Lipid peroxidation products in fry of *Labeo rohita* and *Cyprinus carpio* on exposure to sub lethal cadmium: A comparative study

SURYA KUMARI VADLAMANI  
Department of Zoology, Andhra University  
Visakhapatnam, India  
CHARAN KUMAR BASURI  
Department of Zoology, Andhra University  
Visakhapatnam, India.  
PRABHAKARA RAO YALLAPRAGADA¹  
Department of Zoology, Andhra University  
Visakhapatnam, India

Abstract:  
Lipid peroxidation is one of the several physiological mechanisms of the organism by which reactive oxygen species (ROS) may be released which is toxic to cells and tissues. In general, the formation of lipid peroxidation products in tissues may infer the extent of oxidative damage and decline in anti-oxidant defense mechanism. In the present investigation, lipid peroxidation products were measured by determining the malondialdehyde (MDA) concentrations in the tissues of control and exposed fry of *Labeo rohita* and *Cyprinus carpio*. The fish fry were exposed to their sub lethal concentration of cadmium (1/5th of 96hrs LC₅₀ i.e., 0.1998ppm for *Labeo rohita* fry and 4.98ppm for *Cyprinus carpio* fry respectively) for a period of 20days. Parallel controls were maintained without metal toxicant throughout the experimental period. Samples were collected both from control and exposed fish fry of each species at intervals of 24hrs, 48hrs, 96hrs, 10days and 20days for the estimation of lipid peroxidation products. A

¹ Corresponding Author: Prof. Y. Prabhakara Rao, Emeritus Professor, Department of Zoology, Visakhapatnam, India; yprabhakararao@yahoo.com
maximum amount of lipid peroxidation products was observed at 20 days exposure (3.01n moles of MDA/gm wt of the tissue in Labeo rohita fry and 2.7029 n moles of MDA/gm wt of the tissues in Cyprinus carpio fry). From the data, it was known that more amount of the lipid peroxidation products are found in the fry of Labeo rohita exposed to cadmium than in the fry of Cyprinus carpio indicating the fry of Labeo rohita is more sensitive than Cyprinus carpio.

**Key words:** Lipid peroxidation, malondialdehyde (MDA), fish fry, cadmium, MDA.

**INTRODUCTION:**

Aquatic organisms are often chosen as experimental tools because they possess good filtration capacity, easy caging facility and are sensitive to oxidative stress when exposed to sub-lethal concentration of toxicants. They also provide a model system for investigations to study the production of reactive oxygen species (ROS) and their damage to cellular components. In addition, they can be used to detect the disease occurrence through oxidative stress and subsequent repair mechanisms through amelioration process (Brucka–Jastrzebska, 2010).

Every living organism in nature requires molecular oxygen (O₂) for oxidation of food and generation of energy. In this process some portion of O₂ undergoes tetravalent reduction of H₂O and transforms into ROS. All aerobic organisms generate ROS as super oxide radical (O⁻²), hydroxyl radical (OH) and non-radical species including H₂O during cellular metabolism. The ROS are continuously produced as undesirable toxic byproducts of normal metabolism from various endogenous processes and it has been estimated that about 1-3% O₂ consumed in animal system is converted to ROS (Livingstone, 2001). Free radicals are the molecules having an unpaired electron in the outer orbit (Gilbert, 2000). As they are
unstable and very reactive, they biologically affect at physiological and pathological levels. At physiological level they serve as signaling and regulatory molecules and at pathological level, they are highly deleterious and cytotoxic oxidants (Fridovich, 1999). Lipid peroxidation being one of the several mechanisms by which ROS released may be toxic to cells and tissues. Enhanced lipid peroxidation and a consequence of oxidative deterioration of membrane lipids generally indicate oxidative stress (Kappus, 1983). ROS are capable of damaging major biological macro molecules such as DNA, proteins and membrane phospholipids (Mates, 2000) which can ultimately lead to cell death. Although there exists antioxidant defense mechanism to protect against ROS, they can be overwhelmed and cellular damage may ensure. Thus, the imbalance between ROS production and neutralization by antioxidant defenses and the resulting damage to cellular macro molecules has been termed as ‘oxidative stress’ (Kelly et al. 1998).

