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Alterations in Hepatic Antioxidant Systems and Lipid Peroxidation Products in a Freshwater Fish, *Labeo rohita*, Exposed to 4-Nonylphenol, an Endocrine Disruptor

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Abstract

4-Nonylphenol (4-NP) is a well-known endocrine-disrupting chemical that could be present in the aquatic environment, but little is known about its oxidative stress effects. An emerging literature suggests that early life exposure to 4-NP may increase the risk of metabolic syndrome. Due to its hydrophobicity and long half-life, 4-NP can easily accumulate in living organisms, including humans, where it inflicts a series of toxic effects. In the present study, the oxidative stress and antioxidant parameters of *Labeo rohita* after exposure to various sub-lethal concentrations of 4-NP for 5, 10 and 15 days were examined. The level of glutathione content and the activity of glutathione peroxidase and glutathione-s-transferase were significantly inhibited, whereas the activity of superoxide dismutase, catalase and glutathione reductase and the lipid peroxidation products such as malondialdehyde and conjugated diene were significantly elevated, indicating the occurrence of oxidative stress. The results demonstrate that 4-NP in aquatic systems can affect antioxidant responses.

Keywords: Antioxidants, Endocrine-Disrupting Chemical, 4-Nonylphenol, Oxidative Stress

1. Introduction

Nonylphenol ethoxylates (NPEs) represent the most critical metabolite of Alkylphenols (APs) and Alkylphenol ethoxylates (APEs), which are widely used in the formulation of domestic and industrial products. Quite recently, 4-Nonylphenol (4-NP) has been described as a priority hazardous compound[15,46]. The most contaminated sites are estuaries, lagoons and coastal areas close to sewage treatment plants or industrial and municipal wastewater discharges. These non-ionic surfactants are used in cleaning products, plastic and elastomer manufacturing, textile processing, pesticide emulsifiers, pulp and paper production, and personal care products. Due to its hydrophobicity and long half-life, 4-NP can easily accumulate in living organisms, including humans, where it displays a series of toxic effects[48].

Many studies are focused on the effect of 4-NP on the reproductive impairments including developmental and physiological abnormalities related to estrogenecity. Laboratory experiments replicating the adverse effects of exposure to the potent synthetic estrogen Diethylstilbestrol (DES) during critical periods in development[29] have focused attention on the potential of chemicals with estrogenic properties to cause developmental and reproductive hazards. Increased or decreased activities of antioxidant enzymes serve as good indicators of pollutant-mediated oxidative stress[2]. 4-Nonylphenol has the potential to bring about all these alterations in mammalian species and a few aquatic

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species, but the oxidative potential of 4-NP has not been much reported in sub-mammalian species, including fish. In short, the present study is focused on the investigation of the oxidative stress and antioxidant defences in the liver of *Labeo rohita* (*L. rohita)*[23] when exposed to sub-lethal doses of 4-NP. Notwithstanding their ecological importance, the phenomenon of oxidative stress in fishes to 4-NP still needs in-depth studies.

The enzymatic activity of Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GP_x), glutathione reductase (GR) and glutathione-s-transferase (GST) and the non-enzymatic antioxidant glutathione (GSH) were used as biomarkers on exposure to 4-NP, while the lipid peroxidation (LPO) products such as malondialdehyde (MDA) and conjugated diene (CD) concentration were used to express severe oxidative damage, if any. Pollution impact on ecosystems and human health is an urgent and worldwide issue since there are an ever-increasing number of examples of environmental disturbances, and is likely to affect the biota and humans by both natural and anthropogenic stress[39]. Hence, it was thought worthwhile to investigate the oxidative potential of 4-NP, if any, in an aquatic species animal model, *L. rohita*.

2. Materials and Methods

2.1 Experimental Design

2.1.1 Test Chemical

Based on the acute toxicity studies, 96 hr LC_{50} value of the fish was found to be 3.14 µL/L according to Organisation for Economic Co-operation and Development (OECD) Guidelines[34] by using probit analysis. Based on this, sub-lethal doses of 4-NP such as 1.0 µL/L, 1.5 µL/L and 2.0 µL/L were selected for exposure.

2.1.2 Fish and Aquaria

The fish, *L. Rohita,* used in the experiment were obtained from local suppliers. Prior to experiment, the fish were acclimatized for 30 days in large cement tanks containing well aerated dechlorinated tap water under laboratory conditions such as natural photoperiod and temperature (26±2^{0}C). After acclimatization fish having 30±5 g were selected and divided into 10 groups of ten each in separate glass tanks. The first group of fish were maintained in dechlorinated tap water and served as control. Fish in groups II to X were exposed to 1.0 µL/L, 1.5 µL/L and 2.0 µL/L of 4-NP mixed in the medium (water) for a period of 5, 10 and 15 days, respectively.

2.1.3 Sampling and Sample Preparation for Enzyme Assay and Protein Measurement

The fish were starved for 24 hr prior to sacrifice for getting optimum and stable physiological state. After the stipulated periods of 4-NP exposure, fish were sacrificed by spinal concussion. A portion of fresh liver tissue was carefully dissected, surface-dried with filter paper, and thoroughly washed with an appropriate buffer for assaying all antioxidant enzymes. 150 mg of liver was homogenised in 1 mL Tris HCl buffer (pH 7.2). Homogenates were centrifuged at 10,000 rpm for 10 min at 4^{0}C (Eppendorf, Germany). After centrifugation, the supernatant was collected and kept in a deep freezer (Rotek) maintained at -20^{0}C until biochemical analysis.

