Journal of Global Biosciences
ISSN 2320-1355
Volume 6, Number 4, 2017, pp. 4908-4917
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

FULLERENE (C_{60}) INDUCED ALTERATION IN THE BRAIN ANTIOXIDANT SYSTEM OF THE CICHLID FISH, *Pseudetroplus maculatus* (BLOCH, 1795)

Sumi, N. and K.C. Chitra

Endocrinology and Toxicology Laboratory,
Department of Zoology,
University of Calicut, Malappuram District, Kerala,
India.

Abstract

Fullerene (C_{60}), a pure carbon, sphere-like molecule, acquiring unique physicochemical properties is increasingly used in biomedical, cosmetic and industrial applications. In the present study an attempt has been made to evaluate the role of fullerene in the brain antioxidant system of the cichlid fish, *Pseudetroplus maculatus.* Fish was exposed to fullerene at 0.1 mg/ L concentration for 24, 48, 72 and 96 h maintaining control groups. The results showed no significant changes in the weight of the brain. The activities of antioxidant enzymes superoxide dismutase, catalase, and glutathione reductase significantly ($P<0.05$) decreased with the concomitant significant ($P<0.05$) increase in the levels of hydrogen peroxide generation and lipid peroxidation after 48 h of fullerene treatment. Acetylcholinesterase, a brain marker enzyme showed a significant ($P<0.05$) reduction following fullerene exposure at the end of 48-96 h. These observations indicate that fullerene induced alteration in the brain antioxidant system and also acetylcholinesterase activity of the fish. Therefore, it is understood that fullerene can generate reactive oxygen species in the brain tissue and this could lead to potential adverse effects on fish population.

Key words: Fullerene, Antioxidant, Acetylcholinesterase, Brain, *Pseudetroplus maculatus*.

INTRODUCTION

From the past few decades, there is a great concern on the release of variety of contaminants into the aquatic environment. Nanomaterials, one of the environmental contaminants, at the size ranging from approximately 1 to 100 nm are manufactured and released in large scale in the aquatic ecosystems. The main source of exposure is due to enormous use in various fields, large scale production, industrialization and its unique physico-chemical properties. Some of the nanomaterials are known to be very stable and form suspended aggregates in aquatic ecosystem; therefore, there is

possibility of accumulation in the aquatic organisms. In the recent years, the nanomaterial fullerene C_{60} have attained much attention due to its exceptional properties and versatility, high penetration ability, large surface area and chemical activity that makes more attractive for various applications ranging from consumer products to drug delivery systems. The various other biological properties of fullerenes include antioxidant, neuroprotective and antimicrobial activity (Lyon *et al.,* 2006), anti-HIV, antiapoptotic, enzyme inhibition activities and also widely used in osteoporotic therapy (Bosi *et al.,* 2003). In contrast, it has been reported that manufactured nanomaterials exerts adverse effects on aquatic organisms that induce oxidative stress and could result in severe lipid peroxidation in brain tissue of fish (Oberdorster *et al*., 2004).

Once when nanomaterials are released into the environment, they can easily enter into the body of organisms through the skin, airborne route, or through water and food (Jain *et al.,* 2007; Oberdorster, 2007). There are several studies reporting the entry of nanomaterials in the body of aquatic invertebrates and vertebrates (Zhu *et al.,* 2006a, 2006b; Templeton *et al.,* 2006; Elias *et al.,* 2007). The structure and modification of carbon nanomaterial is one of the main factors responsible for the ability to penetrate into the body of aquatic organisms. Fullerene C_{60} was more toxic than its hydroxylated forms and the artificial modification of such engineered nanomaterial increases its hydrophilicity (Usenko *et al.,* 2008; Isaacson *et al.,* 2007; Zhu *et al.,* 2007). The modification has been shown to occur naturally in the aquatic environment, which include agglomeration or aggregation, partial or complete degradation, chemical and microbial transformation and surface modification (Jafvert and Kulkarni, 2008). Such modifications either artificially or naturally make the nanomaterials more accessible to aquatic organisms.

