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Oxidative Stress in Muscle Tissue of the Freshwater Fish, *Pseudetroplus maculatus* (Bloch 1795): a Toxic Response from Exposure to Fullerene (C_{60}) Nanoparticles

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Abstract

Fullerenes are the manufactured nanoparticles that possess lipophilic properties so that they are able to permeate through the cellular membranes. The goal of this study was to examine the consequences of fullerene nanoparticles on the induction of oxidative stress in muscle tissue of the freshwater fish *Pseudetroplus maculatus* (Bloch 1795). In the present study, the exposure of fullerene at 0.1 $mg.L^{-1}$ concentration for 24, 48, 72 and 96 h indicates that the nanoparticles have a potential role to induce oxidative stress in muscle tissue of fish. The activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione reductase decreased significantly ($p<0.05$) after 72 and 96 h of toxicant exposure. However, the levels of hydrogen peroxide and lipid peroxidation increased significantly ($p<0.05$) after 72 and 96 h of treatment in muscle tissue. The activities of acid and alkaline phosphatases in muscle tissue were also evaluated. It was found that the activities of both enzymes showed significant ($p<0.05$) reduction at the end of 72 and 96 h of fullerene exposure. The present findings provide good evidence that fullerene nanoparticles induced oxidative stress in the muscle tissue of fish. In addition, the changes in the phosphatase enzymes indicate that fullerene also altered the metabolic processes in the muscle tissue of fish.

Keywords: Fullerene C_{60}, *Pseudetroplus maculatus*, oxidative stress, muscle, acid phosphatase, alkaline phosphatase.

Introduction

Recently several hundreds of commercial products available in the market use the science of nanotechnology for the production of nanoscale materials with at least one dimension less than 100 nm. Nanotechnology has extensive potential benefits in a wide range of areas such as biomedical,

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cosmetic and industrial applications. With the emergence of nanotechnology as a recent advance in science, the potential harmful effects of nanomaterials also increased dramatically. The largescale production and use of nanomaterials raised much concern over the possible adverse impacts of its release into the environment. Out of the concern, emerged another area of research, nanotoxicology, that refers to the study of destructive effects of nanomaterials in the environment and its impact on organisms exposed to it (Donaldson et al. 2009).

Nano-scale materials constitute a diverse range of products like electronics, optics, textiles, medical devices, catalysts, biosensors, cosmetics, food packaging, water treatment technologies, telecommunications, pharmaceuticals, fuel cells, etc. (Aitken et al. 2006). These nanomaterials can be nanofilms, nanowires and nanotubes or nanoparticles. Among the nanoparticles, fullerenes are spherical, cage-like molecules formed of pure carbon atoms, also called "buckminsterfullerene" or "bucky balls". Fullerene C_{60} possesses distinct physicochemical properties that make it more robust and versatile compared to other fullerene nanoparticles. It acquires unique physical and chemical properties for use in many applications such as superconductors, cosmetics, electronics and also drug and gene delivery (Bosi et al. 2003).

The fullerene C_{60} molecule has been shown to absorb the UV and visible range of the light spectrum and, in the presence of molecular oxygen, generate reactive oxygen species, particularly singlet oxygen and superoxide anion (Hotze et al. 2008). Therefore, when fullerene C_{60} is released into the environment and activated by sunlight, it has been shown to induce reactive oxygen species. Production of oxygen-free radicals interact with proteins, nucleic acids and the double bonds of membrane phospholipids, which leads to downstream detrimental effects, such as protein and DNA adduction, lipid peroxidation, membrane rupture and, eventually, cell death (Halliwell and Gutteridge 2007). However, several other studies have reported that fullerene is a powerful antioxidant with no acute or subacute toxicity (Gharbi et al. 2005) and also lacks cytotoxic effects (Levi et al. 2006). Therefore, the molecular mechanisms underlying the toxicity of fullerene C_{60} remain a controversial issue. Considering the controversy of fullerene toxicity, the present study was aimed at evaluating the toxic effects of fullerene nanoparticles in the muscle tissue of the fish *Pseudetroplus maculatus* (Bloch 1795).

