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Impact of Nonylphenol on Antioxidant System and Acetylcholinesterase Activity in the Brain of *Etroplus Maculatus* (Bloch, 1795)

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ABSTRACT

Nonylphenol, an environmental contaminant, is widely released into the aquatic ecosystem are also known to affect non-target animals. The present study focused on the impact of nonylphenol on the antioxidant system and acetylcholinesterase activity in the brain of *Etroplus maculatus*. Fishes were exposed to sublethal concentrations ($1/5^{th}$ and $1/10^{th}$ of LC_{50}) of nonylphenol for 24, 72 and 96 h. The results showed that nonylphenol treatment significantly ($P<0.05$) increased the activities of superoxide dismutase and catalase, however, glutathione reductase activity was decreased significantly in all treatment groups when compared to the control groups. The level of hydrogen peroxide generation and lipid peroxidation increased significantly ($P<0.05$) in concentration- and time-dependant manner. Acetylcholinesterase activity was used as biomarker to assess the toxicity effect of nonylphenol and it was found that the enzyme activity was decreased significantly at both sublethal concentrations in time-dependant manner, which revealed the neurotoxic effect of the contaminant. The results hence confirmed that nonylphenol caused significant disturbances in the antioxidant enzyme system and acetylcholinesterase activity in the brain of *Etroplus maculatus*. Thus, the current study provides better information on the potential toxic effects of nonylphenol on aquatic animals, especially to fish.

Keywords: Nonylphenol, *Etroplus maculatus*, brain, antioxidant system, lipid peroxidation, acetylcholinesterase

1. INTRODUCTION

In recent past years, several industrial and agricultural chemicals used for various purposes have coupled with multiple mechanisms of action, which often pose a threat to non-target organisms, including aquatic animals and humans. The risk to exposed animals ranged from sublethal to adverse effects, and the toxicity depends upon the sensitivity of the species to the exposed toxicants as well as the physical and chemical properties of the contaminant concerned. Exposure to environmental toxicant cause high risk to aquatic organisms, particularly fish, as most of the toxicants are leached directly into the

aquatic bodies. Fishes are generally used as a bio-indicator to detect the health status of an aquatic ecosystem because chemicals can accumulate into the body of fishes from water, sediment as well as through the food chain.

Nonylphenol, one of the environmental contaminants, is of great alarm in the recent years. Nonylphenol ethoxylates, one of the commonly used non-ionic surfactants, are biodegraded anerobically to highly toxicant product, nonylphenol. Nonylphenol ethoxylates are typically employed in industrial, domestic and industrial cleaning agents, cosmetics, plastics, paints, and also as dispersing agents in pesticides and herbicides (Klecka *et al.,* 2010). Most of the aquatic organisms are highly exposed, consumed and bioaccumulate nonylphenol which are likely passed to the body of higher organisms through food chain (Soares *et al.,* 2008).

Nonylphenol is an endocrine disruptor that possesses an ability to mimic endogenous estrogens and binds with estrogen receptors (Vivacqua *et al.,* 2003). Nonylphenol treatment has been shown to decrease the epididymal sperm count and induced oxidative stress in epididymal sperm of rat (Chitra *et al.,* 2002). Early exposure to nonylphenol has been shown to cause direct and delayed mortalities as well as non-lethal malformations in the embryos of zebrafish, *Danio rerio* (Ali and Legler, 2011). Sublethal concentration of nonylphenol induced genotoxicity as evidenced by the formation of micronucleus along with other nuclear abnormalities such as binucleated cells, fragmented apoptotic and sticky adherent cells in the erythrocytes of freshwater fish, *Oreochromis mossambicus* (Balakrishnan *et al.,* 2014). There are several evidences suggesting that exposure to environmental contaminants initiates peroxidation of free radicals which would ultimately leads to induction of oxidative stress and cell death (Mates, 2000). Free oxygen radicals have been shown to damage almost all macromolecules of the cell or tissues including membrane polyunsaturated fattyacids (PUFA) causing impairment of cellular functions (Halliwell and Gutteridge, 1985).

