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SHORT-TERM EXPOSURE TO NONYLPHENOL ALTERED MUSCULAR ANTIOXIDANT SYSTEM IN CICHLID FISH, *ETROPLUS MACULATUS* (BLOCH, 1795)

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ABSTRACT

Indigenous adult freshwater cichlid fish, *Etroplus maculatus* was used in the present study in order to assess the short-term effects of one of the environmental contaminants, nonylphenol in muscular tissue of fish. Treatment groups were exposed to nonylphenol at sublethal concentrations (one-fifth and one-tenth of LC_{50}) for short-term duration, ie., 24, 72 and 96 h. Antioxidant parameters were analysed in muscle tissues of both control and treatment groups. Superoxide dismutase activity was significantly ($P<0.05$) increased at both concentrations in time-dependent manner when compared to control groups. However, the activities of catalase and glutathione reductase decreased significantly ($P<0.05$) with concomitant increase in the levels of hydrogen peroxide generation and lipid peroxidation in time-dependent manner at both sublethal concentrations of nonylphenol. In addition the activity of one of the marker enzymes, alkaline phosphatase showed significant ($P<0.05$) reduction than that of control groups. The present observations clearly indicate that short-term exposure to nonylphenol altered muscular antioxidant system in fish and this could probably induced oxidative stress in muscle tissues of *Etroplus maculatus*.

KEY WORDS: Nonylphenol, muscle, *Etroplus maculatus*, oxidative stress, ROS.

INTRODUCTION

Nonylphenol is a toxic environmental contaminant possessing estrogenic properties that are principally the degradation product of nonylphenol ethoxylates. Nonylphenol is known to accumulate in the environmental compartments with high organic content, such as sewage

sludge and river sediments. The persistence of nonylphenol in the environment is mainly due to its physico-chemical properties, for instance low solubility and high hydrophobicity. Anthropogenic activities due to the extensive use of the toxicants in industrial, institutional, commercial and household appliances such as detergents, emulsifiers, wetting and dispersing agents, antistatic agents, demulsifiers and solubilisers are few major sources of nonylphenol exposure in the environment.[1,2]

Nevertheless, the concentration of nonylphenol contamination in the environment is considered to be low where the most detected concentration range of nonylphenol in freshwater ranged from 0.00001 to >0.1mg/ ml.[3] Nonylphenol is mainly found in the aquatic environment as streams, rivers, lakes, estuaries and oceans and appears to be closely related with the discharge of effluents from sewage treatment plants, proximity of industrialized/urban areas and other related anthropogenic activities such as storm water discharges and run-off.[4] Humans are exposed to nonylphenol through drinking water apart from cleaning products and various skin care products, which has been estimated to be more important since the concentrations of nonylphenol are several orders of magnitude higher than in drinking water.[5]

Nonylphenol has been shown to mimic the natural hormone, 17β-estradiol, a natural female hormone, that maintain development and maintenance of female sex characteristics, maturation and function of accessory sex organs.[6] Nonylphenol not only disrupt the endocrine system, several literatures have reported that nonylphenol is highly toxic thereby causing developmental, behavioural, immunological, cytological, genetical and reproductive modifications to the exposed organisms. Due to the demonstrated aquatic toxicity, estrogenic properties and its persistence in the environment, Environmental Protection Agency (EPA) has prepared a guideline for ambient water quality that recommends nonylphenol concentrations in freshwater to be below 6.6 μg/L and, in saltwater, below 1.7 μg/L.[7] However, many other countries, including China, India and several South American countries use and produce nonylphenolic compounds in large amounts and no action has been taken to reduce or eliminate their usage.[8]

An emerging recent trend in the field of ecotoxicology is to evaluate the role of environmental contaminants in the production of reactive oxygen species (ROS). All biological systems are constantly exposed to intrinsic and extrinsic sources of free radicals and reactive oxidants. The partial reduction of oxygen during oxidative phosphorylation has

been known to generate ROS. Singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radicals are the most common ROS and these pro-oxidants are counteracted by endogenous antioxidant defense system in the cell/ tissue. Whenever there is imbalance of pro-oxidants and antioxidants in cells/ tissues of the organism leads to oxidative stress.

