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Acute exposure to fullerene (C_{60}) altered antioxidant defence system in hepatocytes of the cichlid fish, *Pseudetroplus maculatus* (Bloch, 1795)

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ABSTRACT

Fullerene (C_{60}) has attained an important role in nanoscience as it acquires unique physicochemical properties and is used widely as radical scavenger and powerful antioxidant. The present study was aimed to investigate the role of fullerene in the antioxidant system of liver tissue in the cichlid fish, *Pseudetroplus maculatus.* Fullerene at 0.1 mg/ L concentration, dissolved in DMSO as vehicle, was exposed to fish for 24, 48, 72 and 96 h maintaining control groups. At the end of every treatment, fish was sacrificed and liver tissue was collected. It was observed that fullerene treatment did not caused changes in the weight of the liver when compared to the control groups. The activity of superoxide dismutase increased significantly ($P<0.05$) at 72 and 96 h, however, the activities of catalase, and glutathione reductase significantly ($P<0.05$) decreased after 48 h of fullerene exposure. The level of hydrogen peroxide increased significantly only at the end of 96 h and the lipid peroxidation was increased significantly ($P<0.05$) after 48 h in time-dependent manner. From the present findings it was clearly demonstrated that acute exposure to fullerene alters the antioxidant defense system in the liver of fish. On contrary as an antioxidant, fullerene is known to generate free radicals, and, therefore induces oxidative stress in fish hepatocytes.

Keywords: Fullerene, Liver, Antioxidant, Oxidative stress, *Pseudetroplus maculatus*

1. INTRODUCTION

Fullerene C_{60}, the most popular nanomaterial, is produced worldwide and known to have a unique cage-like molecular structure consisting of purely carbon atoms which is the third allotropic form after diamond and graphite. Fullerene possesses sixty carbon atoms arranged into a hollow sphere of cyclopentenes and cyclohexenes named bucky ball (Kroto *et al.*, 1985). This structure favours the stability and persistence of fullerene in water as colloidal aggregates, which along with a substantial fraction forms a size of nanometer range (Andrievsky *et al.*, 1999). Apart from the hydrophobic properties, fullerene due to its electronic configuration has been known to form strong C_{60}-H_2O bonds in the colloidal water suspensions (Khokhryakov *et al.*, 2006). It, therefore, could lead to the formation of stable nano-aggregates that can promote deleterious effects in the biological systems (Murdock *et al.*, 2008). It is well known that nanoparticles possess the ability to carry and release the drug in the right place and at the required dose together with greatly reduced problems

associated with direct treatment with the drugs. Owing to the small size of fullerenes, it was widely used in medicine and pharmaceuticals as it can be employed to facilitate the distribution of drugs into the body. In addition, some nanoparticles were also widely used in the production of several cosmetics, healthcare products and textiles (Nohynek *et al*., 2007).

Consequently, the toxicity and bioaccumulation of nano-C_{60} aggregates have been identified in aquatic ecosystem, with unique reference to algae and crustaceans (Baun *et al*., 2008). Fullerene also contains some paradoxical biological effects since it possesses antioxidant or pro-oxidant properties (Zhu *et al*., 2006; Spohn *et al*., 2009). The pro-oxidant nature of fullerenes has been determined by the production of reactive oxygen species, which may affect the macromolecules such as lipids, proteins, and DNA thereby inducing oxidative stress (Monserrat *et al*., 2007). It has been reported that fullerene C_{60} induced lipid peroxidation in the presence of UV light by increasing the levels of reactive oxygen species thus inducing oxidative stress (Yang *et al*., 2007). Previous study from our laboratory had observed that exposure to fullerene C_{60} has been shown to alter the activities of antioxidant defense system and is known to induce oxidative stress in the gill of the fish, *Etroplus maculatus* (Sumi and Chitra, 2016). Therefore, nanoparticles have unexpected impacts on aquatic organisms, particularly to fish population.

Pseudetroplus maculatus is most common species found in both freshwater and brackish water in south India. The sensitivity of the species to the toxicant exposure and its dominance in the natural environment favours the organism as the most suitable model to evaluate the toxicity effects of nanoparticles. The present study is designed to evaluate if the acute exposure to fullerene (C_{60}) could alter antioxidant defense system in hepatocytes of the cichlid fish, *Pseudetroplus maculatus*.