All living cells maintain a reducing environment by the action of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and peroxidase, thereby prevent free radical mediated cellular damage. Any disturbance in the redox state and exhaustion of antioxidants in the cell by exposure to contaminants lead to oxidative stress and/ or oxidative damage (Bayir, 2005). Brain, the most complex master organ, controls all effector organs of the body because of its structural complexity and functional diversity. For the proper functioning, brain requires high and constant supply of oxygen to meet its energy needs, which in turn generates more free radicals than any other organ. Therefore, brain is highly potential target to generate reactive oxygen species and considered as the most susceptible organ to oxidative stress (Dringen, 2000). The enhanced oxidative stress has been shown to be responsible for neurodegeneration in the brain (Srinivasan, 2002). Therefore, the present study was aimed to focus on the impact of nonylphenol on antioxidant system and acetylcholinesterase activity in the brain tissue of *Etroplus maculatus.*

2. MATERIALS AND METHODS

Etroplus maculatus weighing 7 ± 0.5 g and length 7 ± 1.5 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India. Fishes were acclimatized to the laboratory conditions in well-aerated cement tank (40 L capacity), prior to experiments and were properly dechlorinated. Preliminary test were conducted by maintaining water temperature as 28 ± 2°C, oxygen saturation of water (70 and 100 %), and pH 6.5 to 7.5 using standardized procedures as per APHA guidelines (1998).

Technical grade Nonylphenol, 4-(2, 4-dimethylheptan-3-yl) phenol of 97% purity was purchased from SISCO Research Laboratories Pvt. Ltd., Mumbai, India. Malondialdehyde, NADPH, glutathione oxidized, thiobarbituric acid, pyrogallol, acetylthiocholine iodide and dithiobisnitrobenzoic acid were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

After acclimatization, adult healthy fishes were selected for the experiment and they were maintained in different tanks, each group with 10 fishes. Nonylphenol was dissolved in 1% DMSO; therefore, it is used as a solvent (vehicle) control in the experiment. The median lethal concentration (LC_{50}-96 h) of nonylphenol in *E. maculatus* was determined in our laboratory by using probit analysis, which is 890 µg/ L (Asifa *et al.,* 2016). Two sublethal concentrations, such as one-fifth (178 µg/ L) and one-tenth (89 µg/ L) of LC_{50} of nonylphenol for three durations ie., 24, 72 and 96 h were sustained.

At the end of every experiment, fish was caught very gently using a small dip net, one at a time with least disturbance, weighed and decapitated. Brain tissue was dissected out from both control and treatment groups and stored at 4ºC until the biochemical analysis were performed. A 1% (w/ v) homogenate of whole 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 analysis.

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), level of hydrogen peroxide generation (Pick and Keisari, 1981), level of lipid peroxidation (Ohkawa *et al.,* 1979) and the activity of acetylcholinesterase (Ellman *et al.,* 1961) were measured in 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 group. Data are presented as mean ± SD for ten animals per group and all biochemical estimations were carried out in duplicate.

3. RESULTS

In the current study the data obtained for solvent-free and solvent (vehicle) control groups showed no noticeable differences. Nonylphenol exposure at two sublethal concentrations

showed significant (P<0.05) increase in the activity of superoxide dismutase and catalase in the brain of fish when compared with the corresponding control groups (Figs. 1 and 2). However, the activity of glutathione reductase was decreased significantly (P<0.05) in all the treated groups in time-dependant manner (Fig. 3). Nonylphenol exposure leads to a significant (P<0.05) increase in the level of hydrogen peroxide generation and lipid peroxidation at both sublethal concentrations in time-dependant manner than that of control fishes (Figs. 4 and 5). The activity of acetylcholinesterase showed significant (P<0.05) decrease in concentration and time-dependant manner in response to nonylphenol exposure (Fig. 6).