It is well-known that major portion of the body of an animal is composed of muscles. In fishes, the strong movement of the animal in water is modulated by the muscular system. Exposure of any toxicants ultimately accumulates in the muscles, which is the major body mass. The present study was, therefore, aimed to evaluate if short-term exposure to one of the environmental contaminants, nonylphenol alter the muscular antioxidant system in cichlid fish, *Etroplus maculatus.*

MATERIALS AND METHODS

Animal

Cichlid fish, *Etroplus maculatus* weighing 7 ± 0.5 g and the length 7 ± 1.5 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India. Fishes were acclimatized to the laboratory conditions prior to experiments by exposing the animal to constant supply of dechlorinated water and good lighting system and were maintained in well-aerated aquarium tanks (40 L capacity).

Preliminary tests

The physico-chemical features of the tap water were estimated as per APHA [9]. Standard water temperature (28 ± 2°C), oxygen saturation of water (70 and 100 %), pH (6.5 to 7.5) was maintained throughout the experiment in both control and treated groups.

Chemicals

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 and p-nitrophenol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Treatment

After acclimatization for 2 weeks, fishes were housed in different tanks for the experiment maintaining ten animals per group. Nonylphenol was dissolved in 1% DMSO and therefore used as a solvent (vehicle) control in the experiment. Previous studies from our laboratory determined the median lethal concentration (LC_{50}-96 h) of nonylphenol in *E. maculatus* by using probit analysis, which is 890 µg/ L.[10] In the treatment groups, two sub-lethal concentrations such as one-fifth (178 µg/ L) and one-tenth (89 µg/ L) of LC_{50}-96 h concentrations were used. Treatment was given for 24, 72 and 96 h maintaining negative and positive controls and the experiment designed as follows:

Control Groups: Group 1 – Solvent-free; Group 2 – With solvent (1% DMSO)

Treatment Groups:

Nonylphenol at one-fifth of LC_{50}-96 h (178 µg/ L)

Group 3 – maintained for 24 h

Group 4 – maintained for 72 h

Group 5 – maintained for 96 h

Nonylphenol at one-tenth of LC_{50}-96 h (89 µg/ L)

Group 6 – maintained for 24 h

Group 7 – maintained for 72 h

Group 8 – maintained for 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. Muscles were dissected and stored at 4º C until the biochemical analyses were performed. A 1% (w/ v) homogenate of muscle 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 4º C to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry et al[11] with BSA as the standard. Activity of superoxide dismutase[12], catalase[13], glutathione reductase[14], level of hydrogen peroxide generation[15], level of lipid peroxidation[16] and alkaline phosphatase [17] were measured in crude homogenate.

Statistical analyses

Statistical analyses 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. All biochemical estimations were carried out in duplicate.

RESULTS

Exposure of *E. maculatus* to nonylphenol for 96 h resulted in significant (P<0.05) increase in the activity of superoxide dismutase at both sublethal concentrations in time-dependent manner when compared to control groups (Fig. 1). However, the activities of catalase and glutathione reductase decreased significantly (P<0.05) in all treatment groups (Figs. 2 and 3). There was a significant (P<0.05) increase in the levels of hydrogen peroxide generation and lipid peroxidation in time-dependent manner at both sublethal concentrations of nonylphenol exposure (Figs. 4 and 5). Besides the activity of one of the marker enzymes, alkaline phosphatase showed significant (P<0.05) reduction than that of control groups (Fig. 6).