Materials and Methods

Animals

Healthy adult fish of *P. maculatus*, weighing 8.5±1.5 g and length 9±1 cm, were collected from a fish farm Kaloos Aquarium in Kottakkal, Kerala. Fish were transported with as little disturbance as possible to the laboratory and acclimatised for two weeks to the laboratory conditions prior to the experiment. Animals were maintained in dechlorinated water and good lighting system (12 h light :12 h dark) throughout the experiments.

The health status of fish was continuously monitored and unhealthy fish were removed immediately. The physico-chemical features of the tap water were estimated as per APHA (1998) by using standardised measures where water temperature was 28±2 °C, dissolved oxygen saturation between 70 and 100 % and pH 7.6.

Chemical

Fullerene C_{60} (CAS No. 99685-96-8) of 99.9 % purity was a generous gift obtained from Suzhou Dade Carbon Nanotechnology Co. Ltd., China. DMSO (1%) was used as a vehicle to dissolve fullerene which was sonicated in Sonics-Vibracell VX-400 at 35 Hz for 30 min at 3 sec pulse intervals to attempt uniform dispersion before adding to the exposure tanks to reach 0.1 $mg.L^{-1}$. It is also important to point out that the present study was specifically designed to evaluate the interactions between the nanomaterial fullerene and the biological system as the fish model, not to mimic, for example, an environmental exposure scenario. Therefore, the above concentration was chosen for the present study.

Experimental design

Experiments were carried out for 96 h at 24 h intervals maintaining 10 animals per group at 0.1 $mg.L^{-1}$ (i.e. 100 $\mu g.L^{-1}$) concentration of fullerene along with control and vehicle group.

Group 1: Control group maintained for 96 h.

Group 2: Vehicle group (1% DMSO) maintained for 96 h.

Group 3: Fullerene-treated group maintained for 24, 48, 72 and 96 h.

Preparation of tissue homogenate

At the end of every experiment, fish were caught very gently using a small dip net, one at a time, and were decapitated. Muscle tissue was dissected and 1 % (w/v) homogenate of muscle tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogeniser on crushed ice for a minute. The homogenate was centrifuged at 800 *g* for 15 min at 4 ºC to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry et al. (1951) with BSA as the standard. Activity of superoxide dismutase (Marklund and Marklund 1974), catalase (Claiborne 1985), glutathione reductase (Carlberg and Mannervik 1985); levels of hydrogen peroxide generation (Pick and Keisari 1981) and lipid peroxidation (Ohkawa et al. 1979); and activities of acid and alkaline phosphatase (Bessey et al. 1946) were measured in the supernatant of crude homogenate.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), the test against the normality assumption of data. The normality of data was analysed by Duncan's multiple range test as post-hoc test using the statistical package SPSS 19.0 in order to manage the experiment-wise error rate. Statistical significance was set at $p<0.05$ against the control groups and data are presented as Mean±SD for ten animals per group. All biochemical estimations were carried out in duplicate.

Results

Exposure of fullerene at 0.1 $mg.L^{-1}$ significantly ($p<0.05$) decreased the activities of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase after 72 and 96 h in the muscle tissues (Fig. 1-3). However, the levels of hydrogen peroxide and lipid peroxidation increased significantly ($p<0.05$) after 72 and 96 h of treatment in muscle tissue (Fig. 4 and 5). The activities of acid and alkaline phosphatases in muscle tissue were found to be significantly ($p<0.05$) decreased at the end of 72 and 96 h of fullerene exposure (Fig. 6).