2.1.4 Determination of Total Protein Content

The total protein content was determined separately using the same tissue extract according to the protocol of Bradford[6].

2.2 Assay of Antioxidant Enzymes

2.2.1 Superoxide Dismutase (EC.1.15.1.1)

Superoxide dismutase activity was determined according to the protocol of Kakkar et al[24]. The reaction mixture consisted of sodium pyrophosphate buffer (0.052M, pH 8.3), phenazine methosulphate (186 µM), nitroblue tetrazolium, nicotinamide adenine dinucleotide (NADH) (780µM) and enzyme extract. The reaction was started by the addition of NADH. After incubation, the reaction mixture was stopped by the addition of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with n-butanol. The mixture was allowed to stand for 10 min, centrifuged at 2500 rpm for 10 min and butanol layer was carefully pipetted out. The color intensity of the chromogen in butanol was measured at 560 nm against butanol (blank) using UV-Visible spectrophotometer (Perkin Elmer, USA). One unit of the enzyme activity was required to inhibit the optical density at 560 nm of chromogen production by 50% in 1 min under the assay conditions. The specific activity was expressed as IU mg protein^{-1}.

2.2.2 Catalase (EC.1.11.1.6.)

Catalase was measured according to Maehly and Chance[27]. The measurement was done spectrophotometrically following the decrease in absorbance at 230 nm. The reaction mixture contained phosphate buffer (0.01 M. pH 7.0), hydrogen peroxide (H_2O_2)(2 mM), and approximately diluted tissue extract. A system devoid of the extract formed the blank. The specific activity was expressed in terms of n moles of H_2O_2 decomposed/min/mg protein.

2.2.3 Glutathione Peroxidase (EC.1.11.1.9)

Glutathione peroxidase was measured according to Lawrence and Burck[26] with slight modification[1]. The assay volume contained 0.01 M phosphate buffer (pH 7.4) with ethylenediaminetetraacetic acid (EDTA), 1 mM sodium azide, 0.1 M nicotinamide adenine dinucleotide phosphate (NADPH), and 1 mM reduced glutathione and tissue extract. Blank contained all the reagents except tissue extract. Change in absorbance was measured at 340 nm at 15 sec intervals for 1 min. The enzyme activity was expressed as IU/mg protein.

2.2.4 Glutathione Content

Glutathione content was measured according to Benke and Cheevar[4]. The reaction mixture contained phosphate buffer, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the tissue extract. A system devoid of the tissue extract served as blank. Change in absorbance was measured at 412 nm within 10 min. The concentration of GSH was expressed as nmol/100 g tissue.

2.2.5 Glutathione Reductase (EC.1.6.4.2)

Glutathione reductase was measured according to David and Richard[7]. A fixed volume of tissue homogenate in phosphate buffer was mixed with a volume containing phosphate buffer, EDTA and glutathione disulfide (GSSG). Nicotinamide adenine dinucleotide phosphate was added prior to assay decrease in absorbance at 340 nm was measured spectrophotometrically. The enzyme activity was expressed as units/mg protein.

2.2.6 Glutathione–S-Transferase (EC.2.5.1.18)

Glutathione-s-transferase was measured according to Habig et al[21]. The cocktail contained phosphate buffer, 2,4-dinitrochlorobenzene (CDNB), reduced glutathione and tissue extract. The increase in optical density of the enzyme was measured against that of the blank at 340nm for 2 min. The enzyme activity was expressed as n moles of CDNB-GSH conjugate formed/minute/mg protein for tissue samples.

2.3 Estimation of Lipid Peroxidation Products

2.3.1 Malondialdehyde Content

Malondialdehyde was estimated according to the protocol of Nichans and Sannelson[30]. The homogenate was added to thiobarbituric acid-trichloroacetic acid-hydrochloric acid (TBA-TCA-HCl) buffer and heated in a boiling water bath for 15 min. Then it was centrifuged at 1000 rpm for 10 min and the absorbance was measured at 535 nm. The blank consisted of a TBA-TCA-HCl buffer. The level of MDA was expressed as µmoles/g tissue.

2.3.2 Conjugated Diene Content

Conjugated diene was measured according to Recknagel and Ghoshal[40]. The reaction mixture contained tissue homogenate and chloroform/methanol (2:1). It was centrifuged at 1000 rpm for 10 min; the lower layer was carefully taken out in a pipette. It was then dried at 45^0C in a water bath till the whole solution evaporated. To this, cyclohexane was added and the absorbance was measured at 233 nm. The enzyme activity was expressed as µmoles/g tissue.

2.4 Statistics

The statistical analysis was carried out using the software SPSS 22.0 package for Windows[43]. Data analysis was done by one-way analysis of variance (ANOVA). The difference in means were analysed by using Duncan multiple range test[13]. Significant level was $P<0.05$. The association of variables was studied by Pearson correlation test.

