BIOCHEMICAL AND GENOTOXIC EFFECTS OF OCTYLPHENOL IN HEPATO-MITOCHONDRIAL FRACTIONS OF FRESHWATER FISH, OREOCHROMIS MOSSAMBICUS

SREEDEVI, N. V. AND CHITRA, K. C.

Department of Zoology, University of Calicut, Malappuram, District, Kerala, 673 635, India.  
E: mail: kchitra@yahoo.com; Cell: 094951 35330

Received: March 12, 2014: Revised: March 24, 2014: Accepted: April 15, 2014

Abstract: The environmental contaminants released into the aquatic ecosystem can disrupt the normal structure and functions of various organs in an organism thereby have an effect on its normal life. Octylphenol, one of the environmental contaminants, is known to perturb the normal functioning of aquatic life. In the present study octylphenol at 75µg/ L was exposed to freshwater fish, Oreochromis mossambicus for 7, 14 and 21 days and the biochemical changes and genotoxic potential was studied. During octylphenol treatment the body weights of the fish remained unchanged but the weight of liver and hepatosomatic index was decreased when compared to the control group. Octylphenol significantly increased mucous deposition all over the body in time-dependent manner. Biochemical estimation of the levels of lipid peroxidation, cytochrome oxidase and xanthine oxidase in hepatic mitochondrial fractions showed a significant increase at the end of treatment. This could be due to the generation of reactive oxygen species and energy demand in the mitochondrial fractions of hepatocytes. Exposure to octylphenol resulted in cytogenotoxicity as evidenced by fragmented apoptotic, bi-nucleated and sticky cells in micronucleus test and an increase in the number of colonies of the bacterium, Salmonella typhimurium, than that of control group. Therefore, octylphenol impairs antioxidant status and affects genotoxic potential when exposed chronically in freshwater fish O. mossambicus.

Key words: Octylphenol, Oreochromis mossambicus Hepatic mitochondria

INTRODUCTION

Several man-made or the natural chemicals present in the aquatic environment are able to interfere with the various systems in the aquatic organisms and has been shown to cause adverse effects on growth, behavior, reproduction and immune function. Many of these environmental chemicals are widely used in plastic and detergent industry and were also used in alternative medicine and food industry, respectively. According to research, approximately 300,000 tones of alkylphenols are produced per year and 60% are known to be released into the environment. This is then converted into the biodegradation products as nonylphenol and octylphenol, which are more toxic than alkylphenol itself [1].

Many studies have investigated the toxicity of octylphenol mainly by demonstrating the estrogen-like effects on wide variety of animals including fish. It has been well established for teleost fishes the toxicological effects of octylphenol can affect through multiple pathways. But there has been no strong evidence to support the genotoxic potential of octylphenol on the freshwater fish, Oreochromis mossambicus. So the significance of the present study
is to investigate the genotoxic effect of octylphenol and also in addition, some of the biochemical parameters to prove the antioxidant status were also done to evaluate the toxic effect of octylphenol on the selected fresh water fish. Liver is considered as the main and important detoxifying organ in fish and is essential for both the metabolism and the excretion of toxic substances in the body; and several categories of hepatocellular pathology are regarded as reliable biomarkers of toxic injury and are representative of biological endpoints of contaminant exposure [2]. It is well known that antioxidants are essential for maintaining the redox status of fish cells and tissues. If the antioxidants are insufficient, oxidative stress may occur, leading to an altered physiological condition of the animal, and ultimately to death if essential tissues are affected [3]. In the present study the effect of octylphenol on the antioxidant status was revealed by analyzing the level of lipid peroxidation in the hepatic mitochondrial fractions of *O. mossambicus*.

Cytochrome c oxidase is a large transmembrane protein complex found in bacteria as well as in mitochondria. It is the last enzyme in electron transport chain of mitochondria; therefore it helps in the synthesis of ATP. The decrease in the activity of cytochrome oxidase results in several mitochondrial disorders as dysfunction of cyclo-oxygenase assembly. However, xanthine oxidase is an enzyme involved in the generation of reactive oxygen species and also plays a role in catabolism of purines. Xanthine oxidase activity is widely used to evaluate the damages in liver tissues. Horecker and Heppel [4] have reported that xanthine oxidase could reduce cytochrome c but only in the presence of oxygen.

