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Effect of Chlorpyrifos on Antioxidant Enzyme Activities and Lipid Peroxidation in Liver and Gills of Zebrafish

Bestha Lakshmi, Rakesh Kullayi Swamy and Gundala Harold Philip*

Department of Zoology, Sri Krishnadevaraya University, Anantapur – 515003, Andhra Pradesh, India; gundalahphilip@gmail.com

Abstract

Chlorpyrifos (CP) is a widely used and studied organophosphorous pesticide for more than a decade to control pests in agriculture, including termites, mosquitoes, flies and other household pests. Because of the extensive use of CP, besides destroying the target pests also affecting the non-target organisms like human, fish and finally finding their way to the aquatic environment. Fish is not only a vital source of food for people but also an important component of ecosystem and is shown to be sensitive bioindicator of external factors, such as pollution. In the present investigative study zebrafish, (*Danio rerio*) an excellent model for toxicological studies was exposed to CP (200 µg/L) and the oxidative parameters like catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Glutathione-s-transferase (GST) and Lipid Peroxidation (LPO) in liver and gill tissues of zebrafish were assessed. The antioxidant enzyme parameters were significantly decreased in the treated groups than in control with concomitant increase in detoxifying processes i.e., GST and LPO. The results are discussed in detail.

Keywords: Antioxidant, Chlorpyrifos, Lipid Peroxidation, Zebrafish

1. Introduction

Chlorpyrifos (CP) (O, O–diethyl O-3, 5, 6-trichloro-pyridin-2-yl-phosphorothioate), is an organophosphate insecticide, acaricide and miticide used to control foliage and soil-borne insect pests on a variety of food and feed crops and other household pests for more than a decade[1]. Occupational exposure to Chlorpyrifos affects various aspects of the peripheral nervous system[2]. Traces of CP and its metabolites were also found in human secretions. A study on Organophosphate Pesticide (OP) exposure in school-aged children living in rice and aquaculture farming regions of Thailand revealed the presence of OP urinary metabolites i.e., Dialkylphosphates [DAPs] and 3,5,6-trichloro-2-pyridinol [TCPy] in significant levels[3]. The CP toxicity is so significant that Dow AgroSciences, with the support of the Environmental Protection Agency (EPA) convened an independent panel of scientists to address these concerns[4].

CP is reported to show various detrimental effects in fish such as neurotoxicity via acetylcholinesterase inhibition[5], biochemical and histopathological alterations[6], oxidative stress[6,7], genotoxicity[8] and olfactory and neurobehavioral injuries[9]. Zebrafish, *D. rerio* is known as an excellent model for toxicological studies. The literature reviews the significant outcomes of the CP and its metabolites effects on zebrafish. Majorly CP is known to effect the developmental stages i.e., larvae of zebrafish with marked morphological deformations, long-term effects on response latency and spatial discrimination, developmental neurotoxicity, dopamine depletion and AChE inhibition in adults and significant reductions in swimming activity[10]. Besides the neurotoxicity and developmental defects of CP, is known to get deposited in zebrafish muscle which is an indicative of induction of oxidative stress and of muscle exhaustion[11].

In light of the toxic effects of CP reported, the present investigation is designed to study the effect of CP

*Author for correspondence

on oxidative parameters like catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Glutathione-s-transferase (GST) and Lipid Peroxidation (LPO) in tissues of zebrafish.

2. Materials and Methods

Maintenance of zebrafish and preparation of stock solution was done as described by Philip and Manjunatha[10].

2.1 Experimental Design

Mature healthy and adult male and female zebrafish in equal ratio (n = 8) were taken from our aquarium stock and kept in 20 L glass aquaria with a stocking density of one fish per 2 liters of water. Fishes were divided into 3 groups where Group 1 acted as control group (pesticide/solvent-free water), fish in Group 2 served as the solvent control (only solvent) and fish in Group 3 were added with toxicant CP, 200 µg/L. The toxicant exposure in the present study was conducted for a period of 96 hrs (4 days). After the stipulated period both control and experimental fishes were dissected to collect the liver and gills and further processed for the assays of antioxidant enzymes.