3. Results

3.1 Activities of Antioxidant Enzymes

There was a significant increase in the SOD activity in hepatic tissue after 5 and 15 days of exposure to 1.0 µL/L 4-NP but the activity decreased after 10 days of exposure when compared to 5 and 15 days. However,

on 1.5 µL/L and 2.0 µL/L 4-NP exposure, SOD activity increased significantly in the 5 days treatment group and the increase was maintained till 10 and 15 days with respect to control (Figure 1A). Catalase activity did not change significantly after 5 days exposure to 1.0 µL/L 4-NP. However, significant elevation was observed after 10 and 15 days of exposure to 1.0 µL/L 4-NP. Catalase activity showed a significant increase in the liver tissue of fish after 10 and 15 days of exposure to 1.5 µL/L 4-NP but there was no perceptible difference in the 5 days exposure group (Figure 1B). Similarly, exposure to 2.5 µL/L 4-NP increased CAT activity in the fish to significant levels after 5, 10 and 15 days. Furthermore, a significant positive correlation was found between SOD and CAT activities after exposure to 1.0 µL/L *(r = 0.7802)* 4-NP (Figure 2A), 1.5 µL/L *(r = 0.9684)* 4-NP (Figure 2B) and 2.0 µL/L (*r = 0.9887)* 4-NP (Figure 2C).

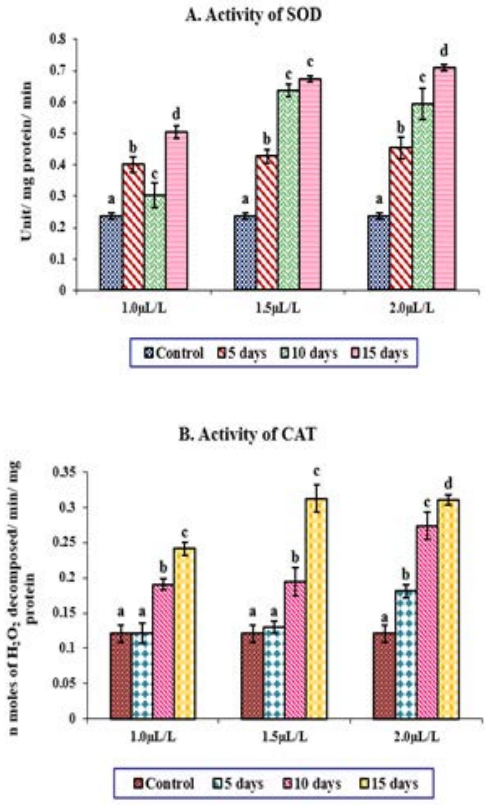


Figure 1. The effect of sub-lethal concentrations of 4-NP (1.0 µL/L, 1.5 µL/L and 2.0 µL/L) on the activities of SOD **(A)** and CAT **(B)** in the liver of *L. rohita*. Each bar is mean ± SEM of six fish. Means with different superscript letters for each parameter is significantly different at P<0.05.

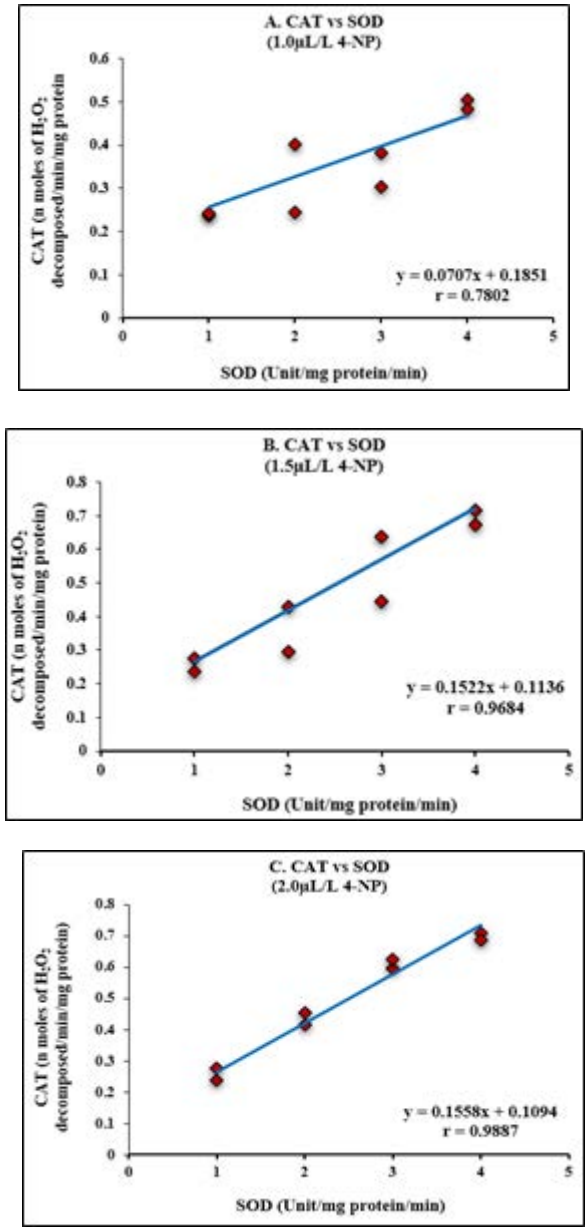


Figure 2. Correlation between CAT and SOD activities in the liver of *L. rohita* exposed to 1.0 µL/ L 4-NP, 1.5 µL/L 4-NP and 2.0 µL/L 4-NP (P<0.05).

