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Response of antioxidant defense system in the muscle tissue of *Oreochromis niloticus* after exposure to octylphenol

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Abstract

The present study was designed based on the hypothesis that if exposure to octylphenol could alter the antioxidant defense system in the muscle tissue of the fish, *Oreochromis niloticus*. Fish were exposed to sublethal concentration of octylphenol i.e., 50.6 µg/L for 24, 48, 72 and 96 h durations along with control groups. Octylphenol treatment showed significant ($P<0.05$) reduction in the activity of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase in time-dependent manner when compared to the corresponding control groups. However, the levels of lipid peroxidation and hydrogen peroxide generation showed a significant ($P<0.05$) increase in all treatment groups and this could be due to generation of free radicals in muscle tissues. The activity of acetylcholinesterase showed significant ($P<0.05$) decrease after 48 h of octylphenol exposure in muscle tissue indicating defect in the neurotransmission at neuromuscular junction. Octylphenol exposure showed alteration in histomorphology of muscle tissues as evidenced by degenerated muscle tissue with split muscle fibres after 24 and 48 h. Complete disorganization of muscle fibres after 72 h, whereas thickened and shortened muscle bundles noted after 96 h of octylphenol treatment. Therefore, the present study suggests that octylphenol altered the antioxidant defense system and also affects the histomorphology of muscle tissues in the freshwater fish, *Oreochromis niloticus*.

Keywords: Octylphenol, antioxidant, oxidative stress, muscle, *Oreochromis niloticus*

1. Introduction

In recent years, freshwater ecosystem is facing a harsh loss of biodiversity that includes climate alterations, nutrient swings, acidification, habitat loss, exploitation, and biological invasions. Apart from this, the main source of water pollution is the discharge of solid or liquid waste products containing chemical contaminants. It includes waste released from industries, agricultural byproducts and sewage waste from domestic households. Increased use of pesticides results in the excess inflow of toxic chemicals into the aquatic ecosystem. Contamination of water with large amounts of pesticides leads to mortality of fish or starvation due to destruction in other food organism in food web. Moreover, many toxicants have been shown affecting the growth parameters and reproduction of fish with evidence of tissue damage. The effects of these chemicals are not only restricted to the aquatic organisms but also affect organisms of higher trophic levels directly or indirectly by means of bioaccumulation and biomagnification through the food chain.

Alkylphenols are a family of organic compounds obtained by alkylation of phenols with alkenes. The long-chain alkylphenols are extensively used as precursors to the detergents, as additives for fuels and lubricants, polymers and as components in phenolic resins. Alkylphenols are xenobiotic compounds that possess the property of weak endocrine disruption (Blackburn *et al.*, 1999) [1]. Octylphenol represents a large number of isomeric compounds, which is branched in a variety of ways or a straight chain located at either 2-, 3- or 4-position of the benzene ring. Of these, 4-*tert*-octylphenol is the most potential isomer having commercial importance. Octylphenol is an organic compound used to manufacture alkylphenol ethoxylates and it is widely used in rubber, pesticides and paints. Octylphenol is released into the environment mainly from the industrial wastes and also possibly from the breakdown of alkylphenol ethoxylates (Klecka *et al.*, 2008) [2]. There is an increasing concern on the exposure of several environmental contaminants to the natural ecosystem.

It not only affects the ecosystem, but also the flora and fauna of the environment. Octylphenol can be transformed both in organisms and in the environment into other metabolites by hydrolysis, oxidation or reduction.

In medaka, octylphenol inhibited spermatogenesis and induced feminization in fish (Gray *et al.*, 1999) [3]. Besides, octylphenol also caused several developmental, hormonal, reproductive, hematological and neuronal abnormalities in different groups of animals in aquatic ecosystem. The toxicity of octylphenol is, therefore, mediated through different toxicological pathways owing to the estrogenicity or lipophilic nature of the toxicant. Octylphenols are metabolized into several metabolites that have more toxic potential than the parent compound, which may either enter into the enterohepatic circulation, or eliminated in feces or get bioaccumulated in the body tissues to elicit the toxicity response (Klecka *et al.*, 2008) [2].