Fullerene C_{60} in unmodified form has been shown to enter into the embyos of zebrafish passing through the chorion whereas hydroxylated fullerenes ($C_{60}(OH)_{24}$), the modified form, failed to penetrate into the embryo (Isaacson *et al.,* 2007). Some studies revealed the toxicity of fullerenes in the brain, gill and liver on the fish large-mouth bass (*Micropterus salmoides*) and zebrafish (*Danio rerio*) (Oberdorster, 2004; Zhu *et al.,* 2007). Fullerene C_{60} and its derivatives have been shown to induce cytotoxicity in human cell lines of skin and liver (Sayes *et al.,* 2004). Therefore, the data available on the toxicity of nanomaterials are rather contradictory and ambiguous.

In our previous study we have reported that fullerene C_{60} induced alteration on oxygen consumption and behavior of fish (Sumi and Chitra, 2015) and in addition, induced lipid peroxidation in the gill tissue of the fish, *Pseudetroplus maculatus* (Sumi and Chitra, 2016). Nanomaterials have the special property to cross the blood-brain barrier to reach the brain and also the cellular membrane in rats and medaka fish (Kashiwada, 2006; Oberdorster *et al.,* 2004). The present study was performed as an attempt to evaluate if fullerene C_{60} alters the brain antioxidant system of the cichlid fish, *Pseudetroplus maculatus*.

MATERIALS AND METHODS

Healthy adult fish, *Pseudetroplus maculatus,* were collected from a fish farm, Kaloos Aquarium, Kottakkal, Kerala with weight of 8.5 ± 1.5 g and length 9 ± 1 cm. Fish with least disturbance were transported to the laboratory and acclimatized for two weeks 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. 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 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 24, 48, 72 and 96 h interval maintaining 10 animals per group at 0.1 mg/ L (ie., 100 µg/ L) concentration of fullerene along with control (solvent-free) and vehicle (1% DMSO) group.

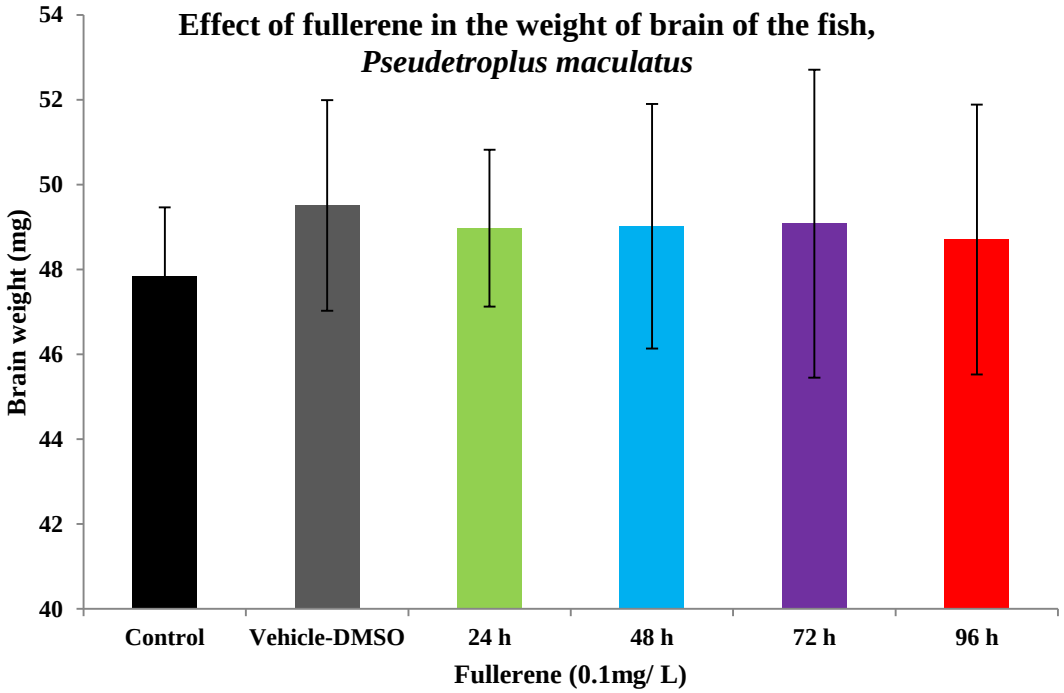
At the end of every experiment, fishes were caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Brain was dissected and 1% (w/ v) homogenate of brain tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer 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 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), activities of acetylcholinesterase (Ellman *et al.,* 1961) 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.