2.2 Sample Preparation and Antioxidant Enzyme Assays

Sample preparation for antioxidant enzyme assays was done as described by Reshma and Philip[12]. Catalase activity was assayed according to the method of Sinha[13], SOD activity was assayed as described by Misra and Fridovich[14], GPx activity was assayed by the Rotruck et al.[15] method, GR activity was estimated by the method of David and Richard[16] and GST activity was assessed by Habig et al.[17] method. LPO levels were studied by the method of Ohkaawa et al.[18].

2.3 Statistical Analysis

Mean, standard deviation and level of significance were calculated for the data obtained from the triplicates of the three groups independently. Data was analyzed using one-way ANOVA with Tukey's test. P-values <0.5 were considered significant.

3. Results

No mortality was recorded in all control and experimental groups during the CP exposure period but reduced feeding, some abnormal spontaneous movements, low touch response and some fishes showed loss of pigmentation on day 3 and 4. After the exposure period of 96 hrs, the activity of CAT, SOD, GPx and GR were found to be significantly lower than the control fishes whereas GST and LPO was increased in the experimental group fishes.

3.1 Effect of CP on CAT

The catalase enzyme activity was found to be significantly lower in both liver and gills, with slightly higher activity in liver. Catalase activity in the control samples was 30.4% and 35% higher than in the exposed liver and gill tissues respectively (Figure 1).

3.2 Effect of CP on SOD

SOD activity was higher in treated liver tissue than the treated gill tissue, whereas both the treated tissues were showing significantly lower SOD activity than the control (Figure 2). Control groups were showing 55.4% and 45.1% higher activity over the treated groups in liver and gills respectively.

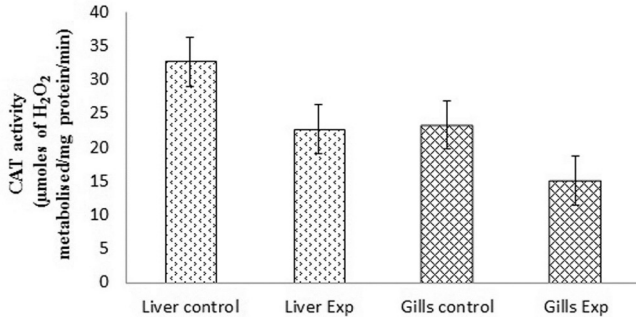


Figure 1. CAT activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean ± SD., n = 8 ($p < 0.05$).

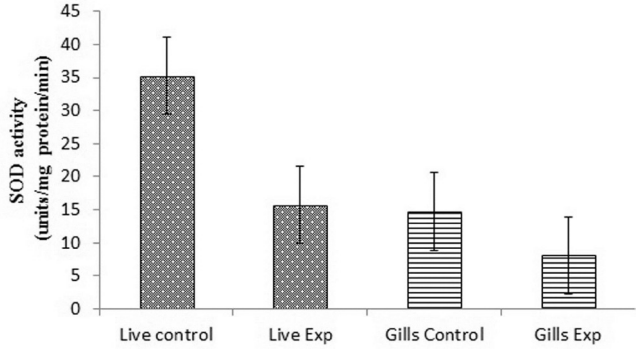


Figure 2. SOD activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean ± SD., n = 8 ($p < 0.05$).

3.3 Effect of CP on GPx

Both the treated liver and gill tissues were showing a significantly lower GPx activity than the control. The GPx activity was higher in liver compared to gill tissue (Figure 3). The significant increase of GPx activity in control over the treated was 38.9% and 34.5% for liver and gills respectively.

3.4 Effect of CP on GR

GR activity was higher in control than the treated and was significantly higher in Liver control (Figure 4). Control groups were showing an increase of 29.9% and 14.2% for treated liver and gill tissues.

3.5 Effect of CP on GST

The GST activity was found to be higher in treated over the control, whereas the activity was higher in treated liver than gill tissues (Figure 5). Treated groups were showing increased GST activity of 43% in liver and 28.6% in gills than the treated.