Discussion

Generation of free radicals in the biological system and the environmental contaminants are a hot point of discussion among researchers in recent years. Aquatic organisms, particularly fish, possess well developed systems for generation and degradation of free radicals (Winston 1991). So the harmful effects of free radicals can be prevented or counter-balanced by antioxidant defence systems having enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase as in the case of mammals (Droge 2002). The present study was aimed at evaluating the induction of oxidative stress after fullerene C_{60} exposure in the muscle of the freshwater fish, *P. maculatus*. The reason for the selection of *P. maculatus* as the test organism is that, among the cichlid group of fishes, *P. maculatus* is the genus endemic to India that breeds throughout the year and has been found in abundance in the backwaters of Kerala, India. The fish has economic importance, but recently, there have been local reports about the decline in the population of *P. maculatus* and this could be due to the frequent release of environmental contaminants into the natural ecosystem. In the present study fullerene, when exposed at 0.1 $mg.L^{-1}$ concentration showed significant ($p<0.05$) reduction in the activity of superoxide dismutase at the end of 72 and 96 h. Superoxide dismutase is the prime antioxidant enzyme that converts superoxide to hydrogen peroxide. In biological tissues, superoxide can be converted non-enzymatically into non-radical species such as hydrogen peroxide and singlet oxygen (Fridovich 1978). The reduction in the activity of superoxide dismutase at 72 and 96 h of fullerene exposure could be due to the failure of elimination of hydrogen peroxide in the muscle tissue, which is further evidenced by the elevated level of hydrogen peroxide generation.

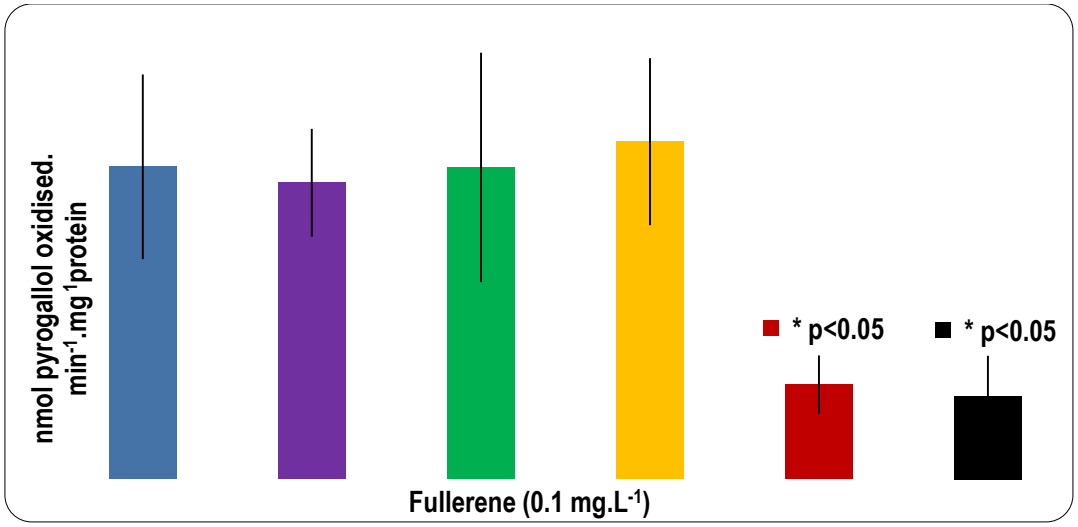

Fig. 1. Effect of fullerene on the activity of superoxide dismutase in muscle of *Pseudetroplus maculatus*