Figure 1

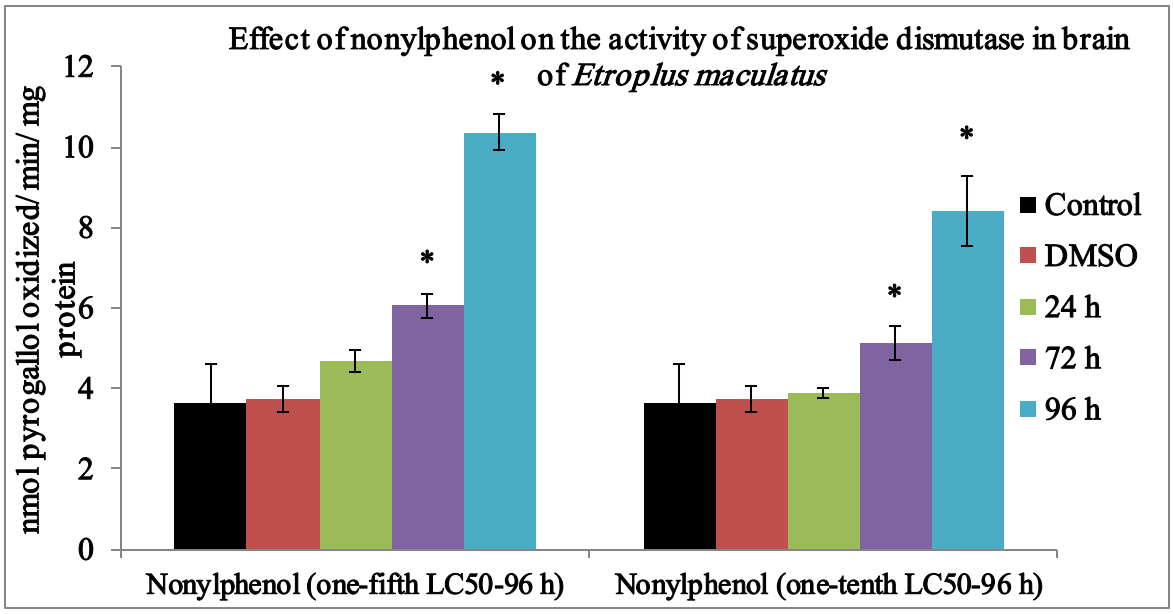
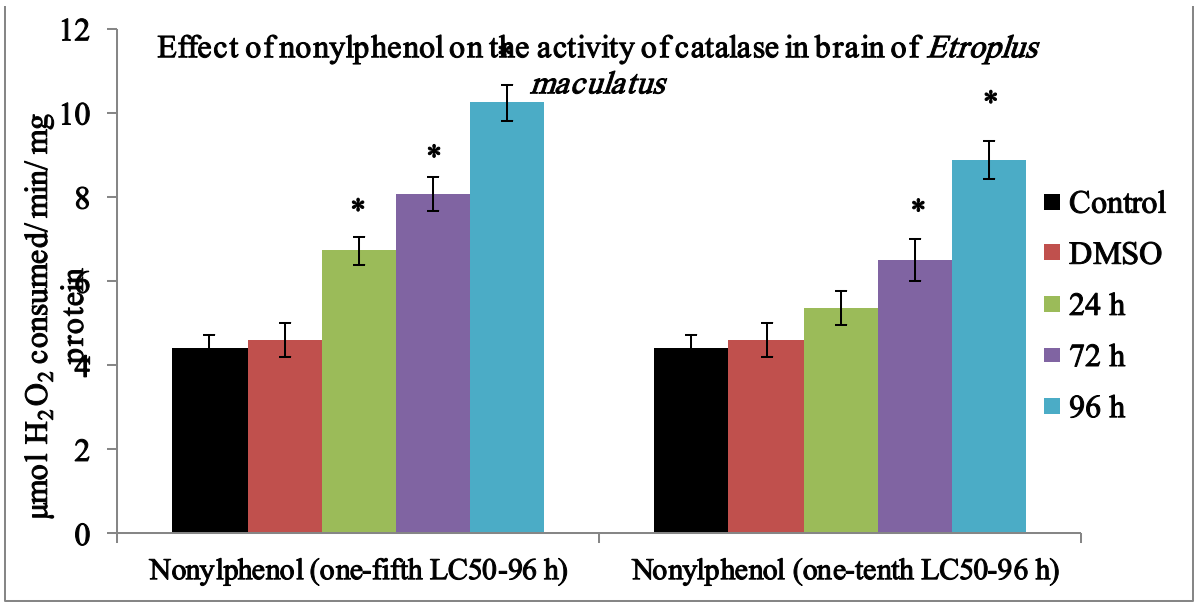