One-way ANOVA showed that there was a significant decrease in GPx activity after 5, 10 and 15 days of exposure to 1.0 µL/L 4-NP (Figure 3A). Glutathione peroxidase activity significantly decreased after 5, 10 and 15 days of exposure to 1.5 µL/L 4-NP. Similarly, fish exposed to 2.0 µL/L 4-NP showed a significant decrease in GP_x activity after 5, 10 and 15 days as compared to control. There was a significant increase of R-value (SOD/GP_x+CAT) in the liver of *L. rohita* with respect to control when exposed to 1.0 µL/L, 1.5 µL/L and 2.0 µL/L 4-NP (Figure 3B).

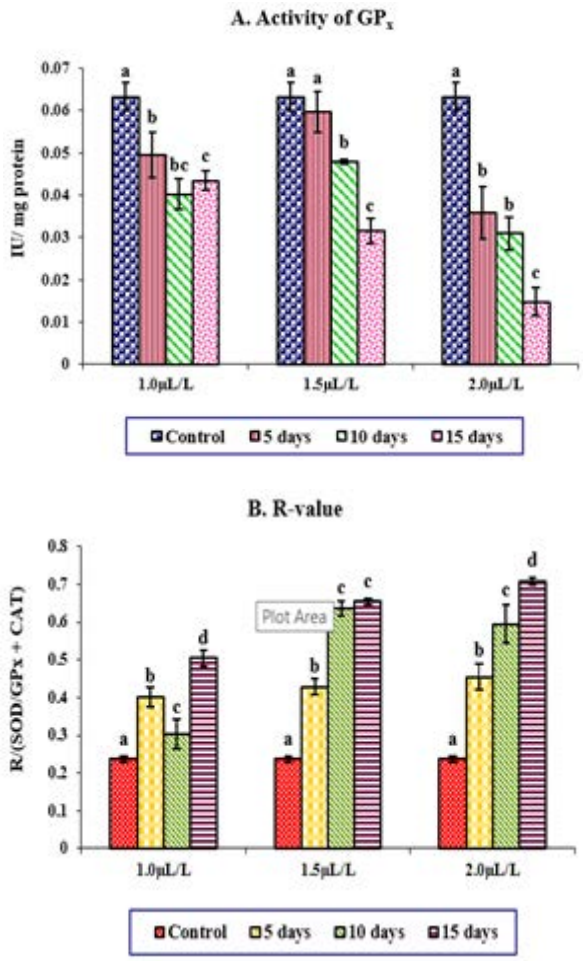


Figure 3. The effect of sub-lethal concentrations of 4-NP (1.0 µL/L, 1.5 µL/L and 2.0 µL/L) on the activity of GP_X **(A)** and on the level of R-value **(B)** in the liver of *L. rohita* in relation to control. Each bar is mean ± SEM of six fish. Means with different superscript letters for each parameter are significantly different at P<0.05

Fish exposed to 1.0 µL/L, 1.5 µL/L and 2.0 µL/L 4-NP had significantly depleted hepatic concentration of GSH after 5, 10 and 15 days in comparison with the control group (Figure 4A). There was a significant increase in the activity of GR to 1.0 µL/L and 2.0 µL/L 4-NP after 5, 10 and 15 days of exposure to 4-NP in a time-dependent manner. The GR activity increased significantly after 5 and 10 days of exposure but showed a significant decline after 15 days when compared to 5 and 10 days of exposure (Figure 4B). The activity of GST measured with CDNB as substrate decreased significantly in hepatic tissue of fish exposed to 4-NP for 5,10 and 15 days at all exposures in a dose- and time-dependent manner (Figure 4C).