Genotoxicity studies using cytogenetic analyses as micronuclei test detecting nuclear abnormalities are the most widely applied methods due to its proven suitability for fish species. Micronuclei are cytoplasm chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes that lagged behind in the anaphase stage of cell division. Their presence in cells is a result of structural and/or numerical chromosomal aberrations arising during mitosis [5]. Micronuclei assay detects both clastogenic and aneugenic effects and therefore can detect the genotoxicity of a wide range of compounds. Nuclear abnormalities, such as micronuclei, and other nuclear malformations as fragmented apoptotic cells, binucleated cells and sticky adherent cells are considered as good indicators of cytotoxicity and genotoxicity, respectively [6].

Micronucleus assay has been widely used to measure genotoxicity, both in vitro and in vivo. The in vivo test is especially relevant to assessing genotoxicity hazard. Micronuclei, also hematologically known as Howell–Jolly bodies, are generally smooth, round remnants of nuclear chromatin seen in erythrocytes. Thus micronucleus assay is devised primarily for evaluating the ability of test agents to induce structural and/or numerical chromosomal damage [7]. Along with micronucleus test Ames test was also conducted in order to identify the cytogenotoxicity of octylphenol. Thus the present study was aimed to examine the toxicity effect of octylphenol on various biochemical and cytogenetic parameters as an end product in the fresh water fish, *Oreochromis mossambicus*.

**MATERIALS AND METHODS**

**Experimental setup:** Fresh water fish, *Oreochromis mossambicus* of weight 9 ± 1 g and length 7 ± 1.5 cm were collected from a nearby local fish farm. The animals were acclimatized to the laboratory conditions for four weeks with constant water supply and good lighting system. They were maintained in well-aerated tubs (40 L capacity). During the period of acclimatization and experiment, fish were fed with standard fish pellets. The LC$_{50}$ for 96h were determined by probit analysis, with a confident limit of 5 % level [8]. The LC$_{50}$ was 750 µg/ L and sub-lethal dosage (75 µg/ L) octylphenol was chosen in the present study.

**Chemicals:** Octylphenol 4-(1,1,3,3-tetramethylbutyl) phenol of 90 % purity was obtained from Merck. *Salmonella typhimurium* was purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. All other chemicals were of analytical grade and obtained from local commercial sources.

**Treatments:** Single dose with different durations were used in the present study. There were four groups, three tanks with toxicant doses of 75 µg/ L maintained for 7, 14 and 21 days, respectively and a tank without toxicant as control fishes. Ten fishes...
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight</strong> (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>570</td>
<td>580</td>
<td>590</td>
<td>600</td>
</tr>
<tr>
<td>7 days</td>
<td>610</td>
<td>620</td>
<td>630</td>
<td>640</td>
</tr>
<tr>
<td>14 days</td>
<td>650</td>
<td>660</td>
<td>670</td>
<td>680</td>
</tr>
<tr>
<td>21 days</td>
<td>690</td>
<td>700</td>
<td>710</td>
<td>720</td>
</tr>
</tbody>
</table>

*Significant difference from control.*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>7 days</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>14 days</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>21 days</td>
<td>120</td>
<td>130</td>
<td>140</td>
<td>150</td>
</tr>
</tbody>
</table>

*Significant difference from control.*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>7 days</td>
<td>1.0</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>14 days</td>
<td>1.8</td>
<td>2.0</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>21 days</td>
<td>2.6</td>
<td>2.8</td>
<td>3.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*Significant difference from control.*

**Fig. 1**: Effect of octylphenol on the body weight of *Oreochromis mossambicus*.

**Fig. 2**: Effect of octylphenol on the liver weights *Oreochromis mossambicus*.

**Fig. 3**: Effect of octylphenol on percentage of mucous secreted in the body of *Oreochromis mossambicus*.

**Fig. 4**: Effect of octylphenol on hepatosomatic index (HSI) of *Oreochromis mossambicus*.

**Fig. 5**: Effect of octylphenol on the level of lipid peroxidation in *Oreochromis mossambicus*.

**Fig. 6**: Effect of octylphenol on the activity of cytochrome oxidase in *Oreochromis mossambicus*.

**Fig. 7**: Effect of octylphenol on the activity of xanthine oxidase in *Oreochromis mossambicus*.
were maintained in each experimental group. At the end of the experiments fishes were caught with least disturbance and decapitated. Liver was dissected out and stored at 4°C until the analyses were performed.