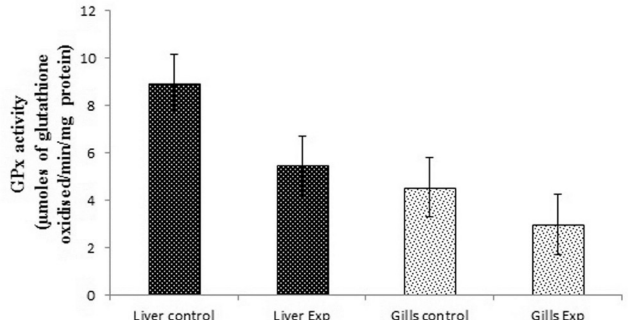


Figure 3. GPx activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean ± SD., n = 8 ($p < 0.05$).

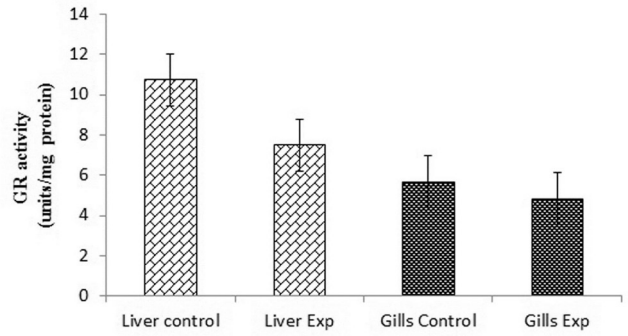


Figure 4. GR activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean ± SD., n = 8 ($p < 0.05$) .

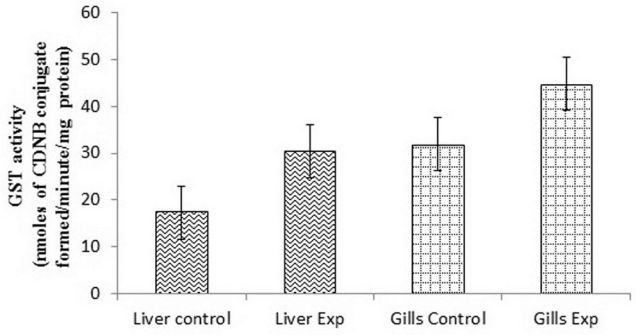


Figure 5. GST activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean± SD., n = 8 ($p<0.05$).

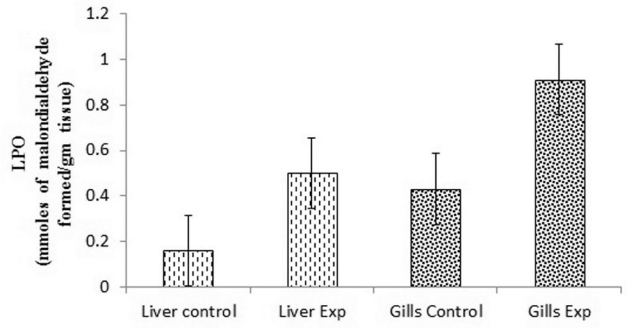


Figure 6. LPO activity in liver and gill tissues of zebrafish exposed to CP at 200 µg/L for 96 hrs. Data is presented as mean± SD., n = 8 ($p< 0.05$).

3.6 Effect of CP on LPO

LPO was significantly higher in treated groups over the control (Figure 6). It was found to be 68% for liver and 52.7% for gill higher than the control.

4. Discussion

Oxidative stress is considered as the most common categories of response in zebrafish when exposed to many pathological factors, particularly in toxicological studies[19]. Generation of Reactive Oxygen Species (ROS) occur in the oxidative stress as a result of the toxicant exposure[20]. Depending upon the toxicant and its varying concentrations the generations of ROS varies, which creates an imbalance between these oxidants (ROS) and antioxidants finally resulting in the failure of antioxidant defense system leading to the damage of cellular components[21].

