Effects of fullerene (C₆₀) on antioxidant enzyme activities and lipid peroxidation in gill of the cichlid fish, *Pseudetroplus maculatus* (Bloch, 1795)

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**Abstract**

Fullerene (C₆₀) nanomaterials possessing unique physicochemical and biological properties are extensively used in the medical and industrial applications. The aim of the study was to evaluate the effects of fullerene on the antioxidant enzyme activities in gill of cichlid fish, *Pseudetroplus maculatus*. Fullerene at 0.1 mg/L concentration was exposed to fish for 24, 48, 72 and 96 h maintaining control groups. The activities of antioxidant enzyme was estimated in gill tissue and it was observed with significant (P<0.05) reduction in the activities of catalase and glutathione reductase. However, the levels of hydrogen peroxide generation and lipid peroxidation was increased in the treatment groups with a significant (P<0.05) increase at the end of 72 and 96 h. Acid and alkaline phosphatases, the reliable indicator of environmental stress to aquatic organisms are also evaluated and the results showed that fullerene significantly decreased the activities of phosphatases in gill of fish in all treatment groups. The present study therefore confirms that fullerene at acute exposure is known to induce oxidative stress and alter the membrane transport across the gill tissue.

**Keywords:** Fullerene, Antioxidant, Acid phosphatase, Alkaline phosphatase, *Pseudetroplus maculatus*.

1. **Introduction**

Fullerenes have gained considerable interest in different fields of science and technology since their discovery in 1985. They are widely applied in diverse areas of biomedical, cosmetic and industrial applications, ranging from drug delivery systems to anti-aging formulations. There is evidence that due to the prompt size, hydrophobicity, three dimensionality and electronic configurations make fullerene an appealing subject in medical chemistry. Also, the unique carbon cage structure coupled with immense scope for derivatization formulated them as a potential therapeutic agent. As a result, fullerenes acquire wide variety of biomedical applications that includes antioxidant, antiviral, antibiotic and anti-cancerous activities, enzyme inhibition, cell signaling, DNA cleavage as well as in imaging and nuclear medicine. Emerging applications of fullerenes in market, widely as creams used on the skin, or as therapeutic drug, has led to widespread concerns about their potential to cause adverse effects to human health. Fullerenes are also known to pollute the aquatic ecosystem which may be from consumer products e.g., sunscreens and cosmetics, as well as spillage from manufacturing and shipping of the nanomaterials. However, the quantity of nanomaterials found in the environment is unknown and difficult to predict, as the number of nanomaterials and its range of applications are increasing tremendously by every year. The toxicity of fullerenes is still a controversial topic, due to their dual property as pro-oxidant or antioxidant in various organisms.
In addition, the lack of toxicological data on fullerenes makes difficult to determine the actual risk assessment of the nanomaterials on animal health and environmental exposure. Thus, there is an immediate need for the scientific society to develop rapid and efficient methods to assess the toxic impact of such nanomaterials in the environment.

The crucial issue on the toxic effects of fullerenes remains as a conflict topic as several literatures provides both pro-oxidant and antioxidant properties in different animal models. Few reports suggested that the manufactured nanomaterials, fullerene C_{60}, induced oxidative stress in the brain of juvenile largemouth bass\textsuperscript{[4]}, developmental toxicity and free radical generation in zebrafish\textsuperscript{[8,64]}. In contrast, several other data have observed that fullerene is a powerful antioxidant with no acute or subacute toxicity\textsuperscript{[7]} and also lacks cytotoxic effects\textsuperscript{[8]}. Therefore, the aim of this study was to elucidate the toxic effects of fullerene on the antioxidant enzyme activities and lipid peroxidation in the gill of the cichlid fish, *Pseudetroplus maculatus*.