**Biochemical estimations:** Hepatic mitochondrial fractions were obtained by the differential centrifugation method as described by Kamath and Narayan [9]. Total protein concentration was estimated by the method of Lowry et al. [10] with bovine serum albumin as standard. The levels of lipid peroxidation were measured using thiobarbituric acid color reaction for malondialdehyde by the method as described by Ohkawa et al [11]. Cytochrome oxidase in the liver mitochondria was determined by the method of Wharton and Tzagoloff [12]. Xanthine oxidase was assayed by the method of Bergmeyer et al [13].

**Genotoxicity analysis:** The micronucleus test was performed according to Heddle [14] and Schmid [15] and nuclear abnormalities were evaluated according to Carrasco et al. [16] with slight modification. The mutagenicity of octylphenol was tested by the method of Ames et al. [17].
Statistical Analysis: Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at p<0.05 against control group. Data are presented as mean ± SD for ten animals per group.

RESULTS

Body weights, liver weights, mucous deposition and hepatosomatic index: Octylphenol at the sub lethal dose of 75 µg/ L showed no significant reduction in the body weight after 7, 14 and 21 days of treatment (Fig. 1) but there was a significant reduction in the weights of liver in all treatment groups when compared with the control (Fig. 2). At the end of every treatment remarkable mucous deposition was observed over the body of fish with the percentage increased to 70% when compared with those of control groups (Fig. 3). The hepatosomatic index significantly (p<0.05) decreased only after 21 days of treatment with a slight decrease at the end of 7 and 14 days of treatment (Fig. 4).

Biochemical parameters: The level of lipid
peroxidation showed a significant decrease at 7 and 14 days of octylphenol treatment, but at the end of 21 days there was a significant (p<0.05) increase in the level of lipid peroxidation as compared with that of control animals (Fig. 5). The activity of cytochrome oxidase was significantly (p<0.05) decreased after 7 and 14 days of octylphenol treatment and showed a significant increase in hepatic mitochondrial fractions after 21 days of exposure as compared with the control group (Fig. 6). A significant (p<0.05) increase in the activity of xanthine oxidase was found in all treatment groups when compared with those of control fishes (Fig. 7).

Genotoxicity: Micronucleus test showed that octylphenol exposure for 7 days resulted in fragmented apoptosis. At 14 and 21 days of treatment showed binucleated and sticky cells (Figs.8,9). Ames test reports more colonies of Salmonella typhimurium in one-tenth (75 µg/ L) of the test dose than one-fifth dose (150 µg/ L) of octylphenol as compared to both positive and negative control groups i.e., without and with DMSO (Figs. 10-13).

DISCUSSION

Toxicity of a chemical refers to its ability to damage an organ system, disrupts biochemical processes, disturb an enzyme system or affect the gene products at some site remote from the site of contact. The most important factor of toxicity testing is the dose-time relationship and it may be either acute toxicity or chronic toxicity. The body weights of fishes were monitored throughout the experiments. In the present study octylphenol showed no significant changes in the body weight after 21 days of treatment, but animals were lethargic immediately after 14 days of exposure. Measures of animal growth are routinely evaluated in toxicology studies and are key to interpret the compound-related effects. The present results suggest that octylphenol exposure did not showed compound-related effect on the body weight of the treated fishes. Octylphenol treatment showed a significant increase in the mucous production when compared with that of control groups. Mucous cells are considered efficient in seizing the toxic agents and thus help to prevent the entrance of these agents into the gills [18]. Hypersecretion of mucous may be the consequence of a chronic defensive mechanism of the fish against the exposure to the environmental toxicant octylphenol. In the present study the weight of liver was significantly decreased in all treatment groups which indicate the toxicity of octylphenol on fish hepatocyte. However, a significant decrease in the hepatosomatic index was observed only at 21 days of octylphenol treatment and this could be possibly due to necrosis or atrophy of hepatocytes.

Xenobiotics in the environment have been shown to stimulate the production of reactive oxygen species which cause changes in the transcription of oxidative stress-related genes in aquatic organisms [19]. In the present study octylphenol increased the level of lipid peroxidation at the end of 21 days, but showed a dramatic decrease at 7 and 14 days of treatment in hepatic mitochondrial fractions of fish as compared with that of control. The result indicates that octylphenol did not alter the status of antioxidant system in hepatic mitochondrial fractions till 14 days of toxic exposure. However, when the reactive oxygen species generation increases tremendously the system could not withstand the oxidative stress generated by octylphenol and thus the level of lipid peroxidation showed a significant increase at the end of 21 days. Our previous report have suggested that nonylphenol treatment elevated the levels of lipid peroxidation and disrupted the pro-oxidant/antioxidant balance thereby causing oxidative stress in the epididymal sperm of rats [20]. Similarly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to increase the lipid peroxidation in mitochondrial and microsomal fractions of rat testis [21]. Lipid peroxidation may alter membrane characteristics and functions, leading to cell death. Thus in the present study increased level of lipid peroxidation revealed that octylphenol disrupts the antioxidant system in hepatic mitochondrial fractions of treated fish.

