



RESEARCH ARTICLE

GENERATION OF REACTIVE OXYGEN SPECIES IN HEPATOCYTES OF TILAPIAN FISH WHEN EXPOSED TO SILICON DIOXIDE: A POTENTIAL ENVIRONMENTAL IMPACT OF NANOPARTICLE

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ABSTRACT

Applications of synthetic nanoparticles in wide variety of industries are identified to be growing at a faster rate. The potential effects of nano-silicon dioxide and its toxic mechanisms remain unclear. In the present study 5 mg/ L concentration of silicon dioxide nanoparticles were exposed to the freshwater fish, *Oreochromis mossambicus* for 24 h, 48 h and 96 h. The body weight remained unchanged whereas the hepatosomatic index showed a significant decrease at 96 h. The percentage of mucous secretion was comparatively high in all treatment groups. The activity of superoxide dismutase, catalase, glutathione reductase altered significantly in hepatocytes after 48 h and 96 h. However, the levels of hydrogen peroxide and lipid peroxidation showed a significant increase in treated groups than the control. Alkaline phosphatase, liver marker enzymes showed a significant decrease throughout the exposure. Thus the study revealed that exposure to silicon dioxide nanoparticle generates reactive oxygen species in fish hepatocytes and this could be due to the potential harmful impact of the nanoparticle.

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INTRODUCTION

Nanotechnology is one of the fastest growing areas of research which has a wide range of commercially available products. As a result several nano-structured materials have been widely used in the fields of biomedicine, pharmaceutical, and other industry. Manufactured nanoparticles with sizes ranging from 1 to 100 nm have been ever more used in a broad range of applications including environmental remediation, cosmetics, material sciences and electronics (Nel *et al.*, 2006). The degree of toxicity of any nanomaterials is based on some critical factors such as dose, route of administration, and duration of exposure. Nanoparticles have been shown to produce an array of diverse toxic effects in many different animal studies as *in vivo* and *in vitro*. Nanoparticles have been recognized to produce toxic effects on the pulmonary, cardiac, reproductive, renal and cutaneous systems, as well as on various cell lines. This is because of the high reactivity of nanoparticles and it has even been reported that naturally nontoxic substances can be transformed into toxic compounds and such particles could be absorbed by plants or any living organisms (Batley *et al.*, 2012). Hence, they can enter the food chain resulting in an exposure to animals and humans. After exposures, significant accumulations of nanoparticles have been found deposited in the lungs, brain, liver, spleen, and bones of exposed species. It

has been well established that the degree of toxicity produced by nanoparticles is linked to their surface properties (Shaw and Handy, 2011). Nanoparticles can enter into human body through three possible absorption paths such as skin, respiratory tract or through gastrointestinal tract. By oral assimilation, nanoparticles can be transported from the intestinal tract into the lymph stream and then into the blood (Hussain *et al.*, 2001). Nanoparticles through breathing can reach the lung and has been reported to cause inflammatory reactions because of their insufficient elimination by macrophages (Oberdörster *et al.*, 1992). Once they have been incorporated into the organism these nanoparticles can reach numerous different areas of the body because of their high mobility. It has been stated that nanostructured materials has the ability to pass through biological barriers such as the blood-brain barrier or cell membranes. Moreover, they can move along the nerve pathways and arrive at organs like liver and kidney. The transfer of nanoparticles from the placenta into the fetus is not only a chance for a selective therapy use but it can also be a risk (Kulvietis *et al.*, 2011). However, the potential risk of such interactions is still widely unknown and the distribution of nanoparticles within the organism depends on their dimension, shape, and material properties.

Nano-sized silicon dioxide (nano-SiO₂) also known as silica nanoparticles is one of the most popular nanomaterials that are

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being used in the fields such as industrial manufacturing, packaging, high-molecule composite materials and ceramics synthesis, disease labeling, drug delivery, cancer therapy and biosensor. Silicon dioxide nanoparticles are highly stable and could bioaccumulate in the environment. Although toxicity studies of this compound to human and mammalian cells have been reported, their effects on aquatic biota, especially on fish, have not been significantly studied.