Many aquatic organisms possess high amounts of lipids with polyunsaturated fatty acid residues which is a substrate for oxidation. These lipids are oxidized through the formation of peroxides and this process is called lipid peroxidation. Metals such as copper, lead, cadmium and mercury are known to cause oxidative stress in fishes indicating an increase in lipid peroxidation products. Therefore, this parameter can be used as a tool to study the oxidative stress in fishes including fry and fingerlings. Our earlier investigations revealed that the fry of *Labeo rohita* was more sensitive to cadmium than that of *Cyprinus carpio* (Suryakumari et al. 2017a). In the present investigation, an attempt has been made to study the comparison of lipid peroxidation products on exposure to sub lethal concentrations of cadmium in the laboratory.
**Materials and Methods:**

**Test compounds and chemicals:**
For all the experimental studies, the toxicant was prepared by dissolving cadmium chloride in distilled water. Cadmium chloride (CdCl₂, 5H₂O) (Analytical Reagent) was purchased from SRL, India. Desired concentrations of cadmium chloride were prepared from fresh stock solutions. All the other chemicals used for the present study were of analytical grade.

**Experimental design:**
*Labeo rohita* and *Cyprinus carpio* are two edible major carps, cultured in East Godavari of Andhra Pradesh. The fish fry available in and around this area are used for culture and they are prone to metal contamination during culture tanks where they use river water without any treatments.

For the present investigation, the fish fry of length 0.8 to 1 cm were collected from fish breeding ponds near Kadium and Dwarapudi of East Godavari District, Andhra Pradesh, India (Suryakumari et al. 2017). In these breeding ponds, processed and treated Godavari river water was used which is devoid of pollutants and metal contaminants particularly cadmium. The two fish fry were acclimated to laboratory conditions (pH: 7; Temp: 29 ± 2°C) for 48 hrs. After acclimatization, healthy fry of *Labeo rohita* and *Cyprinus carpio* were isolated and divided into two groups for each species. The first group without metal toxicant served as control and the second group of each species was the exposed in which the fry of each species were exposed separately to their respective sub lethal concentration of cadmium (1/5th of 96hrs LC₅₀ i.e., 0.1998 ppm for the fry of *Labeo rohita* and 4.98 ppm for the fry of *Cyprinus carpio*) (Suryakumari et al. 2017a) for a period of 20 days. Parallel controls were maintained simultaneously throughout the experimental period. Samples were taken from both the control
and exposed fry of each species separately at intervals of 24hrs, 48hrs, 96hrs, 10days and 20days for the estimation of lipid peroxidation products. The fish fry were fed with rice bran and ground nut oil cake powder. The water was changed every day in both control and exposed groups without causing disturbance to the fry.

**Estimation of lipid peroxidation products:**
Malonaldehyde (MDA), the secondary product of lipid peroxidation, was estimated in the tissue homogenates of fish fry by the method of Hiroshi et al. (1979). A 10% of homogenate was prepared in 1.5% potassium chloride solution. One milliliter of the homogenate was added to 2.5 ml of 20% trichloro acetic acid (TCA). The mixture was centrifuged at 3,500rpm for ten minutes at 4°C. The pellet obtained was dissolved in 2.5ml 0.05M H₂SO₄ and then 3ml of 2M TBA (Thiobarbiturie acid) was added to it. The test tubes were incubated in boiling water bath for 30 minutes at 100°C. The contents were cooled and the color was extracted into 4ml of n-butanol. The colour was read at 530nm using a spectrophotometer (Chemito, 2000) against a blank. The results were expressed as micromoles of malonaldehyde (MDA) formed/gram weight of tissue.