2. MATERIALS AND METHODS

Adult fish, *Pseudetroplus maculatus,* of weight 8.5 ± 1.5 g and 9 ± 1 cm length 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 (1998) by using standardized measures where water temperature ranged from 28 ± 2°C, oxygen saturation between 70 and 100 % and pH 7.6.

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 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.

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

At the end of every experiment, fishes were caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Liver tissue were dissected, weighed and 1% tissue homogenate was prepared for the biochemical analyses. A 1% (w/ v) homogenate of liver 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 4ºC to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry *et al*. (1951) with bovine serum albumin as the standard. Activities of superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), level of hydrogen peroxide generation (Pick and Keisari, 1981), lipid peroxidation (Ohkawa *et al*., 1979) were measured in the supernatant of crude homogenate.

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.

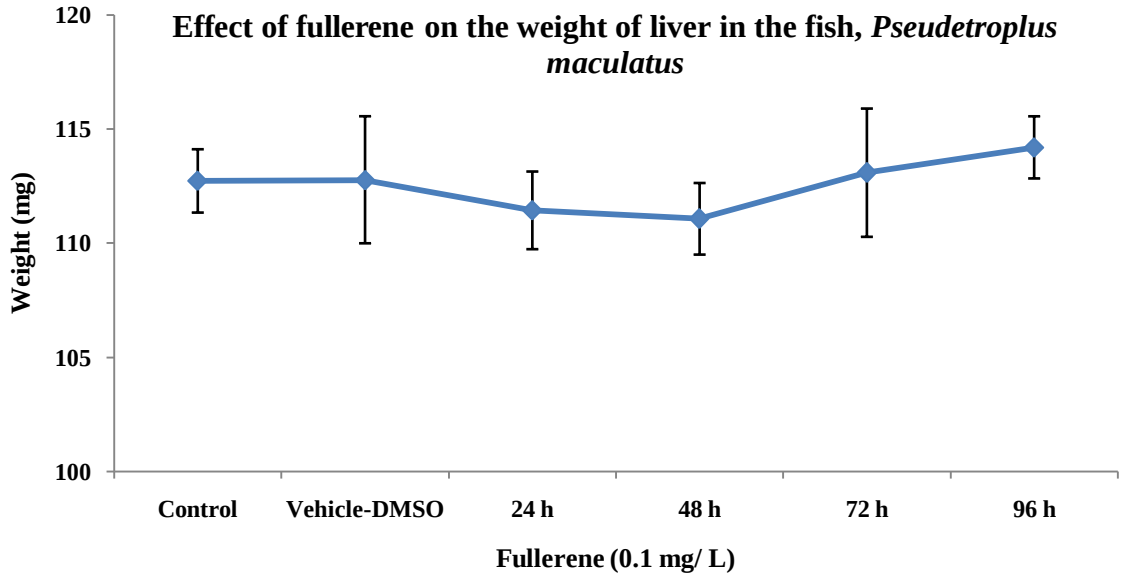
3. RESULTS AND DISCUSSION

Nanoparticles widely used in food, cosmetics, sunscreen and other products have been shown to have prominent effects on the activity of genes expressing enzymes that are responsible for oxidative stress (Runa *et al*., 2016). Cytotoxicity and oxidative stress of any nanoparticles considerably depend on cell type, concentration, diameter, and method of measurement. The present study was designed to study the acute toxic effects of fullerene (C_{60}) in antioxidant defense system of liver tissues in the cichlid fish, *Pseudetroplus maculatus*. Normally, all biological systems are provided with highly efficient antioxidant defense system in order to detoxify the oxygen free radicals. The failure of elimination of potential oxygen-free radicals and other reactive oxygen species (ROS) are known to damage tissues and cellular components and it is termed as oxidative stress. Recently, one of the significant topics of interest in environmental toxicology studies is the evaluation of antioxidant defense system in the biological system. Cellular oxidative homeostasis is achieved by the balance between pro-oxidant and antioxidant defences by the activities of several endogenous antioxidant enzymes, and the imbalance could lead to oxidative damage, especially induced by different classes of chemical pollutants in the environment (Abdollahi *et al*., 2004). Therefore, the activities of antioxidant enzymes are the appropriate measure to evaluate the toxic effects of pollutants.