2. Materials and methods

2.1 Animal

Adult fish, *Pseudetroplus maculatus*, with weight and size ranging from 8.5 ± 1.5 g and 9 ± 1 cm were collected from a fish farm, Kaloos Aquarium, Kottakkal, Kerala. Fishes were then transported to the laboratory with least disturbance and were acclimatized to the laboratory conditions prior to experiment. Animal was maintained in dechlorinated water and good lighting system (12: 12 h; light: dark) throughout the experiments and the health status of fish was also monitored. The physico-chemical features of the tap water were estimated as per APHA\textsuperscript{[9]} by using standardized measures where water temperature ranged from 28 ± 2°C, oxygen saturation between 70 and 100 % and pH 7.6.

2.2 Chemical

Fullerene C\textsubscript{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 interval to attempt uniform dispersion before adding to the exposure tanks to reach 0.1 mg/ L. It is also important to point out that the present study was specifically designed to evaluate interactions between the nanomaterials fullerene and the biological system as fish model, not to mimic, for example, an environmental exposure scenario. Therefore, the above concentration was chosen for the present study.

2.3 Experiment design

Experiments were carried out for 96 h at 24 h interval maintaining 10 animals per group at 0.1 mg/ L (ie., 100 µg/ L) concentration of fullerene along with control and vehicle group.

Group I: Control group maintained for 96 h.
Group II: Vehicle group (1% DMSO) maintained for 96 h.
Group IIIa: Fullerene-treated group maintained for 24 h.
Group IIIb: Fullerene-treated group maintained for 48 h.
Group IIIc: Fullerene-treated group maintained for 72 h.
Group IIId: Fullerene-treated group maintained for 96 h.

2.4 Preparation of tissue homogenate

At the end of every experiment, fish were caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Gill tissue were dissected, weighed and stored at 4°C until the biochemical analyses were performed. A 1% (w/ v) homogenate of gill tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 8000 g for 15 min at 4°C to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry et al.\textsuperscript{[10]} with BSA as the standard. Activity of catalase\textsuperscript{[11]}, glutathione reductase\textsuperscript{[12]}, level of hydrogen peroxide generation\textsuperscript{[13]}, level of lipid peroxidation\textsuperscript{[14]}, activities of acid and alkaline phosphatase\textsuperscript{[15]} were measured in crude homogenate.

2.5 Statistical analysis

Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range test using statistical package SPSS 19.0. Differences were considered to be significant at p<0.05 against control groups. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

3. Results

Exposure of fish at 0.1 mg/ L concentration showed significant (P<0.05) increase in the activity of catalase after 24 h followed by significant (P<0.05) reduction at 48, 72 and 96 h in time-dependent manner (Figure 1). The activity of glutathione reductase showed a significant (P<0.05) decrease at all durations when compared to the corresponding control groups (Figure 2). The levels of hydrogen peroxide generation and lipid peroxidation increased however, significant (P<0.05) elevation was observed only after 48 h of treatment (Figures 3 and 4). Fullerene treatment also showed a significant (P<0.05) decrease in the activities of both acid and alkaline phosphatase in time-dependent manner when compared to that of control groups (Figure 5).
Fig 1: Effect of fullerene on the activity of catalase in gill of *Pseudetroplus maculatus*

Fig 2: Effect of fullerene on the activity of glutathione reductase in gill of *Pseudetroplus maculatus*
Fig 3: Effect of fullerene on the level of hydrogen peroxide generation in gill of *Pseudetroplus maculatus*

Fig 4: Effect of fullerene on the level of lipid peroxidation in gill of *Pseudetroplus maculatus*
Fig 5: Effect of fullerene on the activities of acid and alkaline phosphatase in gill of *Pseudetroplus maculatus*

4. Discussion
The toxic effects of fullerene C_{60} on the activities of antioxidant defense system in gill of fish was evaluated in the present study by using the cichlid fish, *Pseudetroplus maculatus* as animal model. In ecological toxicity studies fish have become the prominent vertebrate model as it represents the largest and most diverse group of vertebrates. Monitoring fish species provides valid information on the effects of exposed pollutants and the health status of the aquatic ecosystem. Fish show high sensitivity to the changes in the aquatic environment than invertebrates and other aquatic animals. Therefore, fish can be used as a good indicator of water contamination in the aquatic ecosystems. *Pseudetroplus maculatus*, commonly known as orange chromid, is an indigenous cichlid fish of India. The preference of *E. maculatus* in the present study is owing to its peculiar distinctive characters, as it is an omnivorous species, continuous breeder throughout the year, substrate spawner showing biparental care, its availability and ease way of maintenance in the laboratory.