SOD is mainly involved in removal of superoxide radicals by converting them into H_2O_2, O_2 and Catalase enzyme degrades the H_2O_2 to water and oxygen[22]. Both the

catalase and SOD enzymes were found to be decreased in the treated over control with higher activity in liver than gills. The decrease in these enzyme activities may be due to the disruption of the enzyme structure due to the high concentration of pesticide and high influx of the superoxide radicals. GPx is another important peroxidase which works together with catalase in eliminating H_2O_2[23], acts through glutathione-dependent reduction of hydroperoxides and hydrogen peroxide. Decrease in GPx activity was found in the treated liver and gill tissues over control in the present study.

GSH is a major cytosolic reducing agent for the SH-group and offer a wide range protection against pollutants[24]. It directly scavenges the oxyradicals and ROS and serves as substrate and cofactor for the detoxifying enzyme, GST[25]. GSH activity was found to be reduced in treated liver and gill tissues with higher activity in liver over gills. Similar results were shown in liver and gill tissues of *C. carpio*[26] and *C. auratus*[24] on exposure to pesticide. Prolonged exposure of *C. idellus* to sub-lethal concentrations of CP also showed decreased GSH activity[27]. The decreased GSH activity can be attributed for excessive utilization of GSH in scavenging the toxic ROS generated in the cells due to the high concentration of CP. Further, the GSH depletion is explained as its exhaustion phase II biotransformation indicating reduced cell protection activity[28].

GSTs are group of multifunctional isoenzymes that catalyze the detoxification reactions by conjugating reduced glutathione to various substrates and protecting the cell from oxidative stress[29]. The activity of GST was higher in CP treated groups indicating increased rate of detoxification. It was higher in liver compared to gills. The decreased GSH activity can be correlated to increased activity of GST, where the GSH was acting as substrate in the GST reactions to eliminate superoxide radicals and hydrogen peroxide.

LPO is another consequent mechanism of oxidative stress and is known as biomarker for ROS production[30]. Increased generation of free radicals results in lipid peroxidation which is a common molecular mechanism[31]. Present experimental study showed an increased LPO levels in treated liver and gills tissues indicating excessive generation of ROS. As liver is the site of detoxification it is showing a higher LPO levels and GST activity.

Similar results were observed in *G. affinis* which was exposed to lethal concentrations of CP for 96 hrs where the activity of SOD, CAT and GR was decreased with elevated levels of LPO[7]. In guppy, *P. reticulate* exposed to sublethal concentrations of CP for 96 hrs showed fluctuated levels of CAT, SOD, GST and GR with increased levels of GSH in brain and gills whereas LPO levels were found to be higher in brain, gills, liver but maximum raise in gills[32]. Elevated GST activity was observed in *O. niloticus* treated with CP, indicating its role in better protection against pesticide toxicity[33].

Increased LPO and SOD activity was observed in *C. carpio* exposed to sublethal concentrations of CP, with decreased activity of CAT, GPx. Significant increased SOD activity was also reported in *O. niloticus* and carp tissues exposed to CP[26,33]. A decrease in the activity of antioxidant enzymes with a concomitant increase in LPO levels in the present study can be explained as the impairment of antioxidant defense mechanisms due to the excessive generation of free radicals as a result of CP exposure. Similar results were reported by Kavitha and Rao[7] in *G. affinis* exposed to CP (297 µg/lit). Prolonged exposure to CP also resulted in similar effects in *C. idellus*, where an increasing trend in LPO activity and a decline in the activity of CAT, SOD, GSH and GST[27].

5. Conclusion

A significant decrease in these enzymes is observed in the present study indicating the excessive generation of ROS which is also strongly supported by increased levels of LPO and GST activity which scientifically explain the mechanism of defense against oxidative stress induced by CP in zebrafish. The high concentration of CP i.e., 200µg/lit induced generation of ROS where the antioxidative enzymes like CAT, SOD, GPx and GSH cannot be able to neutralize their effect resulting in higher GST and LPO levels. Research studies at molecular level including the transcriptomics and metabolite analysis in the Chlorpyrifos exposed liver of zebrafish are under progress.

6. Acknowledgements

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