the nanoform (<100 nm) of silicon dioxide or silica nanoparticles (SiO_2NPs), possesses specific physicochemical characteristics than that of its bulk form; smaller size materials bear a higher surface-to-volume ratio and surface reactivity (Oberdörster, 2011; Napierska *et al.,* 2010). Because of their promising properties, SiO_2NPs are widely used in agriculture, food, consumer productsand cosmetics (Napierska *et al.,* 2010; Khot *et al.,* 2012; Kasaai, 2015; Brinch *et al.,* 2016). Nanoparticles can enter the aquatic environment via manufacturing processes, wastewater sludge, spillsand emissions into the atmosphere. (Scown *et al.,* 2010; Jovanović and Palić, 2012).

The high volume of these particles used in commercial and industrial applications exposes them to a variety of ecological systems (Oberholster *et al.,* 2011) and may pose a threat to aquatic organisms (Baun *et al.,* 2008; Farré *et al.,* 2009). Engineered nanomaterials can take many different physical forms, such as carbon nanotubes and fullerenes, which are carbon spheres (Zhu *et al.,* 2006) and nanoparticles made from metals (Griffitt *et al.,* 2007), metal oxides (Federici *et al.,* 2007), or metal composites (King-Heiden *et al.,* 2009), which have negative effects on fish (Smith *et al.,* 2007). Fish species have long been used as pollution indicators in aquatic toxicity studies due to their sensitivity to stress.

Metal ions and nanoparticles have distinct toxicological effectsand various studies show that metal nanoparticles have significantly higher lethal concentrations than metal ions. Metal nanoparticles have been linked to histological damage, metabolic abnormalitiesand developmental problems in fish, according to various studies. Even though nano-metals are chemically insoluble in water, they release metal ions into the suspension medium, which may have effects similar to those observed in fish (Shaw and Handy, 2011). Fish are an important component of aquatic biotas because they are the most diverse of all vertebrates (Nelson, 1994) and are also the most vulnerable to pollutants (Duncan and Lockwood, 2001). In addition, fish have also been an integral part of diet on regular basis for most of the people

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(Biswas, 2024) due to its high protein and polyunsaturated fatty acids content (Kamble *et al.,* 2023); being a cheap source of nutrients at the same time (Sit *et al.,* 2021). Fish are distributed at various trophic levels (Trujillo-Jiménez and Espinosa de los Monteros-Viveros, 2006), so any toxic effects on this group will have an impact on the entire important structure of the community.

The major purpose of the present study was to investigate the alterations in antioxidant status of *Labeo rohita* fingerlings exposed to SiO_2NPs as stress response.

MATERIALS AND METHODS

The experimental set up was maintained in the Wet lab, College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology (G.B.P.U.A.T), Pantnagar, Uttarakhand from January 2020 to July 2022. The 80-liter tanks were filled with 50-liters of fresh tap water and provided with continuous aeration, regular feeding, waste syphoned from leftover feed and excretaand a daily partial water exchange. In this experiment, 180 fingerlings were randomly distributed in triplicate of three treatments (E_1, E_2 and E_3) and a Control group (E_0 with no added SiO_2NPs) each. The synthesized SiO_2NPs were obtained from the Department of Chemistry, College of Basic Sciences and Humanities, G.B.P.U.A. and T. The total duration of experiment was 90 days, conducted to study the long-term effect of SiO_2NPs toxicity at lower concentration to fulfill the objectives of the study.

Antioxidant-status

Status of catalase activity (CAT), superoxide dismutase (SOD) and lipid peroxidation (LPO) was recorded in the blood hemolysate of exposed fish fingerlings every 15 days up to the experimental period of 90 days.

Statistical analysis

All the experiment's indices were subjected to two-way ANOVA using Statistical Packaging for Social Sciences (SPSS), the Duncan Multiple Range Test, 2013 Versionand Microsoft Excel, 2019.

RESULTS AND DISCUSSION

Experimental groups E_1, E_2 and E_3 showed a significant decrease in CAT activity from 26.14±0.003 to 20.71±0.003, 26.11±0.003 to 14.21±0.003 and 26.17±0.02 to 10.61±0.003 mMH_2O_2 utilized/min/mgHb respectively when compared between initial to 90th day (Table 1).