Another hot point in the last few centuries is the generation of free radicals in the biological systems. There are several findings stating that nano-sized particles also play a potential role in the induction of free radicals in living tissues or organs. Aquatic organisms, particularly fish has developed well equipped systems for generation and degradation of free radicals. Since water bodies are continuously exposed to contaminants and to cope up to the threatened environment the fishes develop a good pro-oxidant and antioxidant homeostasis. However, exposure to some environmental contaminants including nanoparticles may pose a potential impact on the organisms and this could be toxic to the animal. Therefore, the present study was designed to evaluate the toxic effect of one of nanoparticles, silicon dioxide on the elevation of reactive oxygen species in hepatocytes of freshwater fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Chemicals

Silicon dioxide nanoparticle (size 7-14 nm; purity >99.8%) was purchased from Reinste Nano Ventures Pvt Ltd, New Delhi, India. The test solution of silicon dioxide was prepared just before exposure by sonication (100 kHz for 30 min) using double distilled water and was maintained as stock. All other chemicals were of analytical grade and were obtained from local commercial sources.

Test animal

Oreochromis mossambicus weighing 6 ± 1.5 g and length 6.5 ± 1 cm were collected from a fish farm, Safa Aquarium, Kozhikode, Kerala. Fishes were acclimatized to the laboratory conditions prior to experiments and were exposed with constant supply of water and good lighting system. They were maintained in well-aerated tubs (40 L capacity), which was dechlorinated and sustained with fresh water flow and waste water discharge.

Preliminary tests

The physico-chemical features of the tap water were estimated as per APHA (1998). Water temperature in the test ranged from $28 \pm 2^\circ\text{C}$ during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 6.5 to 7.5 which were monitored using a standardized procedures. The specimens were not fed a day prior to and during toxicity tests to reduce faecal and excess food contaminating the test solution. Five specimens were placed in each tub of replicates so that ten fishes were maintained in each test and aerated using tubed

motorized pumps. Monofilament netting was used to cover the tanks to prevent the specimens from jumping out of test solutions.

Median lethal concentration (LC_{50})

The LC_{50} values in the respective time intervals were done by probit analysis, with a confident limit of 5 % level (Finney, 1971). The concentration of the toxicant at which 50 percentages of the test animals dies during a specific period is referred to as median lethal concentration (LC_{50}) or median tolerance limit. For determining LC_{50} concentration separate circular plastic tubs of 40 L of water capacity were taken and different concentrations (25, 50, 75 and 100 mg/ L) of silicon dioxide were added. Then, 10 fishes were introduced into each tub maintaining a control tub without toxicant. No mortality was observed in the above concentrations for 96 h. However, (Ramesh *et al.* 2013) has reported that 50 mg/ L was median lethal concentration for zebra fish, *Danio rerio* and its sublethal concentration (5 mg/ L) has been chosen for their study. Thus in the present study, the same dose (5 mg/ L concentration of nano-silicon dioxide) was chosen to evaluate the antioxidant parameters in *Oreochromis*.

Treatments

There were four groups of 10 fishes each, three tanks with toxicant doses (silicon dioxide dissolved in double distilled water to make the concentration of 5 mg/ L) and a control tank. Single dose (5 mg/ L) with three durations (24 h, 48 h and 96 h) were selected in the present study. No fish mortality was observed throughout the treatment period.

Preparation of tissue homogenate

At the end of each treatment periods the fish was caught very gently using a small dip net, one at a time with least disturbance and the weight of the animal were recorded. Then the mucous on the surface of the body were wiped using filter paper and again the weights were recorded in order to find the percentage of mucous secreted by the animal. Fishes were sacrificed using anesthetic ether and the liver was taken out and the weights were documented and stored in cold normal saline. Crude tissue homogenate (1%) was prepared using cold normal saline by centrifugation of the homogenate at 8000 g for 15 minutes at 4°C to obtain the supernatant and was used for various biochemical estimations.