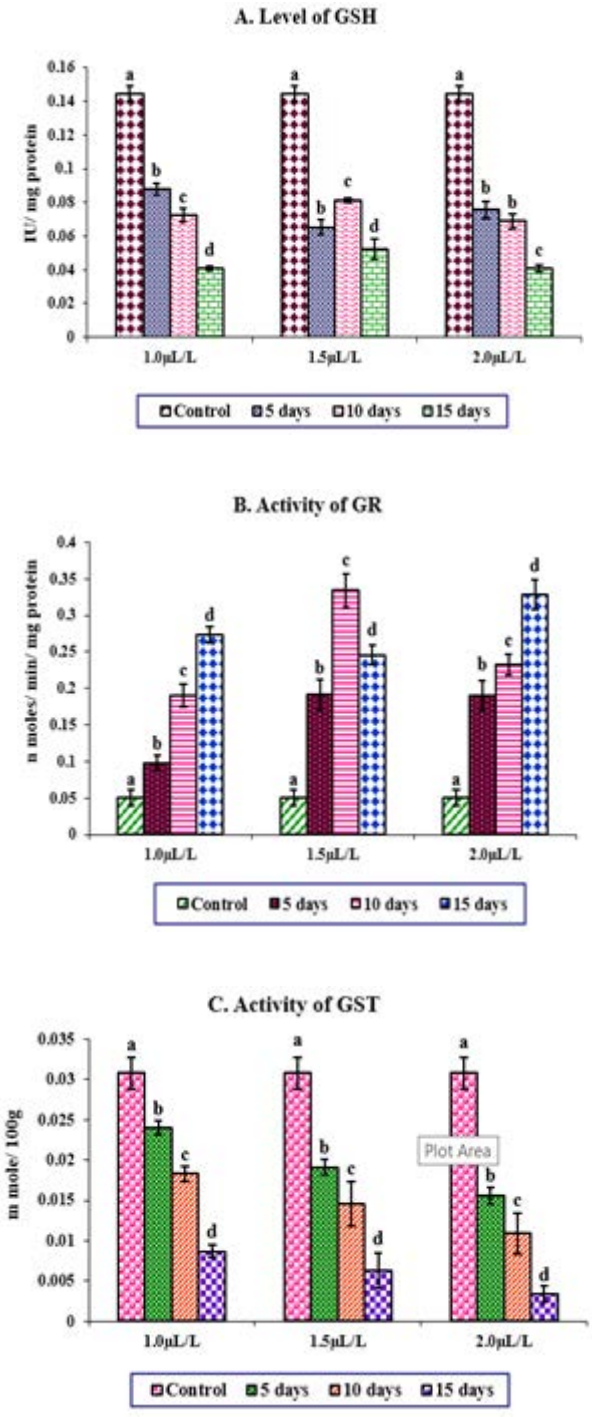


Figure 4. The effect of sub-lethal concentrations of 4-NP (1.0 µL/L, 1.5 µL/L and 2.0 µL/L) on the activity level of GSH **(A)** and on the activities of GR **(B)** and GST **(C)** in the liver of *L. rohita* in relation to control. Each bar is mean ± SEM for six fish. Means with different superscript letters for each parameter are significantly different at P<0.05.

3.2 Levels of Lipid Peroxidation Products

The hepatic concentration of MDA, which reflects the degree of lipid peroxidation illustrated a significant elevation in fish exposed to 1.0 µL/L, 1.5 µL/L and 2.0 µL/L 4-NP after 5, 10 and 15 days of exposure to 4-NP (Figure 5A). A significant positive correlation was observed between the R-value and MDA in fish exposed to 1.0µL/L *(r=0.9043)* 4-NP (Figure 6A), 1.5µL/L*(r=0.9993)*4-NP (Figure 6B) and 2.0µL/L *(r=0.9351)* 4-NP (Figure 6C).

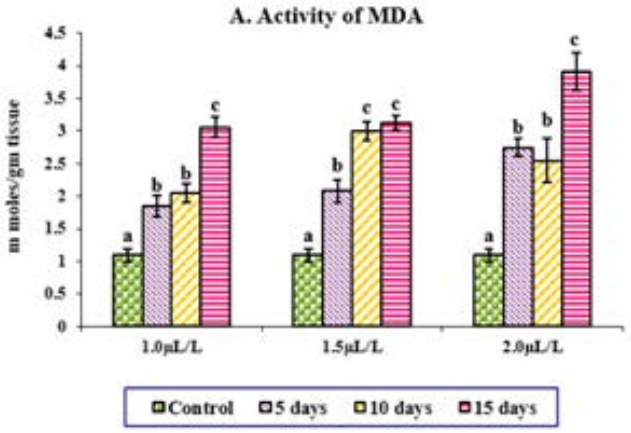


Figure 5. The effect of sub-lethal concentrations of 4-NP (1.0 µL/L, 1.5 µL/L and 2.0 µL/L) on the level of MDA **(A)**, in the liver of *L. rohita* in relation to control. Each bar is mean ± SEM for six fish. Means with different superscript letters for each parameter are significantly different at P<0.05.

Conjugated diene content increased significantly in a dose- and time-dependent manner to 1.0 µL/L, 1.5 µL/L and 2.0 µL/L 4-NP after 5, 10 and 15 days of exposure (Figure 7A). A significant positive correlation was found between the R-value and CD in fish exposed to 1.0 µL/L *(r=0.7803)* 4-NP (Figure 8A), 1.5 µL/L *(r=0.8736)* 4-NP (Figure 8B) and 2.0 µL/L *(r=0.9784)* 4-NP (Figure 8C).