Several environmental contaminants were reported to imbalance pro-oxidant and antioxidant status in cells resulting in the production of reactive oxygen species. The widespread use of octylphenol has been associated with adverse effects on non-target animals including fish. Fish are used as bio-indicator of aquatic ecosystem, so exposure to any chemicals in the environment can be reflected in fish model system. Therefore, fish has become an increasingly popular model in ecotoxicological studies. Research in fish has demonstrated that mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress. The purpose of the present study was to evaluate the response of antioxidant defense system in the muscle tissue of the fish, *Oreochromis niloticus* when exposed to sublethal concentration of octylphenol. The endpoint of octylphenol toxicity in muscle tissue was evaluated by assessing the activity of antioxidant defensive enzymes. In addition, histopathological investigation was also performed to understand the direct effects of any toxicants within target organs of fish in laboratory condition.

2. Materials and Methods

2.1 Animal

Freshwater fish, *Oreochromis niloticus* weighing 14 ± 1 g and length 8 ± 1.5 cm were collected from the fish farm, Aquafish Aquarium, B.H. Road, Kottakal, Malappuram District. Fishes were acclimatized to the laboratory conditions for four weeks with constant supply of dechlorinated water and good lighting system (12h light: 12h dark) in well-aerated tubs (40 L capacity). The physico-chemical features of the tap water were estimated as per APHA [4]. Water temperature (28 ± 2 °C), oxygen saturation of water (70 and 100%), pH (6.5 to 7.5) were maintained and monitored using a standardized procedures.

2.2 Chemicals

Octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol) of 90% purity was obtained from SISCO Research Laboratories Pvt. Ltd., Mumbai, India. Malondialdehyde, NADPH, glutathione oxidized, thiobarbituric acid, pyrogallol and dithiobisnitrobenzoic acid were obtained from Himedia Laboratories, Mumbai, India. Acetylthiocholine iodide was obtained from Alfa Aesar, England. All other chemicals were of analytical grade and obtained from local commercial sources.

2.3 Experimental design

One-tenth of median lethal concentration of octylphenol i.e., $50.6 \mu\text{g/L}$ was selected as sublethal concentration. The test concentration was maintained for four durations i.e., 24, 48, 72 and 96 h, respectively along with control fishes, positive and negative controls. Single concentration with different durations was used in present study and ten fish specimens were used for every test and also in control groups. The first group of fishes was maintained in toxicant-free water and was used as negative control and the second group was treated with vehicle (1% DMSO) and served as positive control. The third group was treatment groups exposed to octylphenol at $50.6 \mu\text{g/L}$ concentration for 24, 48, 72 and 96 h, respectively.

2.4 Preparation of tissue samples for biochemical analysis

The fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure time, fishes were decapitated. Muscle of both control and all treated groups were dissected and stored at 4 °C until the biochemical analyses were performed. A 1% (w/v) homogenate of muscle 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 800 g for 15 min at 4 °C to obtain the supernatant, which was then used for the analyses. Total protein concentration in the tissue was estimated by the method of Lowry *et al.* [5]. The levels of hydrogen peroxide generation [6] and lipid peroxidation [7] were measured in the supernatant of crude homogenate. Activities of antioxidant enzymes such as superoxide dismutase [8], catalase [9], glutathione reductase [10] and acetylcholinesterase [11] were also assayed.

2.5 Histopathology

At the end of treatment, muscle tissue from control and treatment groups were dissected, rinsed in physiological saline to remove blood and debris and fixed in 10% buffered formalin for 24h. Tissues were dehydrated in ascending grades of alcohol and were cleared in xylene till the tissues become translucent. Tissues were then transferred to molten paraffin wax for an hour for complete impregnating with wax. The tissue blocks were made then tissues were cut in sections of thickness 4 to 6 microns using rotary microtome. The sections were double stained with haematoxylin and eosin and mounted in DPX [12]. The slides were carefully examined and photographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioscope-2 plus Trinocular Research Microscope.

2.6 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 the control groups. Data are presented as mean \pm SD for ten animals per group. All biochemical estimations were carried out in duplicate.