Figure 2



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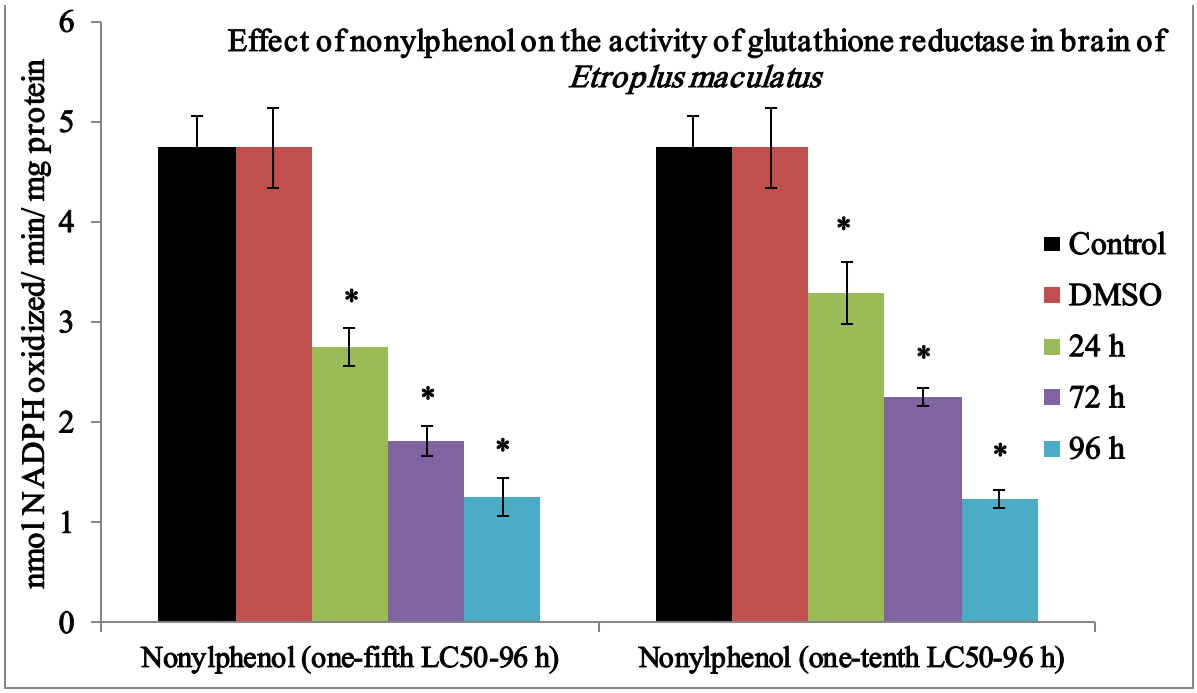