Biochemical parameters

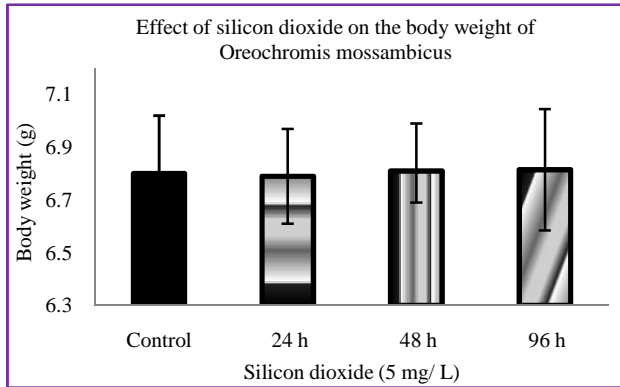
Total protein concentration in the hepatic tissues was estimated by the method of (Lowry *et al.* 1951) using bovine serum albumin as the standard. Activities of antioxidant enzymes such as superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985) and glutathione reductase (Carlberg and Mannervik, 1985) and the level of hydrogen peroxide generation (Pick and Keisari, 1981), lipid peroxidation (Ohkawa *et al.*, 1979) and the liver marker enzyme, alkaline phosphatase (Bessey *et al.*, 1946) were done.

Statistical analyses

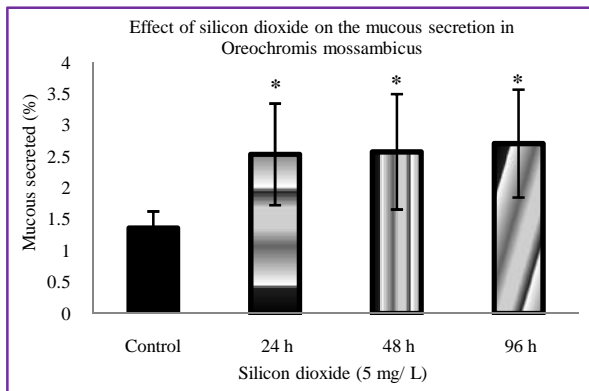
Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p < 0.05$ against control group. Data are presented as mean \pm SD for ten animals per group and all biochemical estimations were carried out in duplicate.

RESULTS

Body weights and mucous secretion

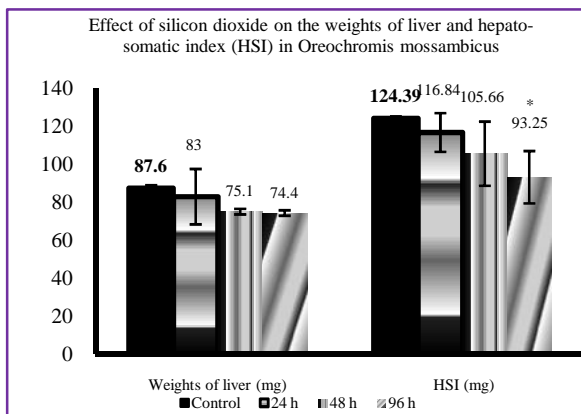


It was evident from the graph that in the present study the body weight of the animal remained unchanged throughout the experiment.