3. Results and Discussion

As a consequence of cellular activity, inside all living organisms, the reactive oxygen species (ROS) or free radicals are naturally and constantly formed. Free radical is

an atom that consists of at least, an unpaired highly reactive electron found in the most outside shell. Free radicals in which oxygen is involved are named reactive oxygen species, which includes superoxide anion, hydroxyl radical, singlet oxygen and hydrogen peroxide. ROS are important regulators of growth, proliferation, differentiation, and adaptation of cells or tissues. However, the imbalance between the physiological production of free radicals and the cells ability to remove those results in a condition called oxidative stress. The cells possess the ability to neutralize the activity of ROS formed by different mechanisms, such as by preventing ROS-generating electron leakage, scavenging ROS by enzymatic activities of superoxide dismutase, catalase, glutathione reductase/ peroxidase system, and by low molecular weight antioxidant species, such as vitamin E, vitamin C, glutathione, or by removal of ROS damaged molecules (Birben *et al.*, 2012) [13]. ROS production is also associated with damage to cellular functions and the intensity of tissue damage depends on the nature of ROS formed. Besides, damages induced by oxidative stress are connected with alteration in physiological activities, metabolism and various diseases. There are increasing evidence that exposure to environmental contaminants impairs the pro-oxidant/antioxidant balance thereby increasing free radical production and decreasing the levels of antioxidant. The present study evaluated the response of antioxidant defense system in the muscle tissue of *Oreochromis niloticus* after exposure to octylphenol for 96 h.

Octylphenol treatment showed significant ($P < 0.05$) reduction in the activity of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase in time-dependent manner when compared to the corresponding control groups (Figs. 1-3). The increase in reactive oxygen and/or nitrogen species in contracting skeletal muscle was first described in the 1980s (Davies *et al.*, 1982) [14] and involves the generation of superoxide and nitric oxide radicals with the formation of secondary reactive oxygen or nitrogen species (Powers and Jackson, 2008) [15]. Oxidative phosphorylation release superoxide radical, which is first converted to hydrogen peroxide and then further reduced to give water. It is important detoxification pathway with the involvement of several enzymes, where superoxide dismutase (SOD) catalyses the first reaction. It is followed by the catalytic activities of other enzymes as catalase and various peroxidases or reductases removing hydrogen peroxide. In the present study, octylphenol exposure resulted in the decreased activities of antioxidant enzymes indicating the failure of antioxidant defense system to remove hydrogen peroxide generated. This is further proved by a significant ($P < 0.05$) increase in the level of hydrogen peroxide after octylphenol treatment (Fig. 4). Hydrogen peroxide is a non-radical ROS having long half-life, permitting its diffusion both within a cell and across cell membranes (Powers *et al.*, 2010) [16]. In addition, hydrogen peroxide also reacts with many different compounds and functions as an important signaling

molecule (Powers *et al.*, 2011) [17]. Therefore, all reducing groups of cellular macromolecules are targeted by ROS and among them the most susceptible macromolecule is lipids. Thus ROS generation has been known to attack polyunsaturated fatty acid lipid residues thereby leading to alteration in the properties of cellular membrane (Trachootham *et al.*, 2008) [18]. The present study proves the above fact by the increase in the level of lipid peroxidation after octylphenol exposure in time-dependent manner (Fig. 5). The result suggests that octylphenol induced oxidative stress by altering the function of antioxidant defense system in muscle tissue. Similar results have been observed when fullerene C₆₀ nanoparticles exposed to cichlid fish, *Pseudotropheus maculatus* (Sumi and Chitra, 2017a) [19].

Acetylcholinesterase is an enzyme concentrated at neuromuscular junctions, which are classified as amphiphilic and non-amphiphilic according to their hydrophobic interactions and as homomeric and heteromeric according to their quaternary structure (Massoulié *et al.*, 1993) [20]. Acetylcholinesterase is a neurotransmitter involved in the transmission of nerve impulse across the neuromuscular junction and cholinergic brain synapses. Exposure to octylphenol at sublethal concentration significantly ($P < 0.05$) decreased the activity of acetylcholinesterase in muscle tissue after 48 h (Fig. 6). Thus the present result indicates that octylphenol inactivated the action of enzyme and proved as acetylcholinesterase inhibitor. The enzyme inactivation induced by octylphenol could lead to acetylcholine accumulation and finally disrupt neurotransmission.