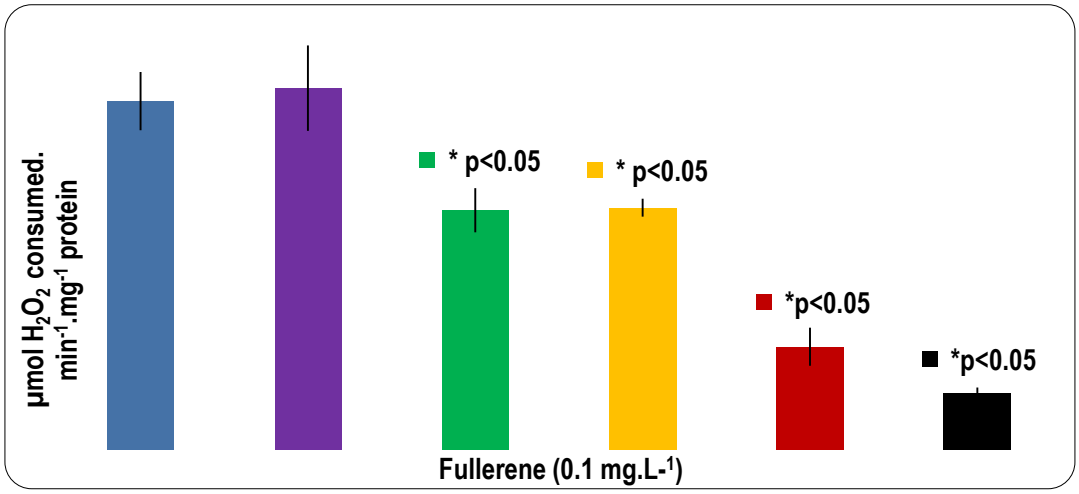

Fig. 2. Effect of fullerene on the activity of catalase in muscle of *Pseudetroplus maculatus*

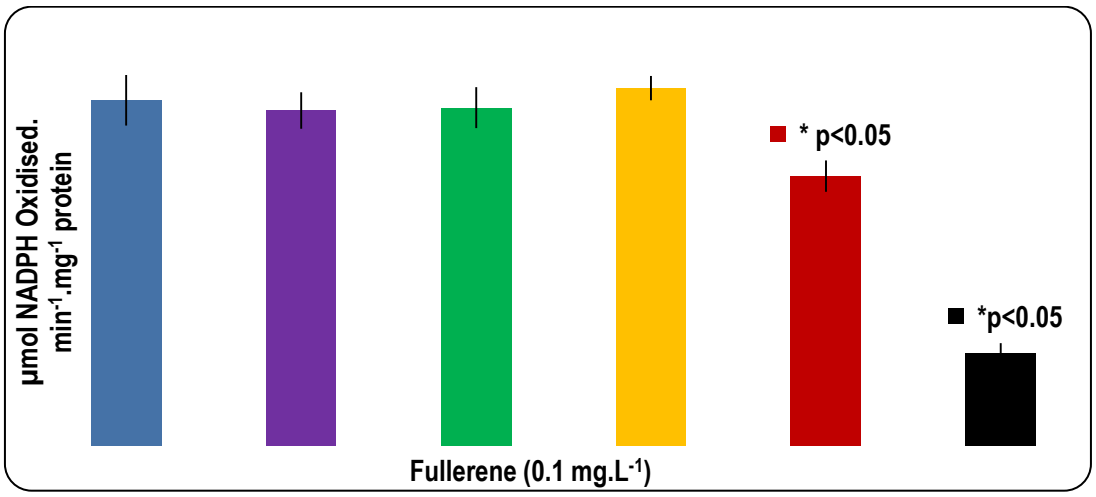

Fig. 3. Effect of fullerene on the activity of glutathione reductase in muscle of *Pseudetroplus maculatus*