RESULTS AND DISCUSSION

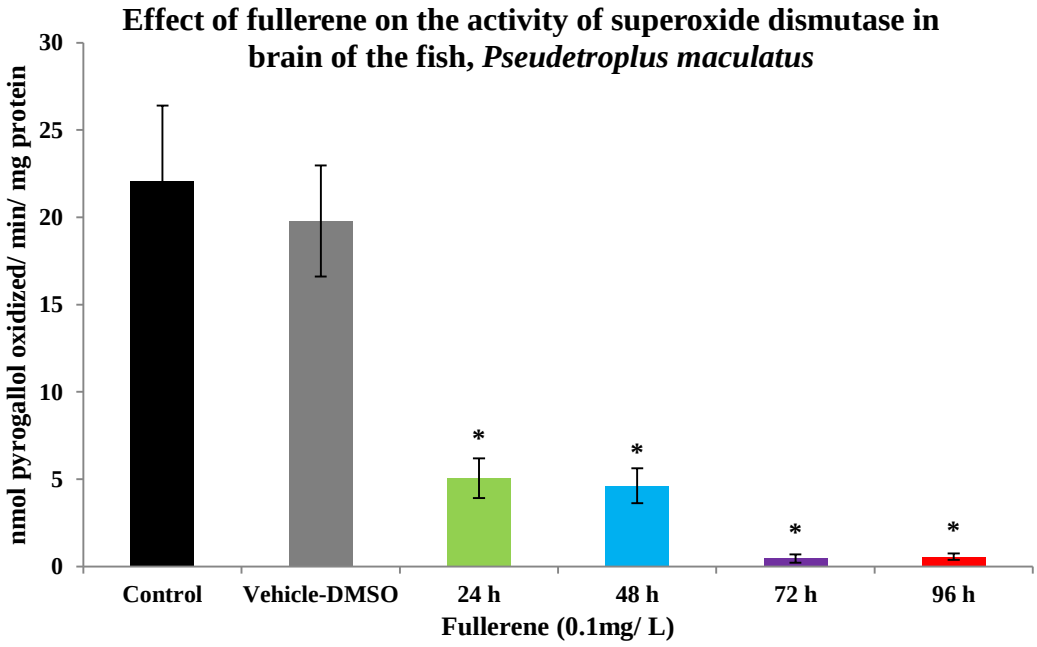
Fish are the widely used laboratory model to evaluate the health status of aquatic ecosystem. Exposure of environmental contaminants in aquatic organisms is associated with the generation of free radicals in tissues thus leading to oxidative stress. Like other higher vertebrates, fish also possess well developed antioxidant defense system in order to overcome the toxic adverse effects of free radicals generated. It includes both enzymatic and non-enzymatic mechanisms, among which the most important antioxidant enzymes are superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase. The present results showed that exposure of fullerene at 0.1 mg/ L for 96 h did not caused dose or duration-dependent significant changes in the weight of the brain (Figure 1).

Figure 1



The evaluation of fullerene induced alteration in the antioxidant defense system in brain tissue of fish revealed that the activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione reductase significantly ($P<0.05$) decreased in all treatment groups when compared to the control groups (Figures 2-4). SOD is a group of metalloenzymes that plays an important role in cellular defense against free radical induced damage by catalyzing dismutation of superoxide anion into hydrogen peroxide and water (Kappus, 1985; Zhu *et al.*, 2008). Decrease in the activity of superoxide dismutase indicates the failure of antioxidant defense system to remove the generated free radicals due to fullerene exposure.

Figure 2



Catalase is the enzyme along with glutathione reductase/ peroxidise scavenges hydrogen peroxide into oxygen and water (Mates, 2000). Fullerene exposure decreased the activities of catalase and glutathione reductase in brain tissue which designate the imbalance of antioxidant system.