There is growing evidence demonstrating that induction of oxidative stress has been shown to direct muscle cells into a catabolic state and accelerates muscle atrophy (Moyle and Reid, 2007) [21]. The alteration in the morphology of muscle tissue was thus evaluated by using histopathology as endpoints that provide complete assessment of the health of organisms and also monitor the effects of pollutants in the aquatic ecosystem. Muscle tissue is inevitable for the motion of the animal and is widely distributed throughout the body. Control tissue showed normal histoarchitecture having clear individual myotomes with smooth myofibrils separated by myoseptum (Fig. 7). Octylphenol exposure showed alteration in histomorphology of muscle tissues as evidenced by degeneration of muscle tissue with split muscle fibres after 24 and 48 h. Complete disorganization of muscle fibres were observed after 72 h, whereas thickened and shortened muscle bundles noted after 96 h of octylphenol treatment (Fig. 7). Muscle is a very sensitive tissue easily affected by the exposure to pollutants and the marked histopathological changes observed after octylphenol exposure reveals that induction of oxidative stress could be one of the reasons for severity of muscle damage. Similarly, exposure to fullerene C₆₀ nanoparticles has been shown to cause disorganized muscle fibres with irregular or absence of nucleus in muscle tissues of the freshwater fish, *Pseudotropheus maculatus* (Sumi and Chitra, 2017b) [22].