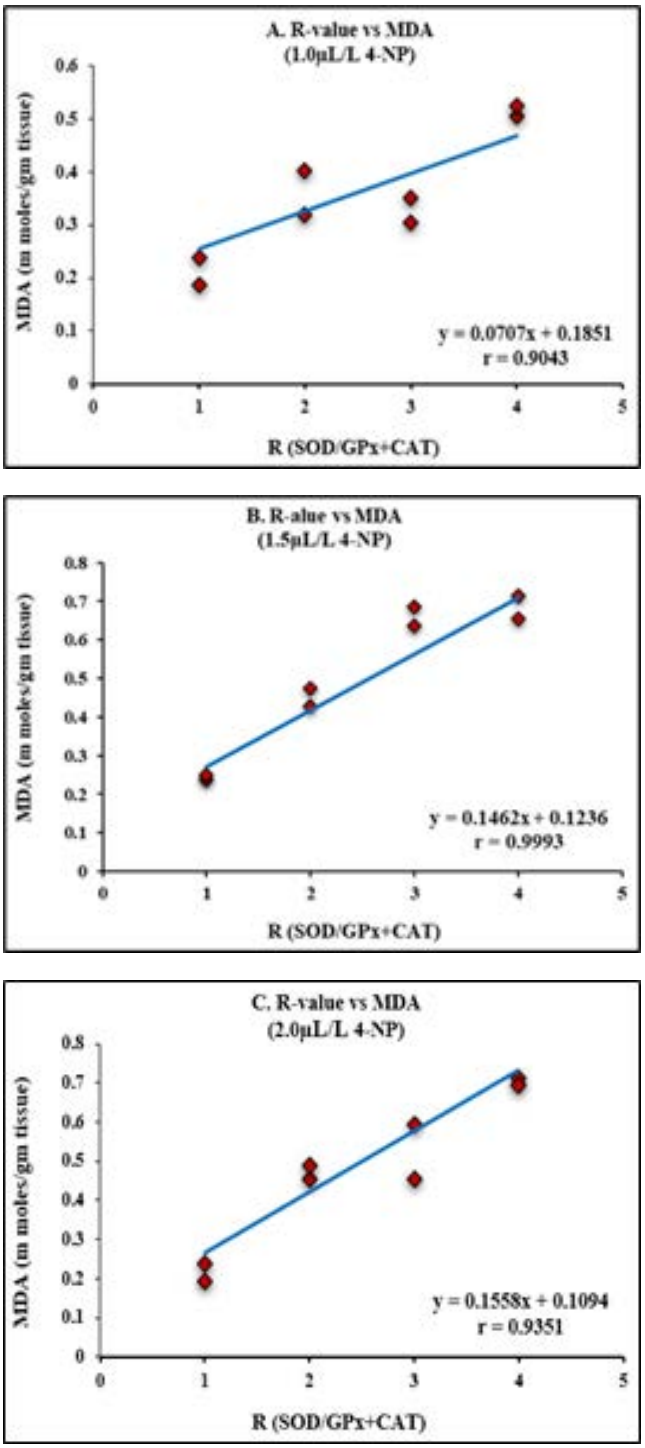


Figure 6. Correlation between the level of R-value and MDA in the liver of *L. rohita* exposed to 1.0 µL/L 4-NP, 1.5 µL/L 4-NP and 2.0 µL/L 4-NP (P<0.05).

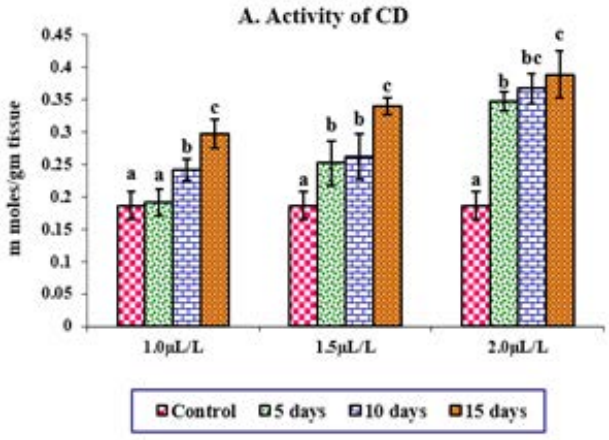
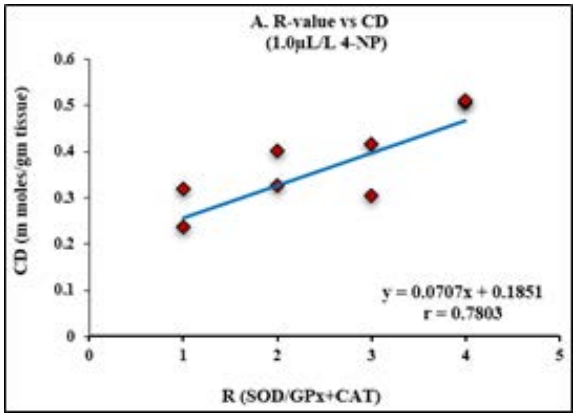
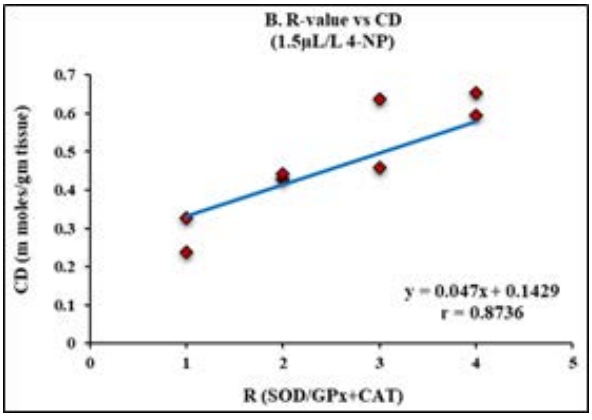


Figure 7. The effect of sub-lethal concentrations of 4-NP (1.0 µL/L, 1.5 µL/L and 2.0 µL/L) on the level of CD (A), in the liver of *L. rohita* in relation to control. Each column is mean ± SEM for six fish. Means with different superscript letters for each parameter are significantly different at P<0.05.





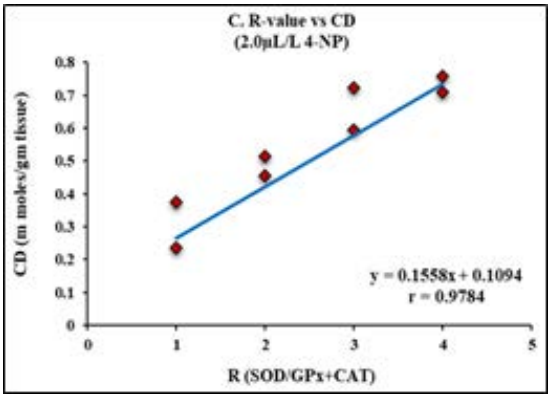


Figure 8. Correlation between the level of R-value and CD in the liver of *L. rohita* exposed to 1.0 µL/L 4-NP, 1.5 µL/L 4-NP and 2.0 µL/L 4-NP (P<0.05).