**Statistical evaluation:**
The experiments on lipid peroxidation products for control and exposed fish fry were repeated five times. Based on the results obtained, the mean values and standard deviation were calculated at each interval for both the exposed and control fish fry. The percent increase over their respective controls was also calculated at each interval. For comparing the results between control and exposed fish fry, Student’s t-test was used (Snedecor and Cochran, 1967).
RESULTS:

Figure 1 represents the data on the lipid peroxidation products of both control and exposed *Labeo rohita* fry at different intervals of exposure. In Figure 2, the lipid peroxidation products of control and exposed *Cyprinus carpio* fry are given at different intervals of exposure. Overall, it is clear from the data that the lipid peroxidation products showed a significant ($P < 0.05$) increase in tissues of both the exposed fish fry with respect to their control values at all intervals of exposure period (Fig. 1, Fig. 2). A comparison of the results on both the fish fry was given in Table 1.

The data on lipid peroxidation products of *Labeo rohita* fry (Fig. 1) indicated a significant ($P < 0.05$) increase at all the six intervals of exposure with respect to their corresponding controls but this increase was not uniform overall. During the short time intervals i.e., between 24 and 96hrs, there was a consistent increase in lipid peroxidation products from 30.13% to 43.86%. However, at long term intervals i.e., 10 and 20days, the lipid peroxidation products showed an increase from 34.45% to 43.32% (Table 1; Fig. 1). A maximum amount of lipid peroxidation products was observed at 20days exposure (3.01nano moles of MDA/gram weight of the tissue) and the lowest was (1.75nano moles MDA/gram weight of the tissue) was observed at 24hrs exposure. The percent increase in lipid peroxidation products was calculated based on their respective control values and it was in the order of 30.13% (24hrs) > 31.09% (48hrs) > 34.45% (10days) 42.32% (20days) > 43.86% (96hrs). Overall, the highest percent of increase in lipid peroxidation products was observed at 96hrs (Fig. 1; Table 1).

Figure 2 shows the data on lipid peroxidation products of control and exposed *Cyprinus carpio* fry at intervals of exposure to cadmium. It is clear from the results (Fig. 2; Table 1) that there was a significant ($P < 0.05$) increase in lipid
Lipid peroxidation products at all intervals of exposure with respect to their control values but this was increase was not consistent and time-dependent. Though an increase in lipid peroxidation products was observed overall, there was no uniformity at least either in short term or long term intervals (Fig. 2; Table 1). Among the values of lipid peroxidation products of exposed fry of *Cyprinus carpio*, the lowest was 1.1036 nano moles MDA/gram weight of tissue observed at 24hrs and the highest amount of 2.7029 nano moles MDA/gram weight of tissue was recorded at 20days. The percent increase at each interval for both the fish fry was calculated based on their respective control values and they were in the order of 11.27% (10days) > 19.33% (24hrs) > 31.32% (96hrs) > 24.68% (20days) > 36.14% (48hrs) (Table 1; Fig. 2).

A comparison between the lipid peroxidation products of both the fish fry indicate that *Labeo rohita* fry are capable of forming more amount of lipid peroxidation products than *Cyprinus carpio* fry though the exposure concentration of cadmium was (0.1998ppm) low than that of its counterpart (4.98ppm). Both the fish fry showed an increase in lipid peroxidation products with increasing exposure period to cadmium but the increase in *Labeo rohita* fry was uniform for the initial short term period up to 96hrs. However, the increase in lipid peroxidation products in *Cyprinus carpio* fry did not show any pattern on exposure to cadmium. The highest percent increase of lipid peroxidation products was also found for *Labeo rohita* fry (43.86% at 96hrs of exposure) compared to *Cyprinus carpio* fry.

DISCUSSION:

Aquatic organisms are known to possess a system for generation and degradation of free radicals and any contamination of water through sewage or industrial water
influx can disturb this balance of generation and neutralization of reactive oxygen species (ROS) causing an oxidative stress (Winston and Di Giulio, 1991; Kelly et al. 1998; Valavanidis et al. 2006). One of the biomarkers of this oxidative stress is the generation of lipid peroxidation products. The results of the present investigation indicated a significant (P < 0.05) increase in lipid peroxidation products for both the species of fish fry. Similar increase in lipid peroxidation products was observed in *Galaxias maculates* on exposure to cadmium (McRae et al. 2018). Yin et al. (2018) while studying a potential biomarker for toxicity of heavy metals namely copper, cadmium, chromium and lead, reported the formation of lipid peroxidation products in zebra fish on cadmium exposure. Rajeshkumar et al. (2017) observed a time-dependent production of lipid peroxidation products in *Cyprinus carpio* on exposure to metal mixture containing cadmium, chromium and lead. Zhang et al. (2017) also reported a significant increase in lipid peroxidation products in the kidney of *Cyprinus carpio* exposed to cadmium. In catfish (*Clarias gariepinus*) embryos, the silver nanoparticles from 25 to 75ng/L caused an increase, then decrease and increase in lipid peroxidation products (Sayed and Soliman, 2017). Other heavy metals like mercury also induced oxidative stress and caused an increase in lipid peroxidation products in *Cyprinus carpio* (Garcia-Medina et al. 2017). Kumar et al. (2017) while studying the protection given by selenium nanoparticles in *Pangasius hypophthalmus* reared under lead and high temperature noticed an increase in lipid peroxidation products indicating lead-induced oxidative stress. Copper-induced oxidative stress was reported by Diaz-de-Alba et al. (2017) with an increase in lipid peroxidative products in different tissues of European sea bass (*Diecentrarchus labrax*) and gills appeared to be very sensitive to copper-induced oxidative stress than other organs in this fish. Environmental pollution-induced oxidative stress with an increase in lipid
peroxidation products was observed in African catfish (*Clarias heterobranchus*) by Ifeakachuku et al. (2014). Lipid peroxidation has been used as a measure of xenobiotic-induced oxidative stress in Atlantic croaker fish (Thomas and Wofford, 1993), Channel catfish (Di Giulio et al. 1993) and Indian catfish (Parihar and Dubey, 1995). Fatima et al. (2000) reported an increased lipid peroxidation in fish exposed to polluted environment. According to Ahmad et al. (2004), exposure to polluted water can induce tissue-specific peroxidative damage in gills, kidney and liver of European eel (*Anguilla anguilla*). Brucka-Jastrzebska (2010) also observed that aquatic cadmium and lead concentrations increased the lipid peroxidation and reduced the superoxide dismutase activity in liver of three freshwater fish species, *Cyprinus carpio*, *Oncorhynchus mykiss*, and *Acipenser baeri*. Sreejai and Jaya (2010) reported an increase in lipid peroxidation products in liver, gill and brain of *Oreochromis mossambicus* on exposure to hydrogen sulphide. Bainy et al. (1996) noticed oxidative stress in Nile tilapia (*Oreochromis niloticus*) from a polluted site. Besides acting as a mediator in oxidative stress, higher levels of lipid peroxidation products can adversely affect the cellular functions (Mates, 2000) and adduct with proteins and DNA which may predispose the cell to mutagenesis and carcinogenesis (Bailey et al. 1996). The metabolism of ROS due to their high damaging capacity and biological activity is under fine cellular control and their concentration usually do not exceed 10^{-8}m (Halliwell and Gutteridge, 1989). Moreover, the production of free radicals is an inevitable part of aerobic life of organisms. A fine balance of free radicals and antioxidant enzymes is maintained by the organisms and any alteration towards production of more free radicals disturbs this balance leading to deleterious effects. Therefore, free radical approach is one of the most commonly used sensitive markers of environmental pollution. Thus, the estimation of lipid peroxidation products in the present...
investigation can be used to evaluate the effects of metal contamination in water.

Oleksiuk and Ianovych (2010) studied the antioxidant enzymes and lipid peroxidation products in the liver of three different species of freshwater fishes namely silver carp, grass carp and common carp and found that these antioxidants are species-specific in nature. This might be the reason for fewer amounts of lipid peroxidation products in *Cyprinus carpio* fry of the present investigation compared to *Labeo rohita* fry. More inhibition of antioxidant enzymes/antioxidants by cadmium in *Labeo rohita* fry might cause less neutralization of reactive oxygen species leading to more oxidative stress. A similar observation of more carotenoid concentration was reported (Surya Kumari et al. 2017b) in cadmium-exposed *Labeo rohita* fry compared to *Cyprinus carpio*. Oxidation stress is caused when there is an imbalance in the biological oxidant to antioxidant ratio which results in oxidative damage to biomolecules of the cell. Abnormal generation of ROS is considered as an indicator of oxidative damage (Barzilai and Yamamoto, 2004).