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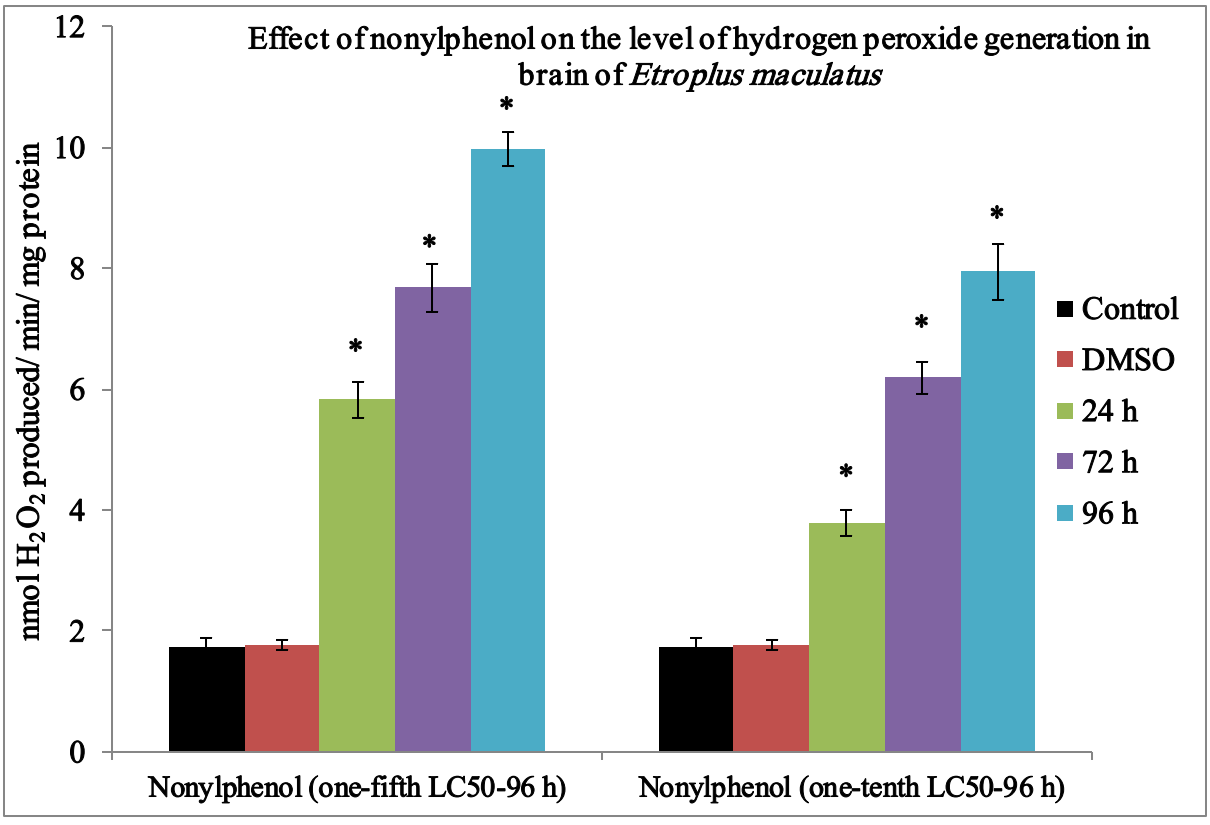