Experimental groups E_1, E_2 and E_3 showed a significant decrease in SOD from 51.06±0.26 to 44.48±0.005, 51.06±0.26 to 30.14±0.01 and 51.03±0.26 to 22.50±0.14 U/mg of hemoglobin respectively when compared from initial to 90th day (Table 2).

Experimental groups E_1, E_2 and E_3 showed a significant increase in LPO from 3.04±0.02 to 14.45±0.34, 3.04±0.02 to 20.05±0.03 and 3.04±0.002 to 23.67±0.003 Nm.MDA.ml^{-1} respectively when compared between initial to 90th day (Table 3).

Throughout the experimental period, SOD and CAT activity decreased (Table 1 and Table 2 respectively), while LPO increased (Table 3). The findings of this study are consistent with the work of P.C Vidya and K.C Chitra, 2018 on *Oreochromis niloticus* exposed to Silicon dioxide nanoparticles. Nanoparticles have easy access to flowing water, which quickly penetrates the gill surface layers and into the secondary lamellae, making fish gills susceptible to nanoparticles (Federici *et al.,* 2007; Sumi and Chitra, 2016). The gill tissue has a powerful radical scavenging mechanism, which includes antioxidant enzymes, to effectively eliminate ROS. Both short-term and long-term exposure to silicon dioxide nanoparticles reduced the activity of antioxidant enzymes in gill tissue, including superoxide dismutase and catalase. Toxicants make fish more vulnerable to the effects of ROS (Lushchak, 2011). Similar findings were reported in other studies that exposed different fish species to different nanoparticles (Linhua *et al.,* 2009) found a similar trend, with a decrease in CAT activity and SOD and an increase in LPO at higher concentrations of Titanium dioxide nanoparticles in fish, *Cyprinus carpio*. Garcia *et al.* (2011) reported a similar significant decrease in SOD and CAT activity, as well as an increase in LPO, in Ag-NP exposed fish *Chapalichthys pardalis*. Nanoparticles' toxicological processes include the production of ROS and the development of oxidative stress (Ahmad *et al.,* 2010).

Small amounts of free radicals, such as superoxide anion, hydroxyl radicaland hydrogen peroxide, are produced within the cell and subcellular compartments during aerobic respiration and energy consumption. ROS is the term used

Table 1: CAT (mMH_2O_2 utilized/min/mgHb) in different experimental groups at different days post exposure.

Days	E_0	E_1	E_2	E_3
Initial	26.02±0.10^{Aa}	26.14±0.003^{Aa}	26.11±0.003^{Aa}	26.17±0.02^{Aa}
0-15	26.14±0.003^{Aa}	25.62±0.01^{Bb}	23.12±0.005^{Cc}	22.15±0.003^{Dd}
15-30	26.21±0.06^{Aa}	24.22±0.003^{Db}	22.11±0.005^{Bc}	21.35±0.003^{Cd}
30-45	26.14±0.003^{Aa}	23.74±0.003^{Cb}	21.67±0.003^{Dc}	20.44±0.003^{Bd}
45-60	26.14±0.003^{Aa}	22.14±0.003^{Eb}	20.18±0.003^{Fc}	19.41±0.003^{Gd}
60-75	26.15±0.006^{Aa}	21.62±0.003^{Fb}	19.34±0.003^{Gc}	18.43±0.01^{Ed}
75-90	26.15±0.01^{Aa}	20.71±0.003^{Gb}	14.21±0.003^{Ec}	10.61±0.003^{Fd}

Small superscript (a-d) indicates significant (p<0.05) difference between groups at a particular day, capital superscripts (A-G) indicate difference between days within particular group.

Table 2: SOD (U/mg of hemoglobin) in different experimental groups at different days post exposure.