Figure 3

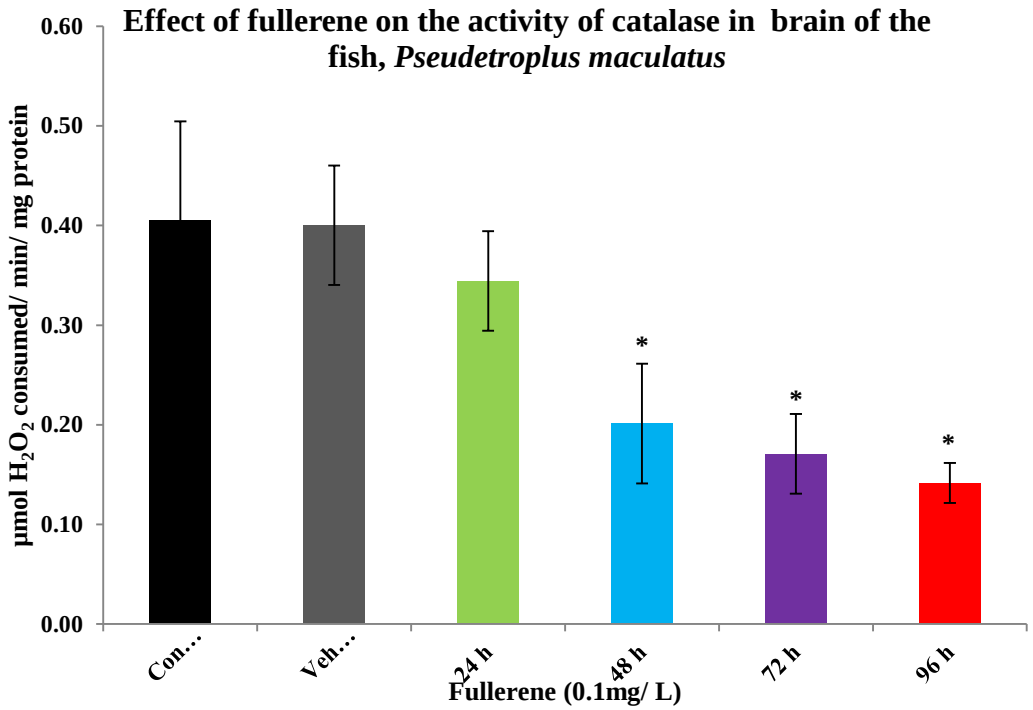
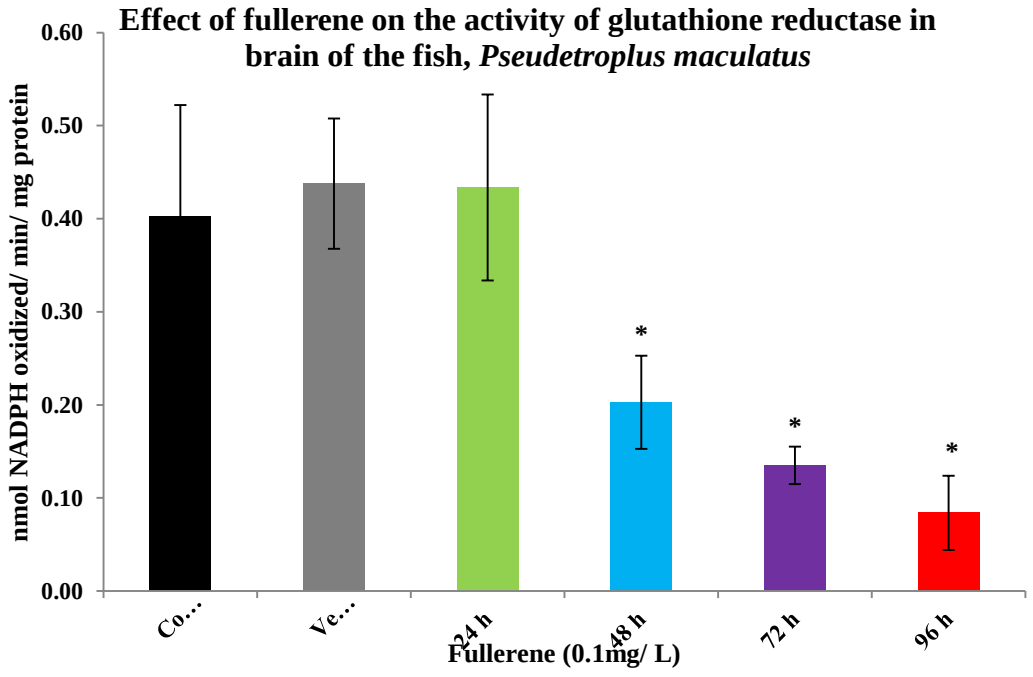