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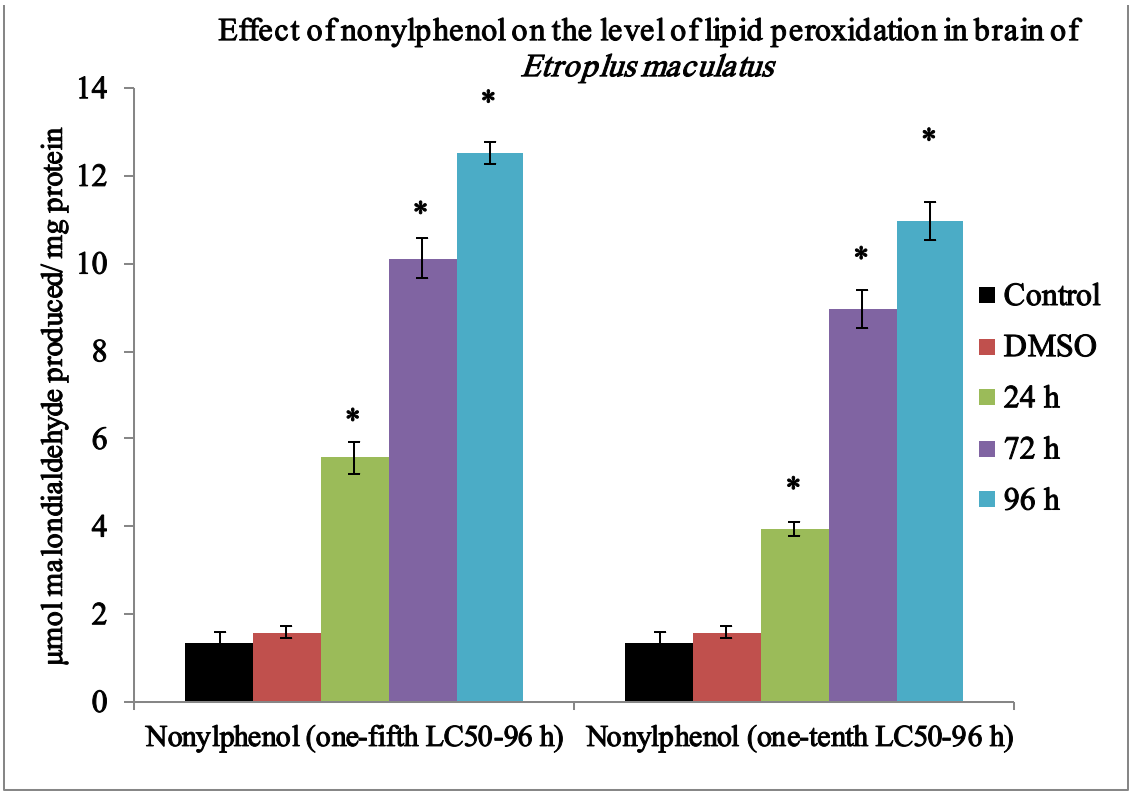
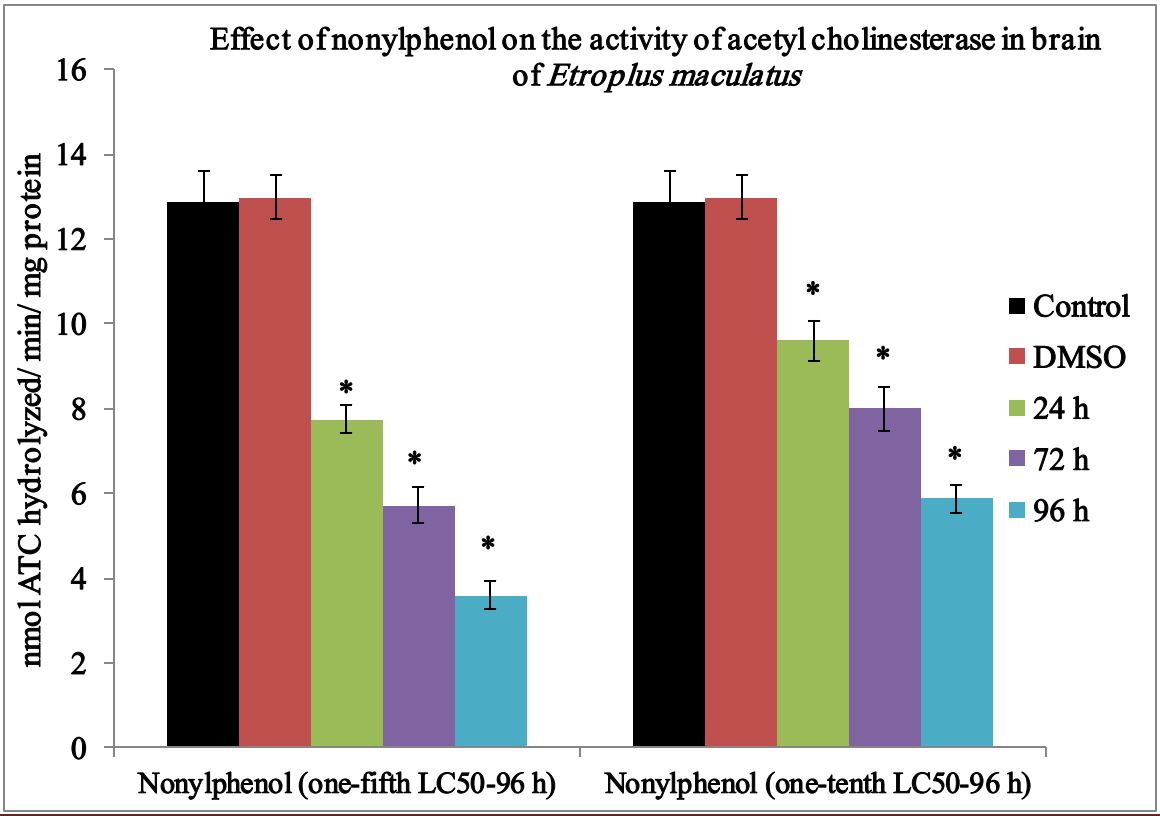