4. Discussion

Liver is one of the main detoxifying organs and is essential for both metabolism and excretion of toxic substances in the body. Hepatotoxic effects are now regarded as reliable biomarkers of toxic injury and are representative of biological endpoints of contaminant exposure. Oxidative stress caused by the imbalance of ROS production and depletion leads to various dysfunctions of cellular biomolecules such as lipids, proteins and DNA[10]. The present study describes the effects of 4-NP and compensatory responses of hepatic antioxidant enzymes, possibly activated to alleviate oxidative stress in *L. rohita*. Of all reported toxicities of 4-NP in animals, malfunction of the endocrine system is the best described[36]. Considerable attention has been paid to oxidative stress in relation to ecotoxicity[20,41].

In the present study, significant elevation of hepatic SOD activity was observed on exposure to various sub-lethal doses of 4-NP as compared to control. Superoxide dismutase catalytically scavenges superoxide radical, which appears to be an important agent of toxicity of oxygen (O_2) and it also provides defence against O_2 toxicity. Superoxide dismutase is the first line of defence against the free radicals where it converts superoxide into hydrogen peroxide. It is then degraded by other antioxidant enzymes such as catalase and glutathione peroxidase or reductase. Similar results were observed in *Cirrhinus mrigala* when subjected to acute and chronic

exposure to methanol[9]. Study conducted by Farombi and Adelowo, in *Clarias gariepinus,* reported SOD activity in the liver and kidney to increase when treated with butachlor[16]. The increased SOD level in liver tissue of *L. rohita* exposed to 4-NP indicates a detoxifying mechanism against the toxicity.

4-Nonylphenol stimulated the antioxidant system as evidenced by an increase in CAT activity. Enhanced CAT activity was found with elevated levels of pollutants, principally hydrocarbons and pesticides[42]. Catalase is the primary scavenger of H_2O_2 in the cell. Increase of CAT activity in this study indicates that pollution stress augments the rate of formation of H_2O_2. The elevation of CAT in the liver is due to its active role in biosynthetic and detoxification activities, which need extensive energy supply provided by oxidative metabolism[32]. The enhanced CAT activity was also verified in a study with the freshwater fish *Channa punctatus*[31] and with the neotropical fish *Astyanax altiparanae* used to assess water quality in an agricultural area[47]. Comparatively, the hepatic tissue of *L. rohita* displayed a higher variation in SOD activity than CAT activity.

It is evident that an abundant protective effect can be exerted by the SOD activity under the synergetic effects of CAT activity. Usually, a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants[11]. Hence, an increase in the activity of these antioxidant enzymes SOD and CAT arose under conditions of 4-NP, as an adaptive response. The antioxidant enzymes that make up the antioxidant defence system are expected to be intrinsically linked and dependent upon the activity of one another. Therefore, one could expect to see correlative changes in the activity of SOD and CAT[17]. Thus, a significant positive correlation was found between SOD and CAT activities after 1.0 µL/L *(r=0.7802)* 4-NP, 1.5 µL/L *(r=0.9684)* 4-NP, and 2.0 µL/L *(r=0.9887)* 4-NP exposure in the present study.

Glutathione peroxidase reduces lipid hydroperoxides into lipid alcohols coupled with GR. Glutathione content serves as a substrate for GP_x. In this study, the decreased GP_x activity in the liver of *L. rohita* was due to the decrease in the levels of GSH and increase in the levels of LPO during 4-NP exposure. The decreased activity of GP_x in the liver indicates its reduced capacity to scavenge H_2O_2 and lipid hydroperoxides, as it decreased the GSH conversion to GSSG[14]. Low GP_x activity may be due to increased CAT activity, both of which use H_2O_2 as substrate[49]. Glutathione peroxidase activity reduced continuously with respect to control values in Arsenic trioxide (As_2O_3)-induced oxidative stress in the liver of *Cyprinus carpio*[5].

There is evidence that a biological optimum exists for the ratio between SOD and GP_x+CAT activities (R), which might be more relevant than the absolute activities of the enzymes[8,12]. Changes in the equilibrium between the formation of H_2O_2 from superoxide dismutation and its decomposition by other enzymes (GP_x, CAT) in erythrocytes is expressed by the ratio R= SOD/(GP_x+CAT)[18]. It has further been postulated that an altered SOD-to-(GP_x+CAT) ratio affects gene expression by affecting the binding and/or availability of transcription factors to DNA[8,12]. There is a significant elevation in the R-value (SOD/GPx+CAT) in the liver of *L. rohita* with respect to control when exposed to 1.0 µL/L (*r*=0.9043) 4-NP, 1.5 µL/L (*r*=0.9993) 4-NP and 2.0 µL/L (*r*=0.9351) 4-NP. This clearly indicates that exposure of 4-NP induced antioxidant enzyme disequilibrium in hepatic cells. Correlations between SOD, CAT and GP_x were studied in the liver of *C. carpio* of Karakaya Dam Lake in Turkey[49]. From the correlation studies of this work, it is revealed that the combined action of SOD, CAT and GP_x in the liver may be responsible for combating the pollution stress and restoration to normalcy.