Figure 4



As a result of the failure of antioxidant enzymes, the free radicals as the level of hydrogen peroxide increased significantly (P<0.05) after 48 h of fullerene treatment (Figure 5). The reactive oxygen species generated in the form of hydrogen peroxide could elicit widespread damage to cells, such as lipid peroxidation in the brain tissue. In the present study the level of lipid peroxidation increased significantly (P<0.05) after 24 h of fullerene treatment (Figure 6). Lipid constitutes a major part of brain tissue and plays a major role in membrane integrity. Fish when exposed to fullerene are subjected to oxidative stresses where the lipid constituent in the brain is more vulnerable to the attack of free radicals and that could ultimately change the properties of biological membranes, resulting in oxidative damage. Thus at high levels of lipid peroxidation could lead to cell degradation and decreases the fluidity of cell membranes (Ates *et al.,* 2008; Talas *et al.,* 2008).

Figure 5

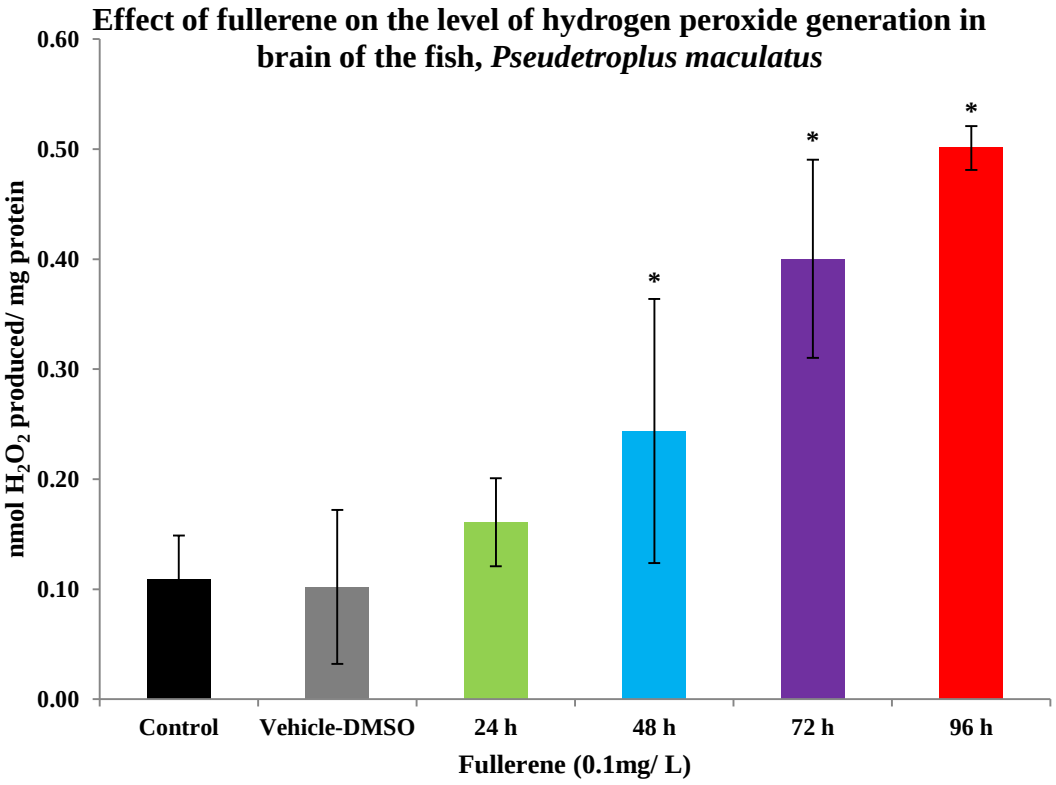
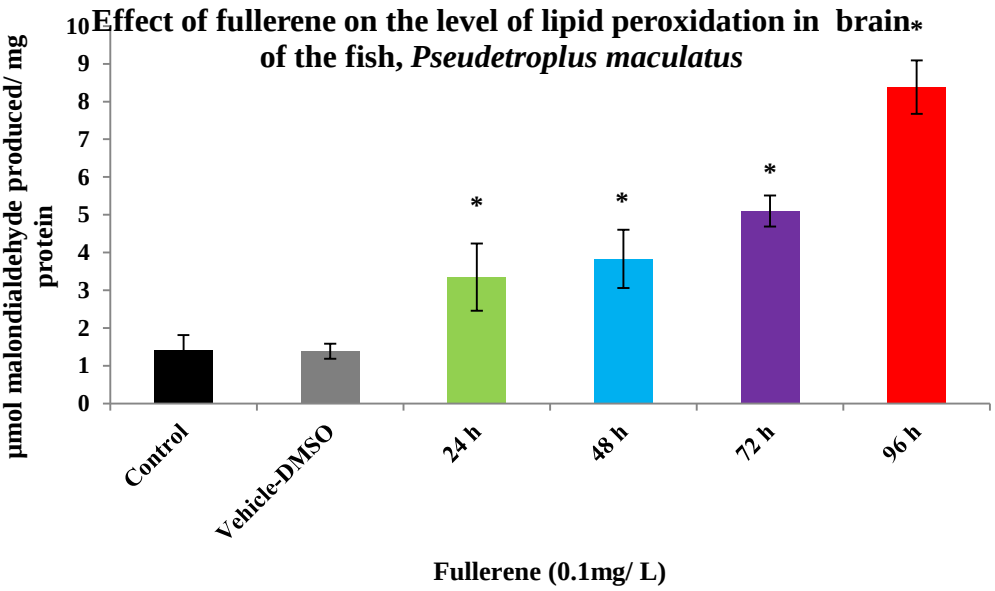
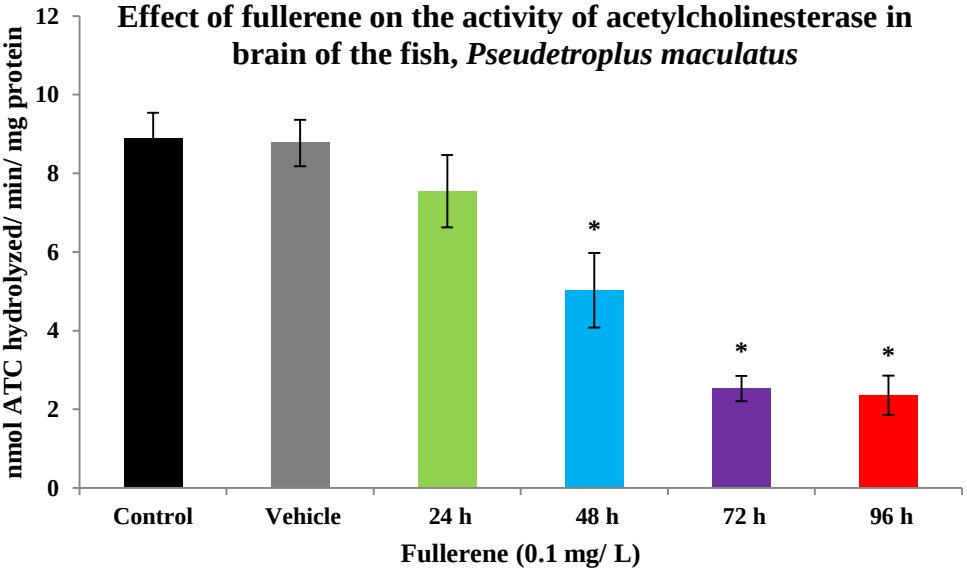


Figure 6



Acetylcholinesterase is the crucial enzyme present in the nervous system of both vertebrates and invertebrates and the highest activity was observed in the muscle and brain of fish. Acetylcholinesterase is responsible for the rapid hydrolytic degradation of the neurotransmitter acetylcholine (ACh) into the inactive products, choline and acetic acid. In the cholinergic transmission the acetylcholinesterase regulates the nervous transmission by reducing the concentration of acetylcholine in the junction by hydrolysis process (Sturm *et al.,* 1999). Acetylcholinesterase activity is widely used as highly sensitive biomarker of neurotoxicity. In the present study the activity of acetylcholine activity was significantly decreased following fullerene exposure at the end of 48-96 h (Figure 7).