Figure 6



4. DISCUSSION

Most of the aquatic organisms possess variety of endogenous defensive mechanisms within the body like detoxification, antioxidant protection, excretion and stress responses in order to survive in the contaminated environment (Franco *et al.,* 2006). Oxidative stress and antioxidant parameter are the most commonly used potential biomarkers of environmental contamination. Superoxide dismutase is one of the major antioxidant enzymes, which provide the first line of defence against free radicals by catalysing the dismutation of superoxide anion radical to hydrogen peroxide (H_2O_2) and oxygen. The H_2O_2 generated is a powerful membrane permeate oxidant that has to be quickly eliminated from the cell otherwise leads to the induction of oxidative damage to lipids, proteins and DNA. The elimination of H_2O_2 is either brought about by the activity of catalase or glutathione reductase/ peroxidase enzyme systems (Hermes-Lima, 2004).

In the present study nonylphenol exposure caused induction of both superoxide dismutase and catalase activities in the brain of *Etroplus maculatus*. The increased activity of catalase is an indication of animal's own effort to fight against the generation of hydrogen peroxide due to the exposure to nonylphenol. It is an adaptive response of brain to reduce the oxidative stress caused by nonylphenol exposure. However, nonylphenol treatment significantly decreased the activity of glutathione reductase, which reflects the inability of brain tissue to regenerates reduced glutathione from its oxidized form, which was required for the functioning of glutathione peroxidase or failure in eliminating hydrogen peroxide from the cell.

Nonylphenol exposure at both sublethal concentrations significantly increased the levels of lipid peroxidation and hydrogen peroxide in the brain in time-dependent manner. Previous study in our laboratory reported that bisphenol A and nonylphenol at acute sublethal concentrations enhanced the production of hydrogen peroxide and lipid peroxidation in the muscle tissues of *Etroplus maculatus* (Thulasi *et al.,* 2015; Asifa and Chitra, 2016). Hydrogen peroxide produced as a result of oxidative stress is known to cause damage to cell membranes, especially membrane lipids, proteins and nucleic acids (Kellogg and Fridovich, 1975). Free radicals generated through oxidative stress leads to a chain reaction called lipid peroxidation. Aldehydes produced as a result of lipid peroxidation forms the DNA adducts and lipid hydroperoxides, which has been reported to cause extensive single and double strand breaks in DNA (Devipriya *et al.,* 2008).

Acetylcholinesterase (AChE) activity is usually used as a biomarker of toxicant exposure. Normally the enzyme catalyses the breakdown of neurotransmitters like acetylcholine to terminates the synaptic transmission. The activity of the enzyme is particularly important for several physiological functions, such as prey location, predator evasion and orientation towards the food (Miron *et al.,* 2005). In the present study, acetylcholinesterase activity in the brain tissue was gradually decreased with

increase in concentrations of nonylphenol. Inhibition of AChE activity was reported in the brain of *Etroplus maculatus* when exposed to 648 µg/ L bisphenol A for short-term exposures (Rejitha *et al.,* 2016).

5. CONCLUSION

The results of the present study demonstrated that nonylphenol is highly toxic to *Etroplus maculatus* at acute sublethal concentration which is evidenced by the alteration in antioxidant defense system in the brain tissue. Therefore, indiscriminate use of nonylphenol derivatives should be controlled in order to conserve the population of fishes and other organisms in natural aquatic ecosystem.

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