Fishes are mainly used as sentinel organisms for ecotoxicological studies because they play several roles in the trophic web, concentrate the toxic substances and also respond to chemicals even at low concentration. Like mammals, fish also possess a well developed antioxidant defense systems designed to neutralize the toxic effects of reactive oxygen species (Pandey *et al*., 2003; Zhang *et al*., 2004). Liver tissues of fish are an important organ of active metabolism and detoxification and are extremely sensitive to pollutants in aquatic ecosystem (Brusle and Anadon, 1996). Fullerene has been shown to diffuse through lipid membrane and affect the structural, elastic and dynamic properties of bilayer of cell (Wong-Ekkabut *et al*., 2008). It can also pass

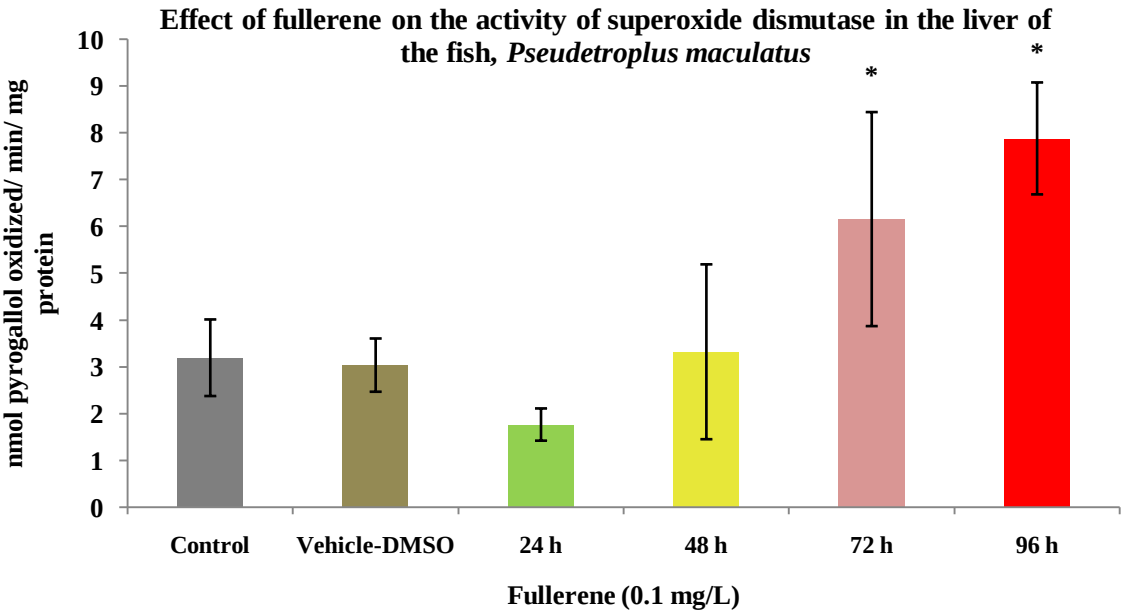
through the blood brain barriers that pass through the body fluids and finally accumulate in the liver tissues (Wang *et al*., 2014). Thus the possible nanoparticle toxicity could be the reactive oxygen generation and cellular oxidative stress (Ahamed *et al*., 2011). In the present study fullerene when exposed at 0.1 mg/ L for 24, 48, 72 and 96 h did not caused changes in the weight of the liver when compared to the control groups (Figure 1).

Figure 1



SOD is a very sensitive enzyme to the pollutants and can be used as early warning signal for the detection of oxidative stress. Fullerene exposure significantly increased the activity of superoxide dismutase at 72 and 96 h than that of control group (Figure 2) and this could be due to the adaptive response of hepatocytes to remove the toxicant (Alves *et al*., 2002).

Figure 2



However, the activities of catalase, and glutathione reductase significantly decreased after 48 h of fullerene exposure (Figure 3 and 4). The inhibition of these enzymes lead to failure in scavenging hydrogen peroxide from the hepatocytes, which was proved by the significant increase in the level of hydrogen peroxide at the end of 96 h (Figure 5). The similar observation has been reported when the nanoparticles silicon dioxide was exposed in hepatocytes of the fish, *Oreochromis mossambicus* (Vidya and Chitra, 2015).