Days	E_0	E_1	E_2	E_3
Initial	51.05 ± 0.26^{Aa}	51.06 ± 0.26^{Aa}	51.06 ± 0.26^{Aa}	51.03 ± 0.26^{Ba}
0-15	51.07 ± 0.26^{Aa}	49.19 ± 0.005^{Bb}	47.70 ± 0.01^{Cc}	42.33 ± 0.11^{Dd}
15-30	50.98 ± 0.22^{Aa}	48.61 ± 0.10^{Cb}	45.11 ± 0.26^{Dc}	39.14 ± 0.008^{Ed}
30-45	51.07 ± 0.26^{Aa}	47.22 ± 0.008^{Db}	42.34 ± 0.07^{Bc}	37.08 ± 0.03^{Cd}
45-60	50.98 ± 0.22^{Aa}	46.11 ± 0.008^{Eb}	40.16 ± 0.11^{Fc}	32.54 ± 0.006^{Gd}
60-75	51.07 ± 0.26^{Aa}	45.12 ± 0.005^{Fb}	39.32 ± 0.006^{Gc}	31.47 ± 0.13^{Hd}
75-90	50.98 ± 0.22^{Aa}	44.48 ± 0.005^{Gb}	30.14 ± 0.01^{Ec}	22.50 ± 0.14^{Fd}

Small superscript (a-d) indicates significant ($p<0.05$) difference between groups at a particular day, capital superscripts (A-H) indicate difference between days within particular group.

Table 3: LPO ($Nm.MDA.ml^{-1}$) in different experimental groups at different days post exposure.

Days	E_0	E_1	E_2	E_3
Initial	3.03 ± 0.02^{Aa}	3.04 ± 0.02^{Aa}	3.04 ± 0.02^{Aa}	3.04 ± 0.002^{Aa}
0-15	3.04 ± 0.02^{Aa}	3.85 ± 0.14^{Bb}	7.42 ± 0.003^{Cc}	9.97 ± 0.04^{Dd}
15-30	3.04 ± 0.03^{Aa}	4.78 ± 0.11^{Cb}	9.31 ± 0.003^{Dc}	12.87 ± 0.003^{Bd}
30-45	3.04 ± 0.02^{Aa}	7.41 ± 0.006^{Db}	12.11 ± 0.003^{Ec}	15.34 ± 0.003^{Cd}
45-60	3.04 ± 0.02^{Aa}	10.07 ± 0.04^{Eb}	15.31 ± 0.003^{Fc}	18.57 ± 0.006^{Gd}
60-75	3.04 ± 0.02^{Aa}	12.43 ± 0.003^{Fb}	18.34 ± 0.01^{Gc}	21.12 ± 0.005^{Ed}
75-90	3.04 ± 0.03^{Aa}	14.45 ± 0.34^{Gb}	20.05 ± 0.03^{Bc}	23.67 ± 0.003^{Fd}

Small superscript (a) indicates significant ($p<0.05$) difference between groups at a particular day, capital superscripts (A-G) indicate difference between days within particular group.

to describe free radicals that, when combined with an insecure intermedia, promote lipid peroxidation. The rate of ROS formation in this study outpaced the antioxidant defence system, as evidenced by a decrease in antioxidant enzyme activity. The enzyme superoxide dismutase (SOD) catalyses the dismutation of superoxide into hydrogen peroxide (H_2O_2) and oxygen. Catalase is responsible for converting H_2O_2 into O_2 and H_2O. Because of their ability to cross biological membranes, SiO_2NPs are commonly used in medication and gene therapy. Thus, oxidative stress results from an imbalance between radical-generating and radical-scavenging processes.

ROS production has several negative consequences, including protein oxidation, DNA damageand peroxidation of unsaturated lipids in cell membranes (Sikka, 2001). According to Miao *et al.* (2024), the widespread use of amorphous silica nanoparticles (aSiNPs) in recent years has resulted in unavoidable human exposure in daily life, raising widespread concerns about aSiNPs safety for human health. The particle size of nanomaterials is an important factor that can influence their toxicity. Because smaller particles have a larger surface area, they may be more active and reactive to biological systems. SiO_2NPs have also been shown to negatively affect the hematological profile of exposed *Labeo rohita* fingerlings suggesting their tendency to affect overall health of the respective animal (Upreti *et al.,* 2021).

CONCLUSION

In conclusion Silica oxide nanoparticle chronic toxicity causes a negative impact on the anti-oxidant status of *Labeo rohita* fish fingerlings when exposed at lower concentration over long time, supporting, a significant decline in the Catalase activity and Superoxide dismutase is clearly observed along with an increase in Lipid peroxidation.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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