Figure 7



Fullerene exposure inactivated the activity of acetylcholinesterase in brain tissue so that the enzyme is no longer available to hydrolyse acetylcholine. It could result in the failure of nerve impulse transmission across the neurojunction due to high accumulation of acetylcholine which proves fullerene as neurotoxic in the fish, *Pseudetroplus maculatus*. Similar results have been observed in brain of fish exposed to environmental toxicants as bisphenol A and nonylphenol (Rejitha *et al*., 2016; Asifa and Chitra, 2016).

CONCLUSION

The present observations conclude that fullerene exposure imbalance pro-oxidant and antioxidant system in the brain tissue and also induced neurotoxicity in the fish, *Pseudetroplus maculatus*. The acute toxicity of fullerene can also be accumulated for longer periods resulting in the disruption of blood-brain barrier in the exposed fish.

REFERENCES

[1] APHA., 1998, Standard methods for the examination of water and wastewater, 20th Edition, Washington, DC.

[2] Asifa, K.P., and Chitra, K.C., 2016, Impact of nonylphenol on antioxidant system and acetylcholinesterase activity in the brain of *Etroplus maculatus* (Bloch, 1795), Int J Res, 3(13), 114-123.

[3] Ates, B., Orun, I., Talas, Z.S., Durmaz, G., and Yilmaz, I., 2008, Effects of sodium selenite on Some Biochemical and Hematological Parameters of Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb2+ and Cu2+, Fish Physiol Biochem, 34, 53–59.

[4] Bosi, S., DaRos, T., Spalluto, G., Balzarini, J., and Prato, M., 2003, Synthesis and anti-HIV properties of new water soluble bis-functionalized[60]fullerene derivatives, Bioorg Med Chem Lett, 2003,13, 4437–4440.

[5] Carlberg, I., and Mannervik, B.J., 1985, Purification and characterization of the flavoenzyme glutathione reductase from rat liver, J Biol Chem, 250, 5474-5480.

[6] Claiborne, A., 1985, Catalase activity. In: CRC Handbook of methods for oxygen radical research. R Greenwald (ed.), CRC Press, Boca Raton, Florida. 283-284 pp.

[7] Elias, A.L., Carrero-Sanchez, J.C., Terrones, H., Endo, M., Laclette, J.P., and Terrones, M., 2007, Viability studies of pure carbon and nitrogen doped nanotubes with *Entamoeba histolytica*: From amoebicidal to biocompatible structures, Small, 3, 1723–1729.

[8] Ellman, G.L., Courtney, K.D., Anders, V., and Featherstone, R.M., 1961, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem Pharmacol, 3, 88–95.

[9] Isaacson, C.W., Usenko, C.Y., Tanguay, R.L., and Field, J.A., 2007, Quantification of fullerenes by LC/ESI MS and its application to *in vivo* toxicity assays, Anal Chem, 79, 9091–9097.

[10] Jafvert, C.T., and Kulkarni, P.P., 2008, Buckminsterfullerene's (C_{60}) octanol–water partition coefficient (K_{ow}) and aqueous solubility, Environ Sci Technol, 42, 5945–5950.

[11] Jain, A.K., Mehra, N.K., Lodhi, N., Dubey, V., Mishra, D.K., Jain, P.K., and Jain, N.K., 2007, Carbon nanotubes and their toxicity, Nanotoxicology, 1, 167–197.

[12] Kappus, H., 1985, Lipid peroxidation: mechanisms, analysis enzymology, and biological relevance. Oxidative Stress. London: Academic Press. pp 273–310.

[13] Kashiwada, S., 2006, Distribution of nanoparticles in the see through medaka (*Oryzias latipes*), Environ Health Perspect, 114, 1697-1702.

[14] Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., 1951, Protein measurement with the Folin phenol reagent, J Biol Chem, 193(1), 265–275.

[15] Lyon, D.Y., Adams, L.K., Falkner, J.C., and Alvarez, P.J.J., 2006, Antibacterial activity of fullerene water suspensions: Effects of preparation method and particle size, Environ Sci Technol, 40, 4360-4366.

[16] Marklund, S., and Marklund, G., 1974, Involvement of superoxide anion radical in antioxidation of pyrogallol and a constituent assay for superoxide dismutase, Eur J Biochem, 47(3), 469- 474.