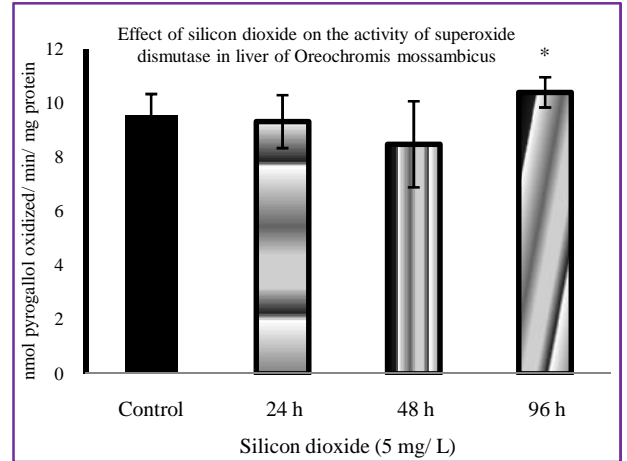
At the end of every treatment, silicon dioxide-treated fishes showed a remarkable deposition of mucous all over the body with the percentage increased to 10% when compared with those of control groups.

Liver weights and hepato-somatic index



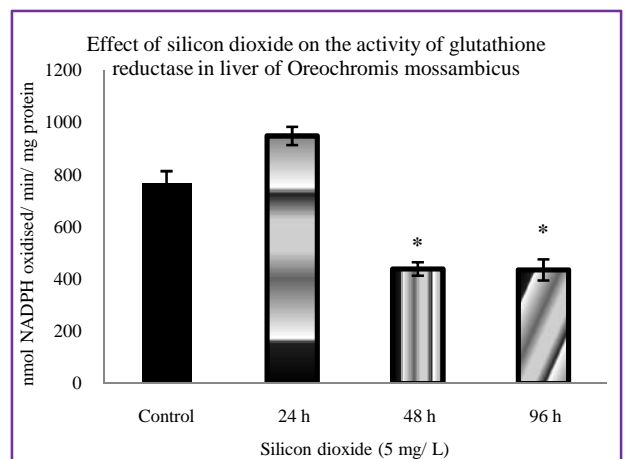
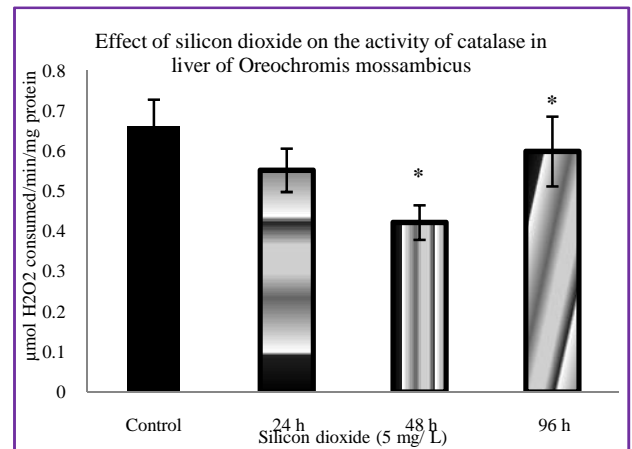
Treatment of silicon dioxide nanoparticle showed no changes in the weights of liver in all treatment groups. However, the hepato-somatic index of 96 h treated group showed a significant ($p < 0.05$) decrease in the weights as compared to control group.

Activities of antioxidant enzymes -Superoxide dismutase



The activity of superoxide dismutase showed a significant increase at the end of 96 h of silicon dioxide exposure whereas no significant changes were observed in 24 h and 48 h of exposure.

Catalase

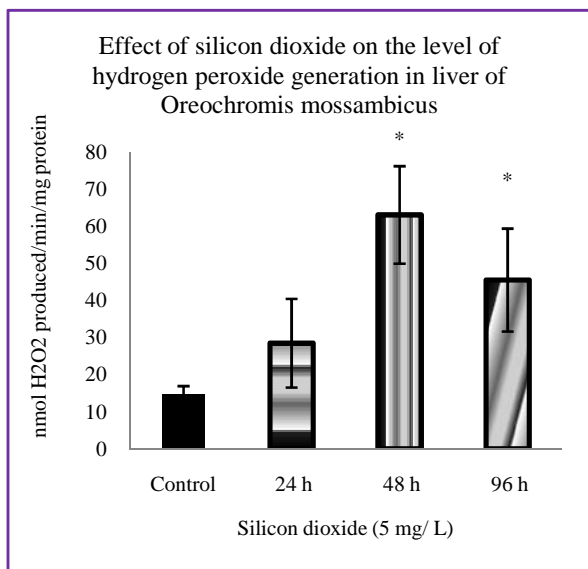


Silicon dioxide treatment significantly decreased the activity of catalase at 48 h and 96 h, on the other hand, the activity remained unchanged at the end of 24 h of treatment.