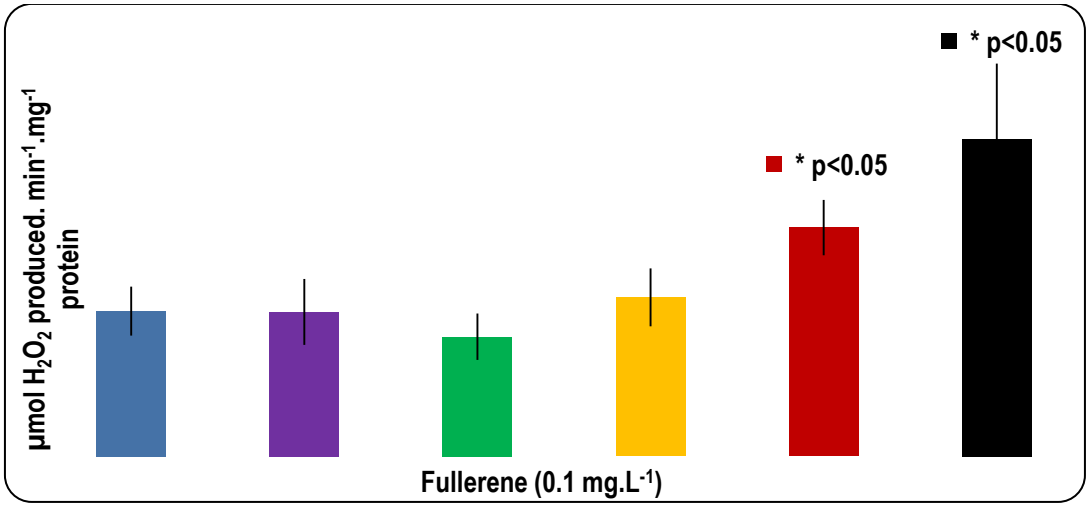

Fig. 4. Effect of fullerene on the level of hydrogen peroxide generation in muscle of *Pseudetroplus maculatus*

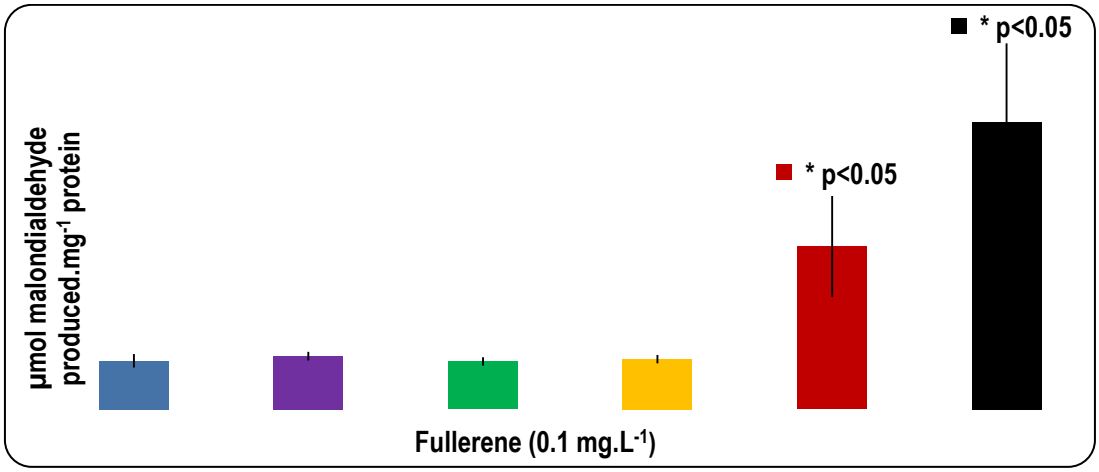

Fig. 5. Effect of fullerene on the level of lipid peroxidation in muscle of *Pseudetroplus maculatus*