Increased reactive oxygen species may induce the mitochondrial inner membrane to dissociate cytochrome c. Once cytochrome c is released from mitochondria the cell is committed to die by activation of apoptotic caspase cascade and nucleic DNA fragmentation, resulting from overproduction of reactive oxygen species and insufficient supply of ATP [22]. In the present study the activity of cytochrome oxidase was significantly decreased after 7 and 14 days of octylphenol treatment whereas a significant increase in the activity of cytochrome oxidase was observed after 21 days of exposure. The result obtained clearly states that as a result of reactive oxygen species generation, mitochondrial membrane dissociates cytochrome oxidase and this could be due to insufficient supply of ATP as a
consequence of octylphenol exposure. Thus the increase in the cytochrome oxidase could be due to the increase in energy demand of toxicity exposure. Similar results have been observed as the effect of diabetes on retinal oxidative metabolism in rats [23].

Another marker enzyme used in the present study is xanthine oxidase. The basic role of the enzyme is to catalyze the biochemical reaction of the oxidation of hypoxanthine and xanthine into uric acid in the latter stages of purine catabolism [24]. In mammals, xanthine oxidoreductase occurs in two interconvertible forms, xanthine dehydrogenase and xanthine oxidase. It exclusively uses \( \text{O}_2 \) yielding reactive oxygen species \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) as reaction by-products. Xanthine oxidoreductase is widely distributed among animal species and tissues with the highest activity in liver, intestine and milk. The capability for xanthine oxidase to generate \( \text{O}_2^- \) has been known since 1968 and its role in mediating oxidative stress [25].

In the present study octylphenol increased the activity of xanthine oxidase in all treatment groups when compared with those of control fishes and this could be due to the generation of reactive oxygen species. The result is supported by various reports, where an increased xanthine oxidase activity in liver of mice has been observed during viral, bacterial and protozoan infection or with Ehrlich ascetic carcinoma [26]. Xanthine oxidase is involved in the production of oxygen-associate molecular reactants (\( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), \( \text{ONOO}^- \)), which can initiate oxidative cell damage and organ failure. Xanthine oxidase, as a generating source of superoxide anion as well as \( \text{H}_2\text{O}_2 \) comprises a rather unique free radical generator because of its capacity to generate its reactants not only intracellularly but also extracellularly. The extracellular generation of reactive oxygen species by xanthine oxidase is temporally commensurate with the significant increase in plasma or serum xanthine oxidase activity, as well as xanthine oxidase gene transcription [27].

The present study also focused on the cyto-genotoxicty of octylphenol by measuring micronucleus and Ames test. In vivo micronucleus assay is devised primarily for evaluating the ability of test agents to induce structural and/or numerical chromosomal damage in the exposed organisms. In the present study administration of octylphenol for 7 days showed fragmented apoptosis and at 14 and 21 days of treatment showed binucleated and sticky cells thereby proving cytotoxicity of the test chemical. The purpose of the Ames test or bacterial reverse mutation assay is to evaluate the genotoxicity of test chemical by measuring its ability to induce reverse mutations in bacterial strains. The test strain *Salmonella typhimurium* TA 100 is most widely used in mutagenicity assay in the field of genetic toxicology [28]. In the present study octylphenol showed more colonies in one-tenth of the test dose than one-fifth dose as compared with controls. Thus octylphenol is considered as mutagenic similar to nonylphenol, another alklyphenol at 1.5 mg/ L dose which is evidenced when the number of revertant colonies in the test plates was doubled than the number of revertants in solvent control [29]. On the basis of the foregoing discussion it can be concise that exposure to octylphenol induces oxidative stress in liver as well as caused genotoxicity in the freshwater fish, *Oreochromis mossambicus*.

**ACKNOWLEDGEMENT**

The authors acknowledge the availability of fund from UGC-SAP/ BSR, Government of India for the study.

**REFERENCES**