Glutathione reductase

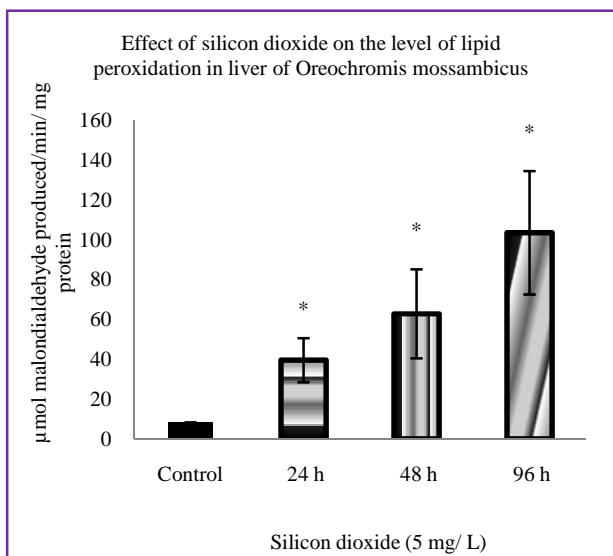
Exposure to silicon dioxide significantly ($p < 0.05$) decreased the activity of glutathione reductase in 48 h and 96 h with no changes in the 24 h when compared to the control groups.

Hydrogen peroxide generation



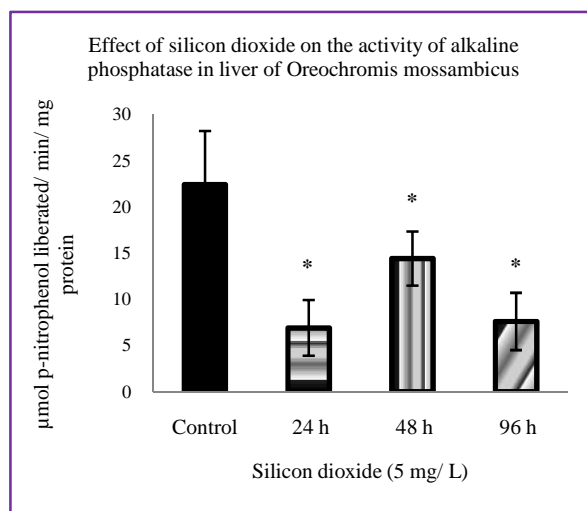
The level of hydrogen peroxide was observed to be increased in all treatment groups, with significant ($p < 0.05$) changes in the 48 h and 96 h of nano-silicon dioxide exposure.

Lipid peroxidation



Level of lipid peroxidation estimated by the production of malondialdehyde showed a tremendous increase in time-dependent manner in the silicon dioxide-treated fishes when compared with the corresponding control group and the increase was proved significant at $p < 0.05$ in all treatments.

Alkaline phosphatase



The activity of one of the liver marker enzymes, alkaline phosphatase decreased significantly in all treatment groups that that of control animal.

DISCUSSION

Recent trends in progressive science are the development of new nanoparticles in various fields. Most of the nanomaterials have been widely used in construction materials including coatings inside drinking water pipes, detergents, food processing, paper manufacturing, agro-chemicals, plant protection products, plastics including food packaging, weapons and explosives etc. (Chaudhry *et al.*, 2006). Most of the nanoparticles exert its mechanism of toxicity by excessive generation of reactive oxygen species or by depletion of antioxidant system in various animals thereby inducing oxidative stress (Ahamed *et al.*, 2010; Wise *et al.*, 2010). The present study was therefore designed to investigate the activity of antioxidant enzymes in *Oreochromis mossambicus* following the exposure of one of the most widely used nanoparticles, silicon dioxide.