Heavy metals including cadmium are known to cause oxidative stress in different species of fish (Valavanidis et al. 2006; Ifeakachuku et al. 2014; Rajasekhar et al. 2017; Sayed and Soliman, 2017; Zhang et al. 2017; Kumar et al. 2017; Garcia-Medina et al. 2017; Diaz-de-Alba et al. 2017; McRae et al. 2018; Yin et al. 2018) as has been observed in the present investigation. Cadmium accumulation in the fry of *Labeo rohita* and *Cyprinus carpio* (Surya Kumari, et al. 2018) might have resulted in the generation of reactive oxygen species and inhibition of antioxidant defense mechanism of the fish fry by decreasing the enzyme activities of superoxide dismutase and catalase as reported by Wang et al. (2005) in zebra fish. Cadmium-induced inhibition of superoxide dismutase and catalase results in the production of lipid peroxidation products.
Cadmium is also known to replace the redox active metals from the binding sites of the cell causing stimulation of reactive oxygen species (Nair et al. 2013).

CONCLUSIONS:

The results of the present investigation show a time dependent and significant (P < 0.05) increase in lipid peroxidation products in exposed fry of both the species. A comparison between the fry of both the species reveals that the formation of lipid peroxidation products is more in Labeo rohita fry than in Cyprinus carpio fry. This indicates that Labeo rohita fry might have shown more inhibition of antioxidants (both enzymatic and non enzymatic) by cadmium leading to a lesser degree of defense mechanism. Therefore, cadmium-induced oxidative stress is more operative in Labeo rohita than Cyprinus carpio. Further investigations on antioxidant enzymes in these fry on exposure to cadmium are in progress.

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REFERENCES


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Table 1: Effect of sub-lethal exposure of cadmium (0.1988 ppm; 1/5th LC₅₀) on lipid peroxidation products of *Labeo rohita* and *Cyprinus carpio* fry. Each value represents the mean ± standard deviation. The values in the parentheses represent percent decrease over their respective controls.

*Significantly different from their respective controls at P < 0.05

<table>
<thead>
<tr>
<th>Period of Exposure</th>
<th><em>Labeo rohita</em></th>
<th><em>Cyprinus carpio</em></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(nano moles MDA/ gram weight of the tissue)</td>
<td>(nano moles MDA/ gram weight of the tissue)</td>
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<tr>
<td>Control</td>
<td>Exposed</td>
<td>Control</td>
</tr>
<tr>
<td>24 hrs</td>
<td>1.2264 ± 0.1802</td>
<td>1.7554 ± 0.0613*</td>
</tr>
<tr>
<td></td>
<td>(30.13)</td>
<td>(31.09)</td>
</tr>
<tr>
<td>48 hrs</td>
<td>1.2537 ± 0.1237</td>
<td>1.8192 ± 0.1565*</td>
</tr>
<tr>
<td></td>
<td>(31.09)</td>
<td>(36.14)</td>
</tr>
<tr>
<td>96 hrs</td>
<td>1.2227 ± 0.1036</td>
<td>2.1778 ± 0.1764*</td>
</tr>
<tr>
<td></td>
<td>(43.86)</td>
<td>(36.14)</td>
</tr>
<tr>
<td>10 days</td>
<td>1.4740 ± 0.1204</td>
<td>2.2487 ± 0.3862*</td>
</tr>
<tr>
<td></td>
<td>(34.45)</td>
<td>(36.14)</td>
</tr>
<tr>
<td>20 days</td>
<td>1.7064 ± 0.2269</td>
<td>3.0106 ± 0.4462*</td>
</tr>
<tr>
<td></td>
<td>(43.32)</td>
<td>(36.14)</td>
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</table>

Figure 1: Lipid peroxidation products in fry of *Labeo rohita* exposed to sub-lethal concentration of cadmium (*Significantly different from their respective controls at P < 0.05)

Figure 2: Lipid peroxidation products in fry of *Cyprinus carpio* exposed to sub-lethal concentration of cadmium (*Significantly different from their respective controls at P < 0.05)