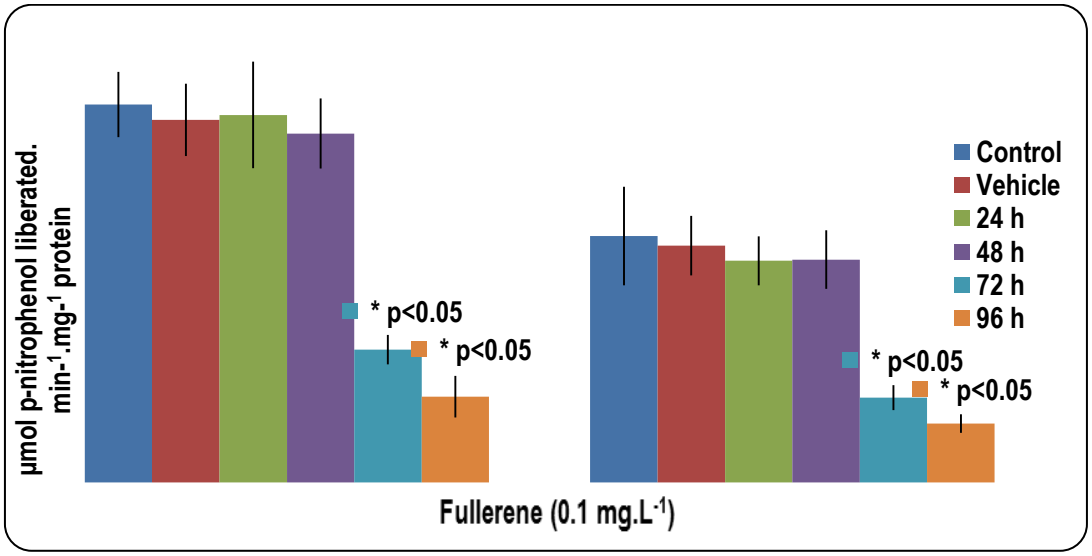

Fig. 6. Effect of fullerene on the activities of acid and alkaline phosphatase in muscle of *Pseudetroplus maculatus*

Catalase detoxifies the hydrogen peroxide and has no electron donor requirement. The reduction in the activity of catalase after fullerene exposure indicates the failure of antioxidant enzymes to defend against the exposed toxicant. The activity of glutathione reductase showed a significant ($p<0.05$) decrease only at 72 and 96 h of fullerene exposure when compared to the corresponding control groups. Maintenance of intracellular level of antioxidant enzymes is a very important defensive mechanism to prevent free radical toxicity. Glutathione reductase is the enzyme that catalyses the reduction of glutathione disulfide (GSSG) to the sulfhydryl form of glutathione (GSH), which is a crucial enzyme in resisting oxidative stress and maintaining the reducing environment of the cell (Galter et al. 1994). In view of this point, the decrease in the activity of glutathione reductase at 72 and 96 h of fullerene exposure indicates the failure of the enzyme glutathione reductase to maintain glutathione (GSH) in a reduced state, which would ultimately lead to disturbance of redox status in muscle tissue.

Evaluation of oxidation of lipids is one of the most commonly used approaches in the field of free radical research because many organisms, especially aquatic animals, contain large amount of lipids with polyunsaturated fatty acid residues as a substrate for oxidation. Lipids are usually oxidised by the formation of peroxides and the process is known as lipid peroxidation (Lushchak and Bagnyukova 2006). Measurement of malondialdehyde as end product of lipid peroxidation is recommended for quantifying the level of thiobarbutiric acid reactive substances (TBARS). In the present study, the levels of hydrogen peroxide generation and lipid peroxidation increased significantly ($p<0.05$) after 72 and 96 h of fullerene treatment. The present results suggest the major contribution of fullerene in the induction of oxidative stress in muscle tissue after fullerene exposure. Similar observations such as alteration of antioxidant defence system in gill, brain and liver tissue has been reported when fullerene was exposed at 0.1 $mg.L^{-1}$ concentration for 96 h in fish (Sumi and Chitra 2016; 2017a,b). Histopathological lesions were also described in gill, liver and muscle tissues of the fish, *P. maculatus* after acute exposure to fullerene (Sumi and Chitra 2017c).

Acid and alkaline phosphatases are important biomarkers of plasma membrane and endoplasmic reticulum of tissue that are widely involved in adaptive cellular response (Leohner et al. 2001). Fullerene treatment showed a significant ($p<0.05$) decrease in the activities of both acid and alkaline phosphatase in 72 and 96 h exposure periods. However, there were no changes in the activities of antioxidant enzymes as well as in the activities of acid and alkaline phosphatases at 24 and 48 h of fullerene treatment when compared to control groups. Results from the study clearly indicate that fullerene (0.1 $mg.L^{-1}$) at acute exposure adversely affect the facilitation of transfer of metabolites across the cell membrane in muscle tissue of the fish, *P. maculatus*.

Conclusion

The present study is a preliminary step undertaken to understand the potential adverse effects of the nanoparticle, fullerene, in biological system. It was evident from the present findings that fullerene induced oxidative stress in the muscle tissue of the fish, *Pseudetroplus maculatus*. Further investigations should be done to clearly define the adverse impacts of nanoparticles in living organisms, particularly on fish.

Acknowledgements

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