In the present study it was observed that the nanoparticles at 5 mg/L did not alter the body weight of the animal if exposed short-term for 96 h. But the mucous deposition was observed throughout the body during the treatment period and this could be a first line of defensive mechanism of the fish to get rid of the exposure to silicon dioxide. Therefore, hypersecretion of mucous may be considered as a defensive mechanism of the fish against the exposure to the nanoparticle. Moreover the presence of mucous is an indicator of existence of toxic substances in the water and this could lead to the functional alterations and intrusion in fundamental process such as osmoregulation (Bernet *et al.*, 1999). The present investigation observed no changes in the weight of liver or hepato-somatic index (HSI) except for 96 h treatment, which showed a significant decrease in HSI. This result could reflect that silicon dioxide exposure does not cause much physical damage to hepatocytes.

Our results showed significant changes in the activity of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase in *Oreochromis* during the exposure of silicon dioxide nanoparticles at 48 h and 96 h, which concludes that free radicals could have generated in fish tissues due to the exposure of silicon dioxide nanoparticles. It is well known that the generation of reactive oxygen species (ROS) plays an important role in producing liver dysfunction.

Reactive oxygen species are molecules like hydrogen peroxide, ions like the hypochlorite, free radicals like the hydroxyl radical, superoxide anion etc. Reactive oxygen species have long been considered as harmful by-products of intrinsic oxygen metabolism or cellular responses to hazardous stimuli. It was well proved fact that formation of one reactive oxygen species usually leads to generation of other compounds, spontaneously or in a reaction catalyzed by superoxide dismutases (SOD) forming hydrogen peroxide. Superoxide dismutase catalysis the dismutation of superoxide to hydrogen peroxide (H₂O₂) and oxygen (O₂).

The conversion of H₂O₂ to 2H₂O is by the enzyme glutathione reductase and glutathione peroxidase and the conversion of H₂O₂ to O₂ and H₂O is by the enzyme catalase (Sikka, 2001). In the present study the activity of superoxide dismutase increased significantly and thus it could have generated the level of hydrogen peroxide. The activity of other antioxidant enzymes as catalase and glutathione reductase was decreased significantly so that the evolved hydrogen peroxide could not be eliminated in the form of water or oxygen molecule.

This could have resulted in the elevated level of hydrogen peroxide and in turn it might have induced lipid peroxidation in the hepatocytes after silicon dioxide exposure, which is evidenced by the significant increase in the production of malondialdehyde. Similar observations have been reported in muscle, liver and gill of zebra fish, *Danio rerio* after 7 days of silicon dioxide treatment (Ramesh *et al.*, 2013). The present study clearly illustrates that silicon dioxide nanoparticle could have upset the balance of pro-oxidant and antioxidant in the hepatic tissues in acute exposure and thus could result in the generation of reactive oxygen species in hepatocyte of the freshwater fish, *Oreochromis mossambicus*.

The liver marker enzyme alkaline phosphatase decreased significantly ($p < 0.05$) in all treatment groups and this might be due to the decreased state of inter and intracellular membrane transport across the hepatocytes owing to the nano-silicon dioxide toxicity in *Oreochromis* (Chitra and Maiby, 2014).

CONCLUSIONS

On the basis of the foregoing discussion it can be summarized that the present study provides unequivocal evidence for a highly significant positive correlation between exposure of *Oreochromis mossambicus* to silicon dioxide nanoparticle and a substantial increase in the production of reactive oxygen species in fish hepatocytes. Thus the study strongly suggests a consequence of imbalance in pro-oxidant and antioxidant system in liver tissue is due to the potential hazardous environmental impact of exposed nanoparticle.

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