Glutathione is responsible for protection against reactive oxygen and nitrogen species and detoxification of endogenous and exogenous toxins of electrophilic nature[33]. Glutathione content was depleted in the liver of *L. rohita* after exposure to 4-NP. It has been reported that severe oxidative stress may suppress GSH levels due to the impairment of adaptive mechanisms[50]. Similar to the present study, Tanu et al[44] have reported a high rate of GSH decrease in the gill, liver, kidney and brain of the freshwater fish *Channa punctatus* exposed to arsenic. Maiti and Chatterjee, reported that after acute exposure to arsenic in rats, GSH concentration was significantly reduced[28].

Glutathione-S-transferase activity decreased significantly in 4-NP exposed fish in a time- and dose-dependent manner. Increased GST activity has been demonstrated in liver tissues following exposure of African Sharptooth Catfish (*C. gariepinus*) to a mixture of 17α-ethynylestridiol and benzopyrene[38]. Teles et al[45] reported that GST activity increases in the liver of the fish *Sparus aurata* L exposed to estradiol (E_2). Decreased GSH level and GPx activity and an increase of GST activity in the liver might suggest a critical role of glutathione-

mediated enzyme function against the deleterious effects of 4-NP.

It was observed that GR, the enzyme that regenerates GSH by reducing oxidised glutathione (GSSG), was increased when exposed to the sub-lethal doses of4-NP. In a study conducted by Kwan,[25] in Far Eastern Catfish, *Silurus asotus*, exposed to 4-NP the GR activity of the liver increased. Glutathione reductase differs from CAT in that it is capable of reacting with both lipid and H_2O_2[22].

Lipid peroxidation is one of the main processes induced by oxidative stress. It concerns oxidative deterioration of polyunsaturated lipids in the membranes of cells and organelles. The results showed high levels of MDA in the liver of exposed fish. Malondialdehyde is produced by LPO and considered as an indicator of oxidative stress, which results from the free radical damage to membrane components of cells[3]. Cadmium treatment increased cellular activities such as MDA production in the clam *Ruditapes decussatus*[19]. A significant increase in lipid peroxidation as MDA formation was observed in the liver, kidney, gills and heart of the fish following exposure to butachlor[16]. 4-Nonylphenol-induced MDA production could be due to the impairment of the tissues' natural protective system and could be directly related to the GSH depletion.

In the present study, CD in the liver of *L. rohita* exposed to 4-NP was found to be significantly increased over control. Conjugated diene represents the initial product of radical attack, a rearrangement of the double bonds in unsaturated fatty acids[37]. Conjugated diene, a lipid oxidation marker, increased significantly in grey mullet, *Mugil cephalus*, exposed to heavily polluted Ennore estuary, Chennai[35].

A significant positive correlation was observed between the R-value and MDA and between R-value and CD in the liver. From these results, it can be conceived that disequilibrium of antioxidant enzymes would lead to increased lipid peroxidation as evidenced by the increase in lipid oxidation markers like MDA and CD levels. Such a correlation between R-value and LPO and increase in ratio of activities of antioxidant enzymes were also reported in humans suffering from Down's syndrome[18] and was also associated with aging[12].

The findings in this study carry importance because the 4-NP concentrations to which the experimental fish were exposed were relatively low compared with those practiced by other investigators. For example, most studies have delivered 4-NP in the range of a few milligrams to several hundred milligrams. On the other hand, the present study used 1.0 µL/L, 1.5 µL/L and 2.0 µL/L of 4-NP. The results indicate induction of oxidative stress and the failure of antioxidant enzymes to remove free radicals from hepatic cells. It was confirmed by the reduction in the activities of some of the antioxidant enzymes and the increase in the level of lipid peroxidation in the liver of *L. rohita*. To sum up, observations of the present study indicate that 4-NP at sub-lethal concentrations when exposed for short durations alters the antioxidant defence system in hepatocytes of fish, and thereby engages as an endocrine disruptor.

5. Conclusion

From the present study, it has been unambiguously revealed that exposure of 4-NP to *L. rohita* induces oxidative stress in the hepatocytes mainly through increasing activity levels of enzymatic antioxidants such as, SOD, CAT, and GR and decreasing the levels of non-enzymatic antioxidants such as GSH which might be related to the down-regulated activity of the enzymes GPx and GST. The present study is mainly focused on the assessment of quantitative changes of the antioxidant enzymes and LPO products in the liver of experimental fish in order to gain insight into the antioxidant ability of the subject towards environmental pollution by the test chemical. Moreover, there was a definite interrelationship between antioxidant enzymes and LPO products which also substantiate that imbalance in antioxidant enzymes leads to increased LPO products such as MDA and CD. The alterations in the level of enzymatic and non-enzymatic antioxidants and LPO products in the present investigation reflect the differential effects of pollution stress, which can be considered as biomarkers of exposure and subsequently as tools for biomonitoring in the assessment of endocrine disrupting environmental pollution by 4-NP. Hence, the delicate balance among the antioxidant enzymes is disturbed which may lead to perturbations of redox status. The results clearly show that 4-NP is a pollutant with oxidative potential.

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7. References

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