Figure 3

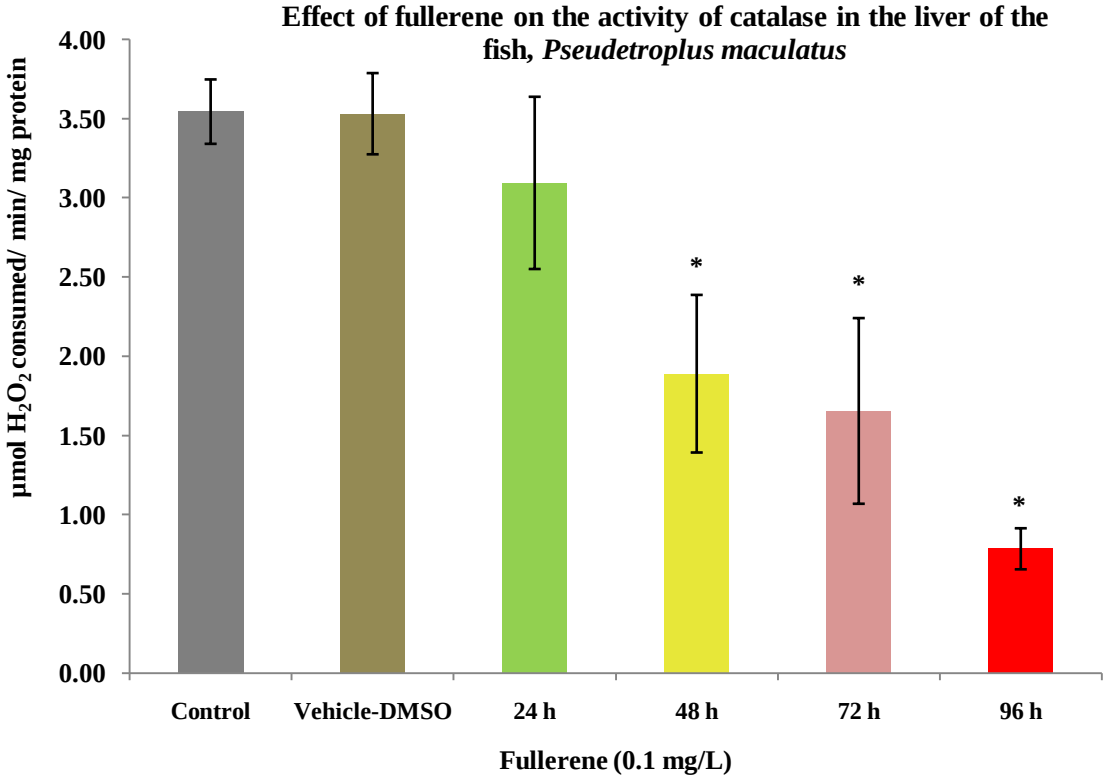


Figure 4

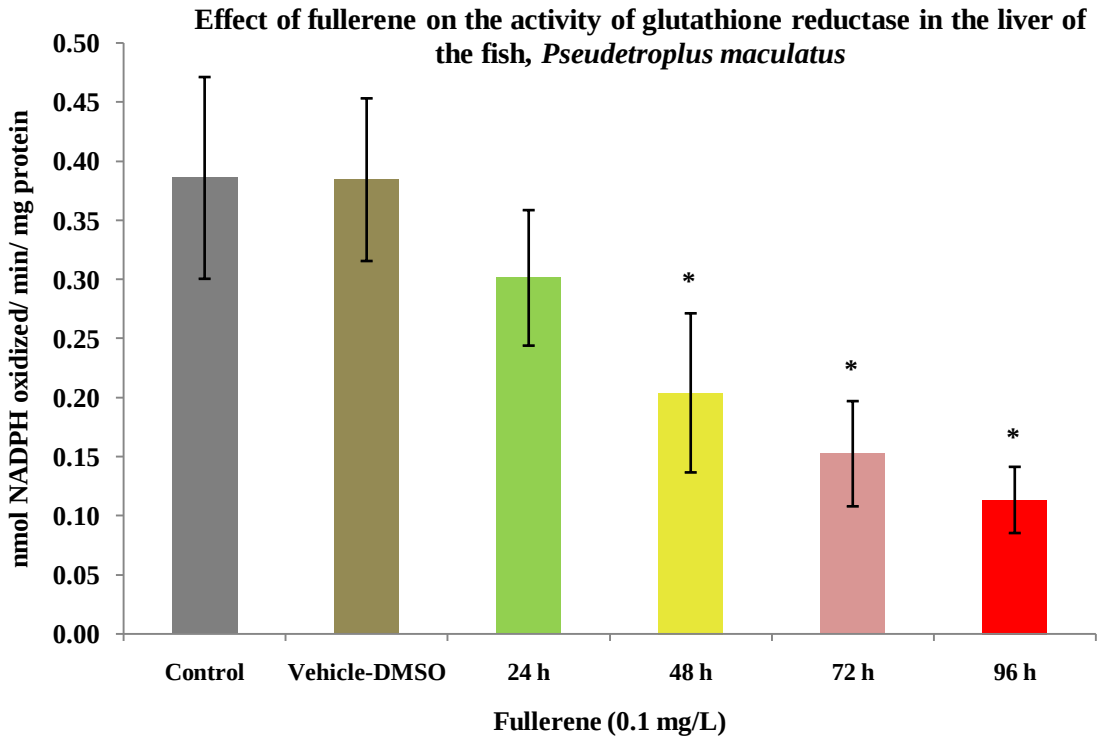