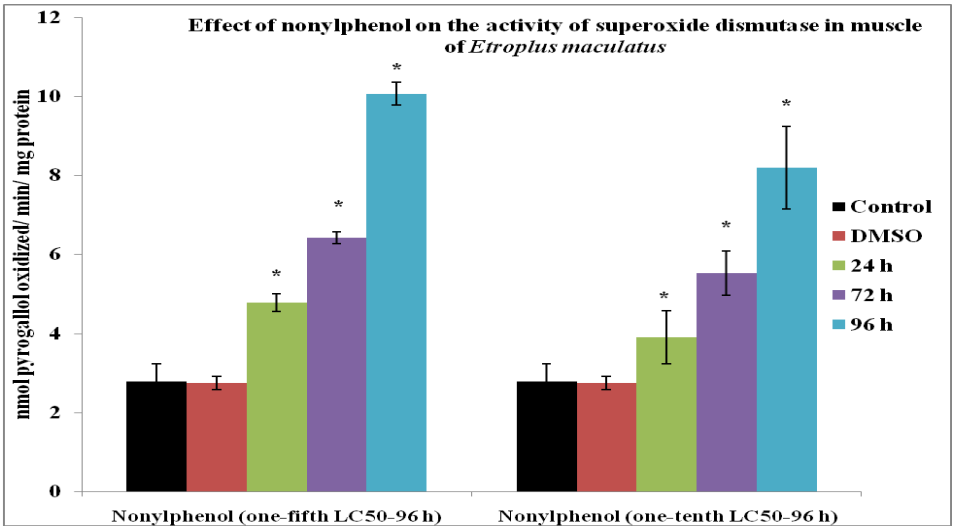
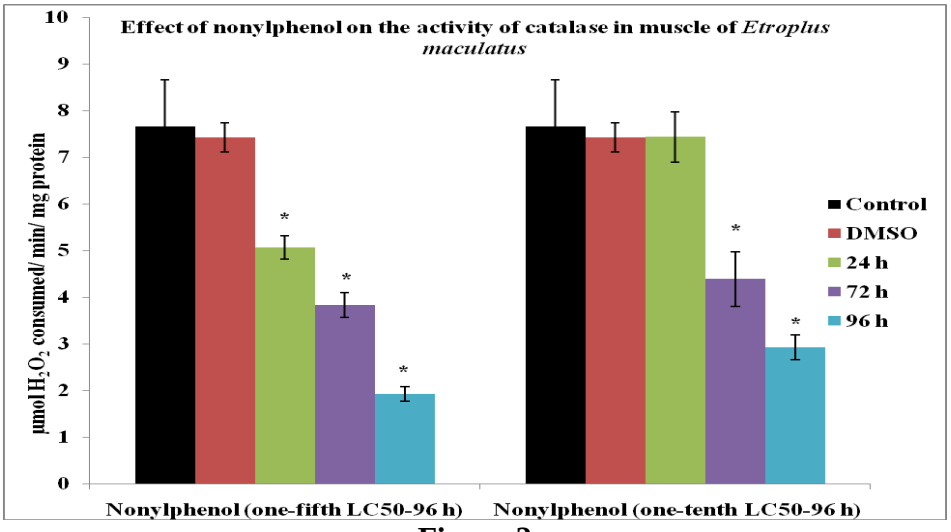


Figure 1



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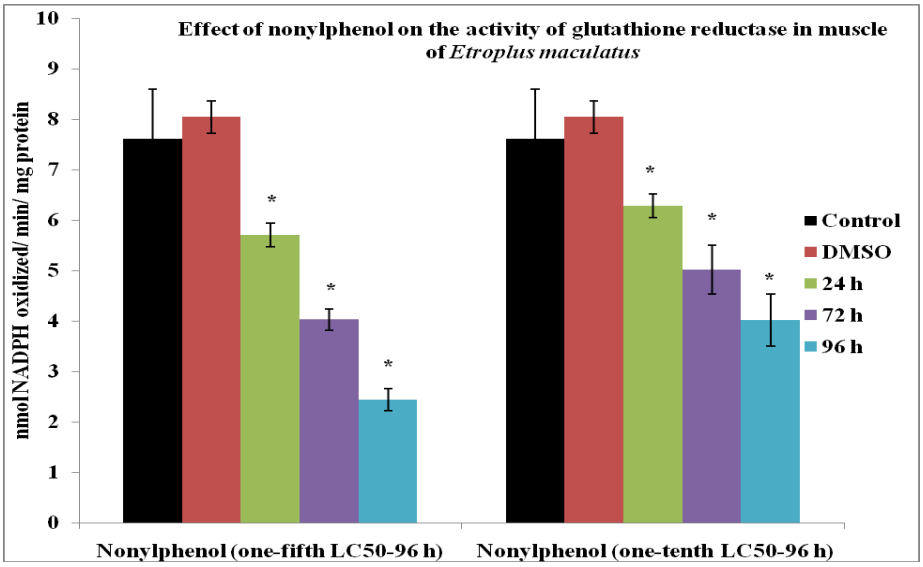