Contamination of aquatic ecosystem leads to the health risk in the dwelling organisms, and some toxicants in aquatic environment can be transferred through food chain into humans. Recently, there is a growing concern that nanomaterials are exposed to aquatic environment in large scale due to its ubiquitous use and existence in the environment. Fullerene C_{60} nanomaterials otherwise known as ‘bucky balls’ or ‘Buckminster fullerenes’ have been selected in this study as it is used extensively in groundwater remediation, cosmetics and drug delivery[16]. Fullerene generated the most controversy regarding its toxicity, therefore, the present study aimed to demonstrate if fullerene act as antioxidant or else it alter the antioxidant defense system in the gill of fish.

To address the concern of negative environmental effects of fullerene, it was exposed to fish for short duration and the activities of antioxidant enzymes were evaluated. The present results showed that fullerene decreased the activities of antioxidant enzymes as catalase and glutathione reductase with concomitant increase in the levels of hydrogen peroxide and lipid peroxidation in gill of fish. Like mammals, fishes also possess a well developed antioxidant defense system to neutralize the toxic effects of reactive oxygen species (ROS)[17]. There are evidence that nanomaterials induced oxidative stress through the generation of ROS those damage lipids, carbohydrates, proteins and DNA[18,19].

Catalase is the key enzyme in antioxidant defense system converting hydrogen peroxide into water and oxygen molecule. Significant reduction in the activity of catalase after fullerene treatment indicates the
failure of elimination of hydrogen peroxide in the gill tissue, which was evidenced by the elevated level of hydrogen peroxide. Glutathione peroxidase/reductase directly act as antioxidant enzymes to inhibit lipid peroxidation\cite{20}. In the present study, the activity of glutathione reductase was also significantly decreased in all duration which reflect the increased oxygen free radical generation.

Free oxygen radicals such as superoxide, hydroxyl radical, singlet oxygen and hydrogen peroxide are generally considered cytotoxic agents because of their ability to induce lipid peroxidation in tissues and cell membrane. ROS has been also shown to cause peroxidation of DNA, proteins and lipids, which can lead to cellular damage, tissue injury or organ failure\cite{21,22}. Among these, lipids are most susceptible to free radical attack, particularly long chain polyunsaturated fatty acids (PUFA), which contain several double bonds. Therefore, lipid peroxidation has often been used as a biomarker of environmental stress, reflecting damage to cell membranes from free radicals and is an important feature in cellular injury\cite{23}. In the present study, the increased level of lipid peroxidation after fullerene exposure indicates the damage to the tissue and the induction of oxidative stress in gill of fish.

Despite the antioxidant enzymes, the study also focused on the activity of gill stress marker enzymes, acid phosphatase and alkaline phosphatase. These are important phosphatases that differ in their function and sub cellular distribution. Acid phosphatase is associated with lysosomes and was distributed among all gill cell types because of the presence of lysosomes within the cells, whereas the activity of alkaline phosphatase was found to be highly concentrated in plasma membrane enriched fraction. In the present study both acid and alkaline phosphatase activities were decreased significantly in fish gill tissue. Similar observation was reported in gill tissue when fish exposed to metanil yellow and diethyl phthalate \cite{24,25}. The present observation clearly indicates that fullerene exposure caused cellular damage and affected metabolic process and transmembrane transport across the gill tissue.

5. Conclusion

This study gives the basic knowledge of toxic effects of nanomaterials, fullerene and its impact on the antioxidant defense system in gill of fish. Acute exposure to fullerene induced oxidative stress and also affected metabolism and membrane transport in gill of fish.

6. References

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