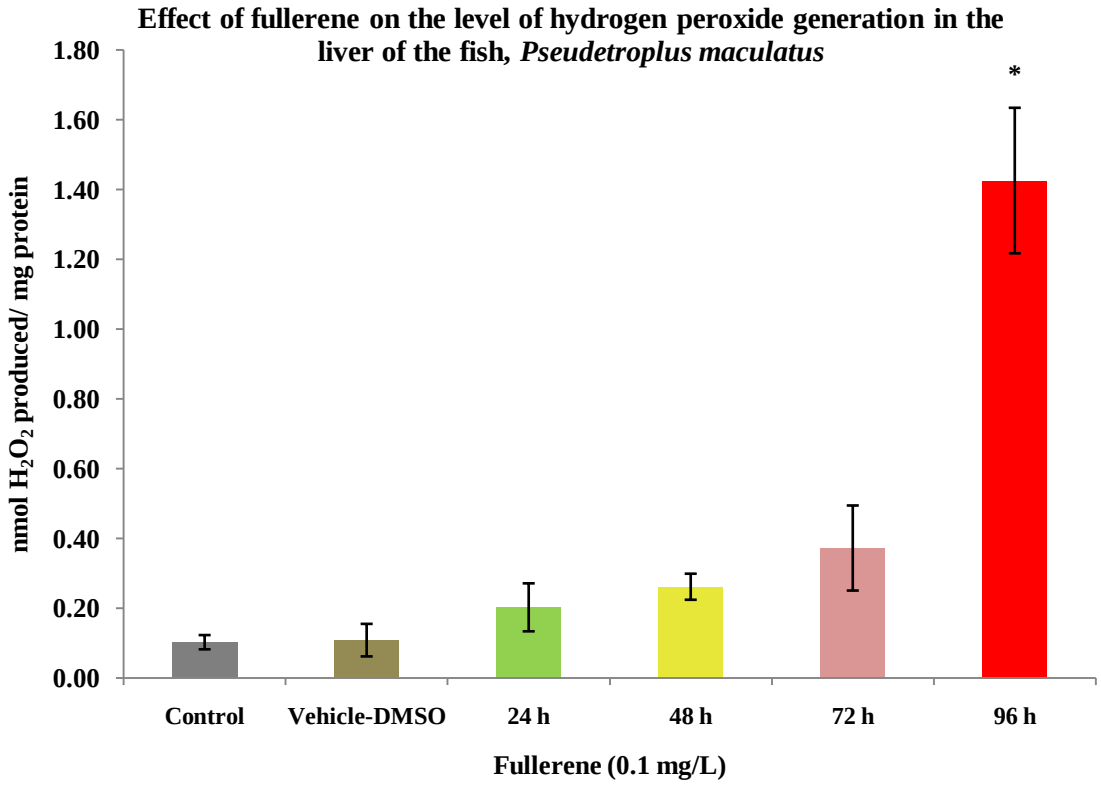
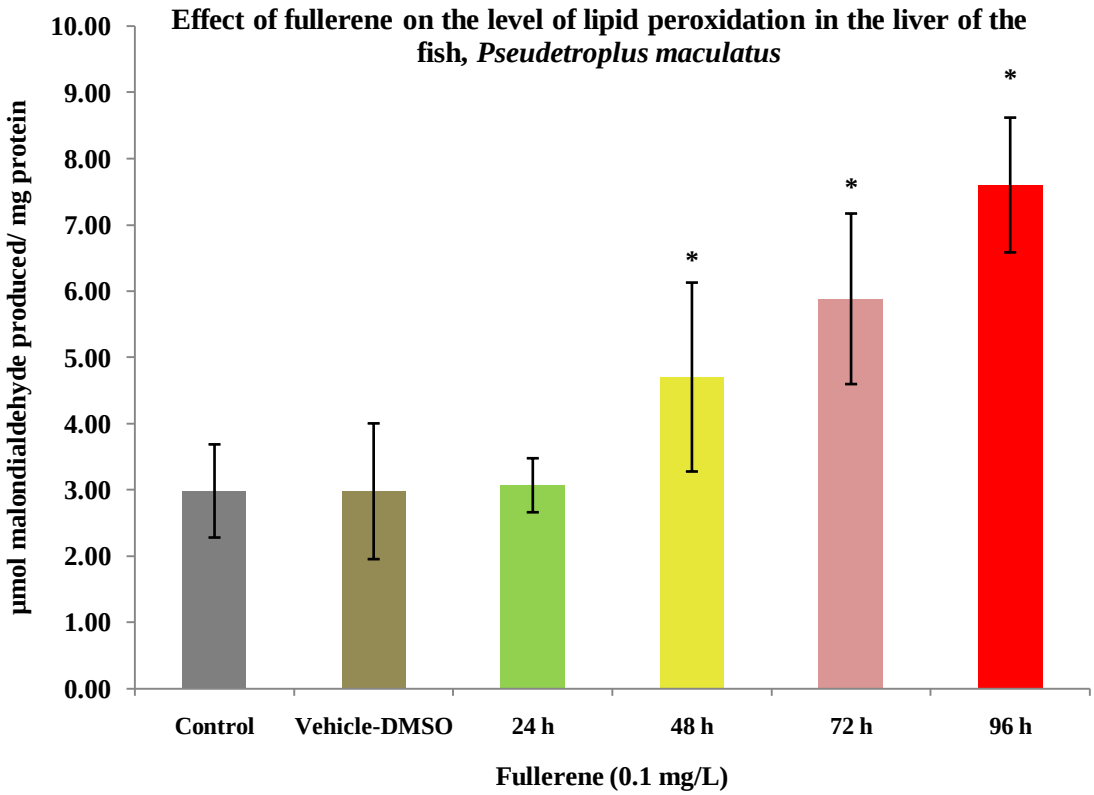