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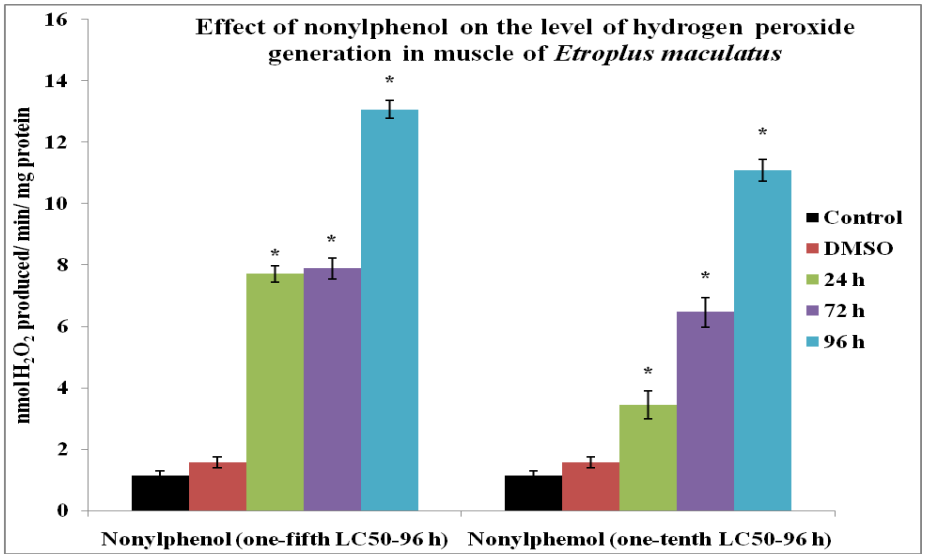