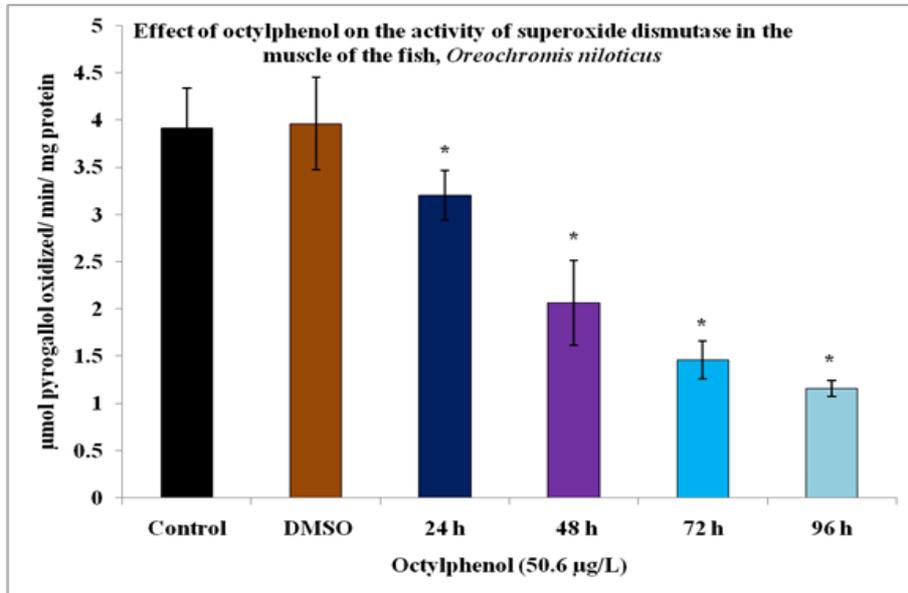


Fig 1

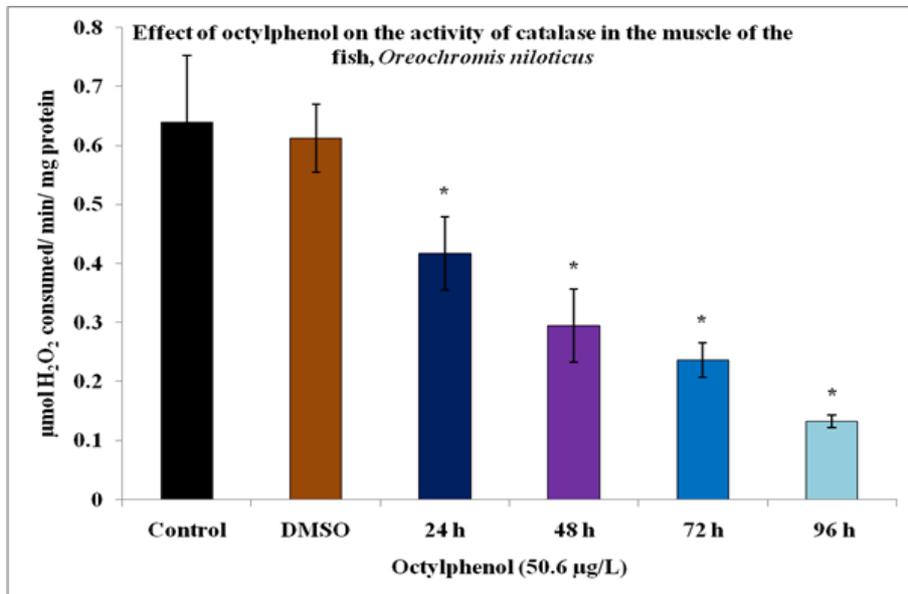


Fig 2

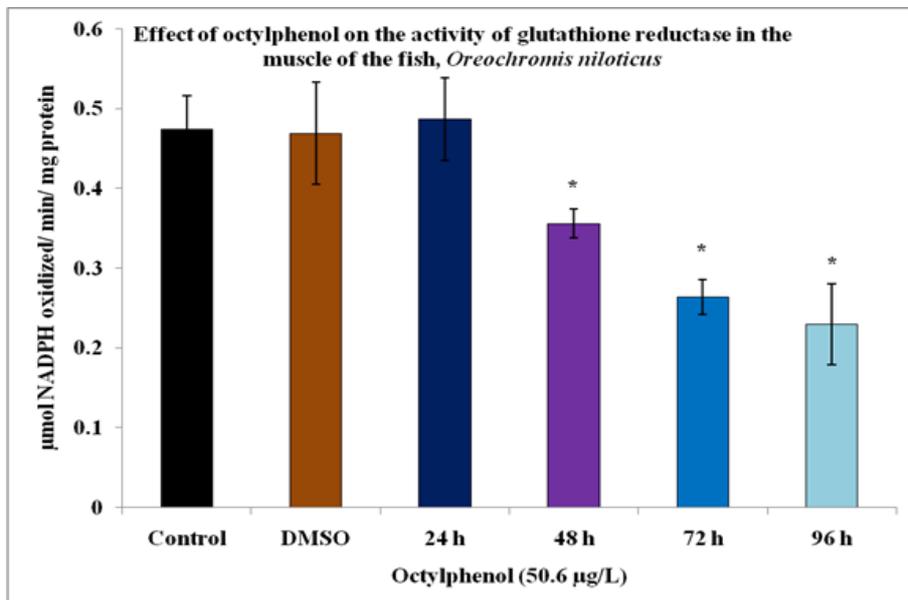


Fig 3

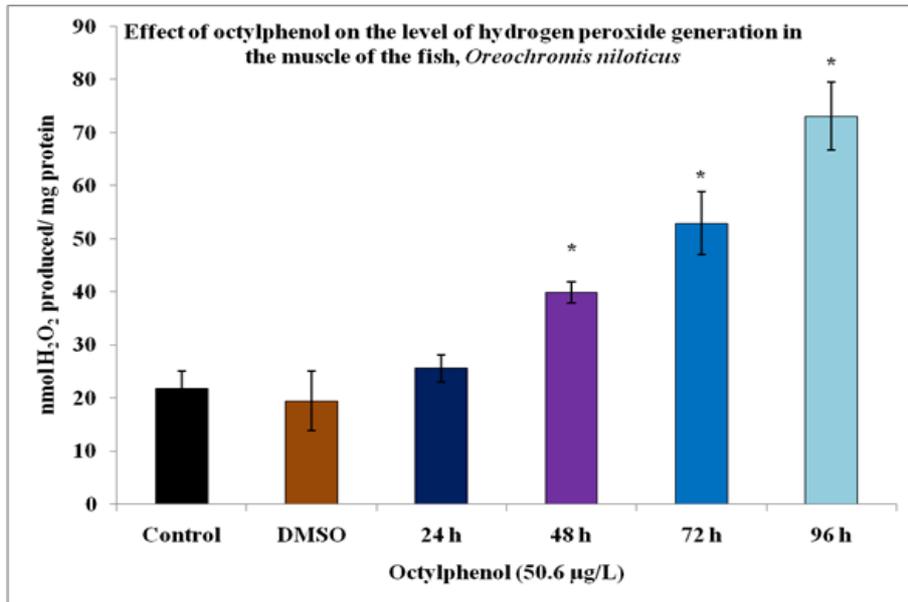


Fig 4

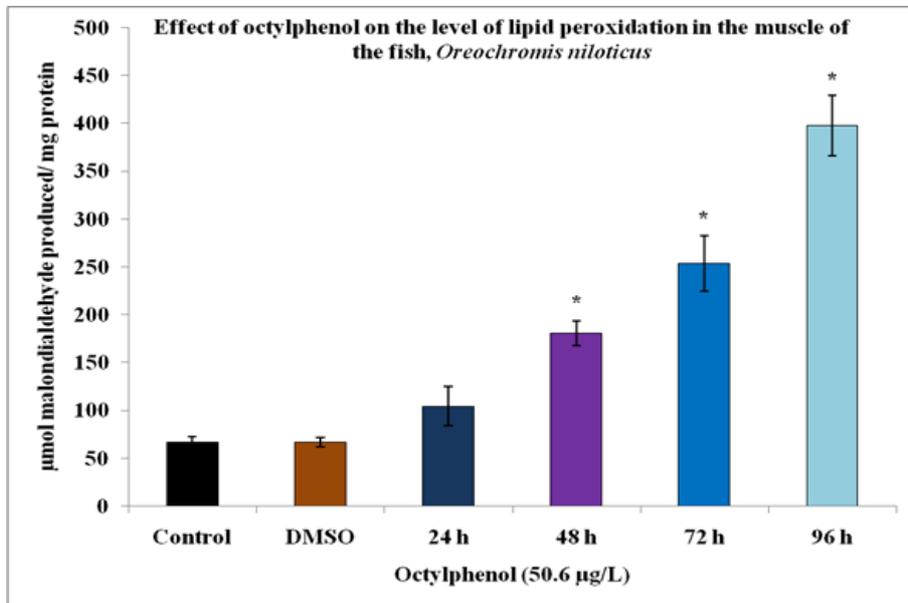


Fig 5

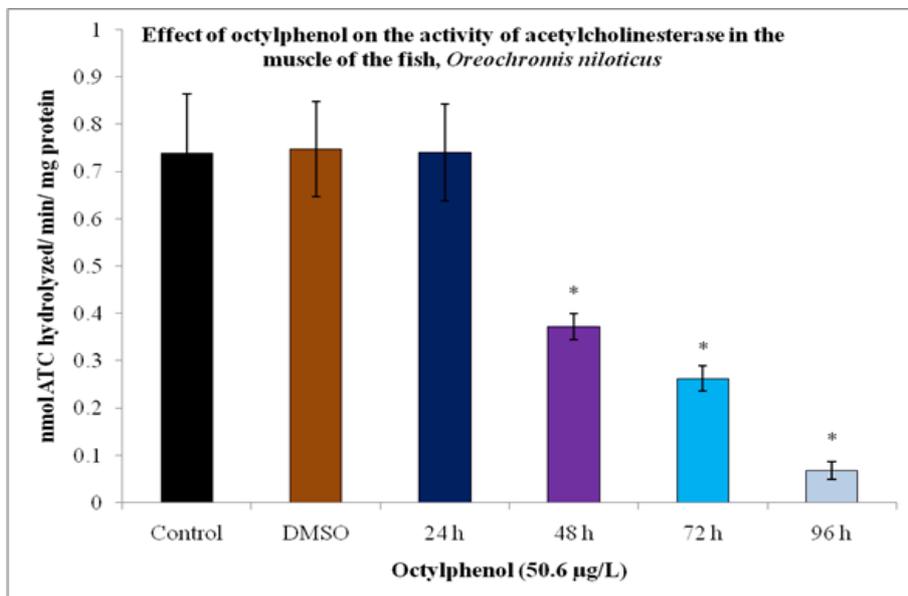


Fig 6

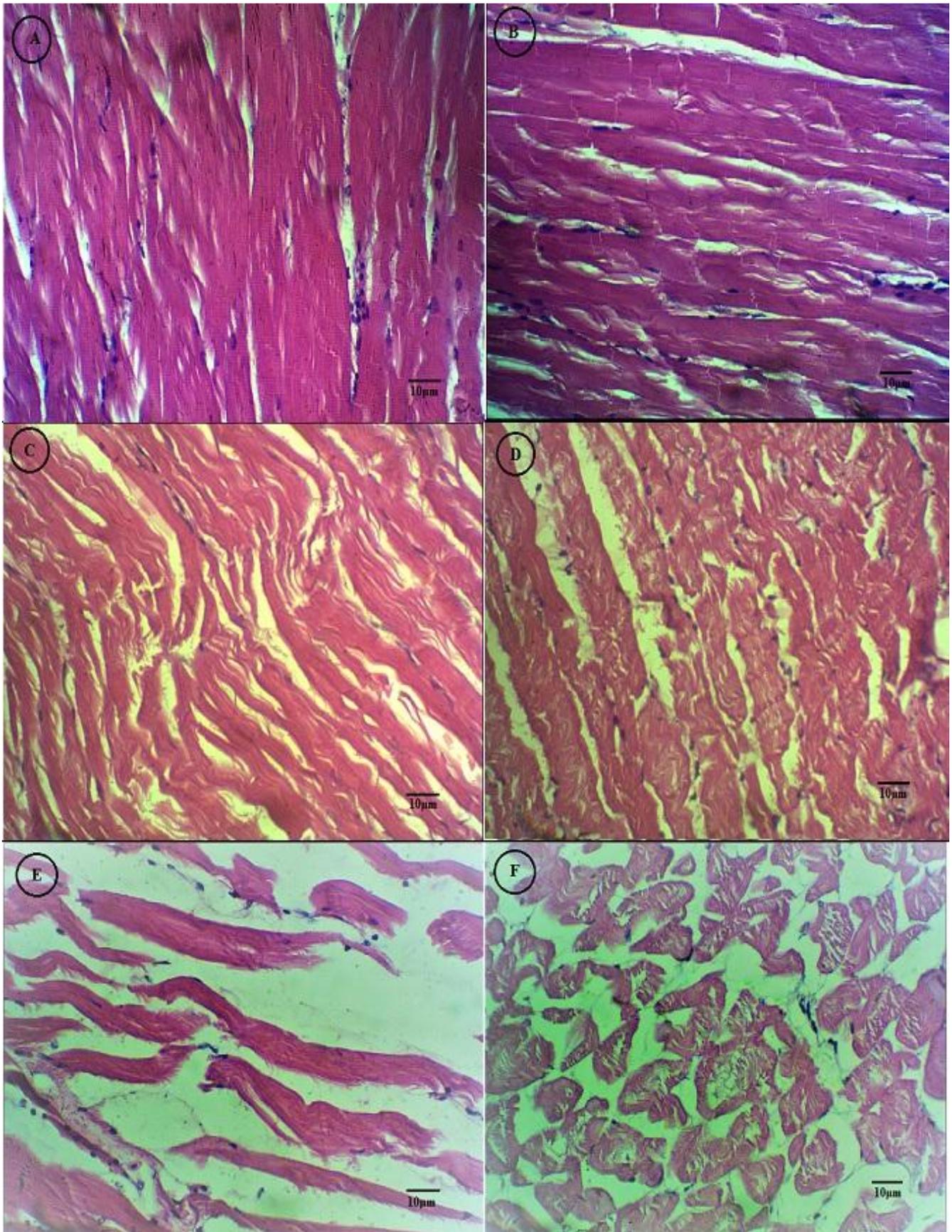


Fig 7a. Photomicrograph showing normal architecture of control muscle tissue with muscle fibre and spindle nucleus in the fish, *Oreochromis niloticus*. **b.** Normal histoarchitecture in vehicle (DMSO-treated) group. **c and d.** Degenerated muscle tissue with split muscle fibres after 24 and 48 h of octylphenol exposure, respectively. **e.** Disorganization of muscle fibres after 72 h. **f.** Thickened and shortened muscle bundles after 96 h treatment.

4. Conclusion

Octylphenol exposure at sublethal concentration altered the antioxidant defense system and also affects the histomorphology of muscle tissues in the freshwater fish, *Oreochromis niloticus*. The present findings suggest that induction of oxidative stress is one of the modulator for the muscular damage.

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