[17] Mates, J.M., 2000, Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology, Toxicology, 153, 83–104.

[18] Oberdorster, E., 2004, Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass, Environ Health Perspect, 112, 1058–1062.

[19] Oberdörster, G., 2007, Biokinetics and effects of nanoparticles. In: Nanotechnology – Toxicological Issues and Environmental Safety, Edited by: P.P. Simeonova, N. Opopol, M.I. Luster. NATO Science for Peace and Security Series – C: Environmental Security, Springer Publishers with NATO Public Diplomacy Division, pp 15-51.

[20] Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C., 2004, Translocation of inhaled ultrafine particles to the brain, Inhalation Toxicol, 16, 437-445.

[21] Ohkawa, H., Ohishi, N., and Yagi, Y., 1979, Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction, Anal Biochem, 95(2), 351-358.

[22] Pick, E., and Keisari, Y., 1981, Superoxide anion and H_2O_2 production by chemically elicited peritoneal macrophages-induced by multiple nonphagocytic stimuli, Cell Immunol, 59(2), 301-318.

[23] Rejitha, R., Asifa, K.P., and Chitra, K.C., 2016, Induction of reactive oxygen species in brain of *Etroplus maculatus* after exposure to bisphenol A, J Appl Nat Sci, 8(1), 386-391.

[24] Sayes, C.M., Fortner, J.D., Guo, W., Lyon, D., Boyd, A.M., Ausman, K.D., Tao, Y.J., Sitharaman, B., Wilson, I.J., Hughes, J.B., West, J.I., and Covlin, V.I., 2004, The differential cytotoxicity of water-soluble fullerenes, Nano Lett, 4, 1881–1887.

[25] Sturm, A., da Silva de Assis, H.C., and Hansen, P.D., 1999, Cholinesterases of marine teleost fish: Enzymological characterization and potential use in biomonitoring of neurotoxic contamination, Mar Environ Res, 47, 389–398.

[26] Sumi, N., and Chitra, K.C., 2015, Consequence of fullerene nanoparticle (C_{60}) on oxygen consumption and behavioural modification in *Etroplus maculatus*, Int J Curr Res Rev, 7(14), 40-44.

[27] Sumi, N., and Chitra, K.C., 2016, Effects of fullerene (C_{60}) on antioxidant enzyme activities and lipid peroxidation in gill of the cichlid fish, *Etroplus maculatus* (Bloch, 1795), J Zool Stud, 3(6), 31-37.

[28] Talas, Z.S., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A., and Yilmaz, I., 2008, Antioxidative role of selenium against the toxic effect of heavy metals (Cd2+, Cr3+) on liver of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), Fish Physiol Biochem, 34, 217-222.

[29] Templeton, R.C., Ferguson, P.L., Washburn, K.M., Scrivens, W.A., and Chandler, G.T., 2006, Life cycle effects of single walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod, Environ Sci Technol, 40, 7387–7393.

[30] Usenko, C.Y., Harper, S.L., and Tanguay, R.L., 2008, Fullerene C_{60} exposure elicits an oxidative stress response in embryonic zebrafish, Toxicol Appl Pharmacol, 229, 44–55.

[31] Zhu, X., Zhu, L., Li, Y., Duan, Z., Chen, W., and Alvarez, P.J.J., 2007, Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials buckminsterfullerene aggregates (nC_{60}) and fullerol, Environ Toxicol Chem, 26, 976–979.

[32] Zhu, Y., Ran, T., Li, Y., Guo, G., and Li, W., 2006a, Dependence of the cytotoxicity of multi walled carbon nanotubes on the culture medium, Nanotechnology, 17, 4668–4674.

[33] Zhu, Y., Zhao, Q., Li, Y., Cai, X., and Li, W., 2006b, The interaction and toxicity of multiwalled carbon nanotubes with *Stylonychia mytilus,* J Nanosci Nanotechnol, 6, 1357–1364.

[34] Zhu, Y.P., Fan, J.F., Cheng, Y.Q., and Li, L.T., 2008, Improvement of the antioxidant activity of Chinese traditional fermented okara (Meitauza) using *Bacillus subtilis* b2, Food Control, 19, 654–661.