Figure 4

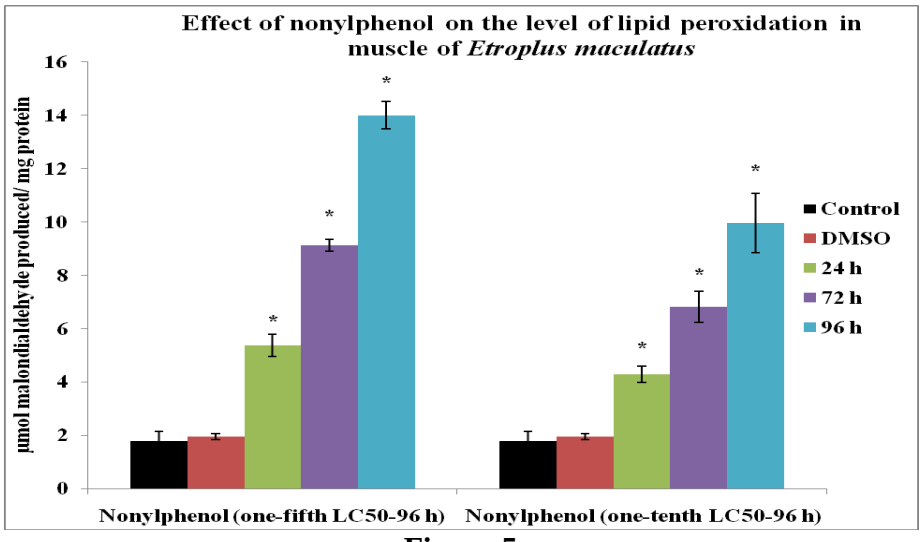

Figure 5

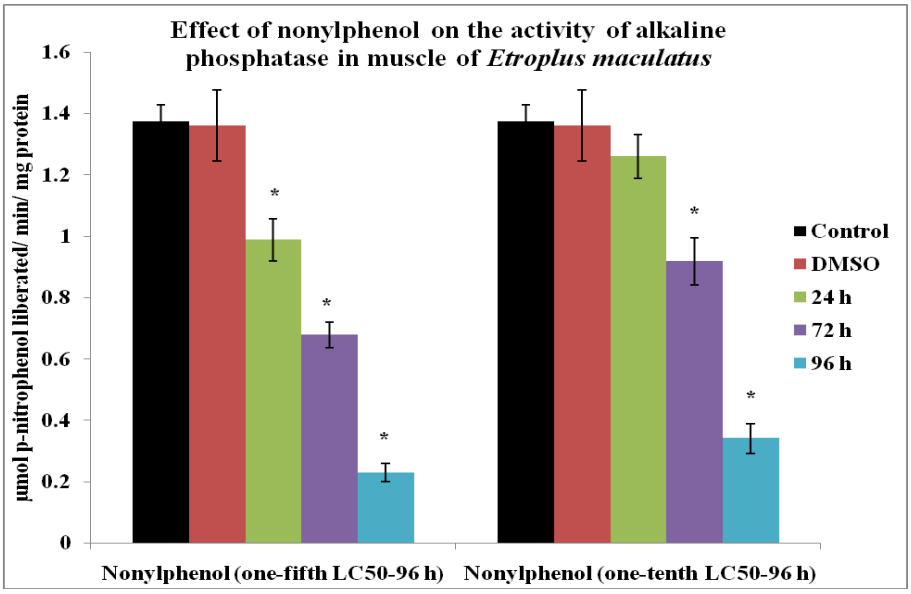

Figure 6

DISCUSSION

The present study demonstrates that the short-term exposure to nonylphenol altered muscular antioxidant system in cichlid fish, *Etroplus maculatus.* Environmental contaminants are the important sources of reactive oxygen species production in the biological system. Aquatic organisms are more sensitive to the exposure of toxicants and several evidences prove that the increased production of reactive oxygen species and exposure of environmental contaminants are positively correlated. Cells/ tissues are equipped with well developed enzymatic and non-enzymatic antioxidant defensive mechanism to overcome the cellular components from the oxidative stress. Superoxide anion and hydroxyl radicals have been known to cause oxidative damage to important cellular biomolecules.[18] The assay of endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase can be served as biomarkers of oxidative stress. The present observation showed a significant increase in the activity of superoxide dismutase after nonylphenol exposure in muscle tissue.

Superoxide dismutase converts the superoxide radical into hydrogen peroxide which is then reduced to water and oxygen by catalase. The increase in the activity of superoxide dismutase could be due to nonylphenol toxicity in order to eliminate superoxide radical. The elevated enzyme activity in nonylphenol exposed fish may be due to the compensatory response of the animal within its tolerance range to overcome nonylphenol-related stress. However, the decrease in the activities of catalase and glutathione reductase reflects the failure of

elimination of the produced hydrogen peroxide. It was shown by the increase in the level of hydrogen peroxide at both sublethal concentrations of nonylphenol treatment in time-dependent manner. Hydrogen peroxide is generally considered as cytotoxic agent because of their ability to induce lipid peroxidation in tissues and cell membrane.

Lipid damage can occur due to oxidative stress or disruption in the balance between pro-oxidant and antioxidant thereby alters lipid structure and function.[19] Membranes of muscle tissues in fish are well equipped with high degree of polyunsaturated fatty acids (PUFA), which are more susceptible to the oxidative stress. Malondialdehyde is the well characterized oxidation product of PUFA, which is measured for observing the level of lipid peroxidation.[20] The present results showed that nonylphenol exposure significantly increased the level of lipid peroxidation in muscle tissues at concentration-dependent and time-dependent manner.

Alkaline phosphatase, a stress marker hydrolytic enzyme, present in almost all tissue of an organism are hydrolytic enzyme released by lysosome involved in the hydrolysis of monophosphate esters, carbohydrate metabolism, growth and differentiation, protein synthesis and transport of phosphorylated intermediates across the cell membranes.[21] In the present study, time and concentration-dependent decrease in the activity of alkaline phosphatase in muscle after nonylphenol exposure may be due to the decreased transmembrane transport. Therefore, fishes could not overcome the toxic stress due to nonylphenol exposure as the decreased activity of alkaline phosphatise indicate disturbed membrane transport system and distressed structural integrity of myocytes. The consequences of the present observation provide a clear conclusion that measurement of oxidative stress markers in fish show suitable evaluation of health status of fish against exposed toxicant.

CONCLUSION

Short-term exposure to nonylphenol altered the antioxidant defense system in muscle of cichlid fish, *Etroplus maculatus*. Imbalance in the antioxidant system is due to the sensitive response of fish against the toxicant-exposed environment.

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