Figure 5



The initial step of cellular membrane damage is by the increase in the level of lipid peroxidation and it is considered as a valuable indicator of oxidative damage of cellular components (Sayeed *et al*., 2003). Thiobarbituric acid (TBA) is a widely using reagent for the determination of breakdown products of lipid peroxides where malondialdehyde (MDA) is the byproduct of the reaction and conjugate TBA-MDA formed are used as index of lipid peroxidation and oxidative stress (Draper *et al*., 1993). In the present study fullerene treatment significantly increased the level of lipid peroxidation after 48 h in time-dependent manner (Figure 6).

Figure 6



The present results were found similar to another study showing the liver tissues when exposed to silicon dioxide nanoparticles in fish *Oreochromis mossambicus* elevated the level of lipid peroxidation (Vidya and Chitra, 2015). Therefore, lipid peroxidation as a result of oxidative stress contributes to the damage in liver tissue thus indicating liver as the target organ of fullerene toxicity.

4. CONCLUSION

The results of the present investigation demonstrate that fullerene elicited acute toxic effects that were observed with altered antioxidant defense system and lipid peroxidation in hepatocytes of the cichlid fish, *Pseudetroplus maculatus*. The acute exposure of fullerene induced oxidative stress in liver tissue, and there is possibility that it could lead to lethal effects when the fish is exposed at higher concentration for prolonged duration.

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