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Short Communication

Sub-lethal Effects of Chlorpyrifos on Glutathione S-Transferase Activity and Total Protein Contents of Fish, *Labeo rohita*

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QS executed the research work. SA planned the research. KA was member of supervisory committee. HN helped in statistical analyses. LS and QL wrote the manuscript.

Key words
Fish, biochemical markers, sub-lethal exposure, protein content, Chlorpyrifos glutathione S-transferase, *Labeo rohita*

ABSTRACT

In this work, sub-lethal (1/3rd, 1/5th and 1/6th of LC_{50}) effects of chlorpyrifos (CPF) on biochemical markers such as glutathione S-transferase (GST) activity and total protein contents in various tissues (gills, hepatic, renal, brain, muscle and cardiac) of *Labeo rohita* was determined. Fish was exposed for 60-day and sampling was done after 7-day. Results showed that the GST activity was considerably increased in *L. rohita* as compared to control. The GST activity was enhanced in various tissues (hepatic, brain, cardiac, gills, renal and muscle) of CPF treated fish as compared to control group. Comparison among different concentrations indicated that 1/3rd of LC_{50} caused greater increase in GST activity followed by that of 1/5th and 1/6th. The GST activity in selected tissues of fish varied with duration of exposure as 28>21>35>42>14>7>49>56. Total protein contents in all selected tissues of CPF exposed fish were decreased with the passage of time. Among all the concentrations, 1/3rd of LC_{50} cause greater decrease in protein contents. The total protein contents in various tissues of *L. rohita* followed the order: muscle>hepatic>brain>gills>renal>cardiac.

Chlorpyrifos (CPF) is a commonly used organophosphate (OP) pesticide, which is applied to kill the pest in agricultural, residential and commercial settings (Rusyniak and Nanagas, 2004; Wu and Laird, 2003). It is more lethal to aquatic species specifically to fish rather than other insecticides (Tilak *et al*., 2001). It is investigated that CPF is involved in various mechanisms like causing genotoxicity (Mehta *et al*., 2008), hepatic dysfunction (Poet *et al*., 2003), and changes in neurochemical and neurobehavioral mechanisms (Slotkin *et al*., 2005; Verma *et al*., 2009; Ojha *et al*., 2011). Oxidative damage caused by pesticides has become a popular toxicological research topic as a potential toxicity pathway (Abdollahi *et al*., 2004; Sharma *et al*., 2005). A defensive system is required to defend biochemical pathways from the damaging consequences of reactive oxygen species (ROS).

Fish are gifted with such defensive systems which diminishes the influence of ROS. The ROS are by-product of various metabolic compounds. There are several antioxidant enzymes viz, glutathione S-transferase (GST), glutathione peroxidase (GPOx), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). In addition to these enzymes, there are low molecular weight organic compounds which act as antioxidant such as vitamin A, ascorbate (vitamin C) and glutathione (GSH) (Mates, 2000; Van der Oost and Beyer, 2003). CPF is widely used in Pakistan for pest control, the aim of current study was to investigate the sub-lethal effects of CPF on biochemical markers such as GST activity and total protein contents in various tissues of *Labeo rohita.*

Materials and methods

The experimental fish (*Labeo rohita*) were procured from Fish Seed Hatchery, Faisalabad and transferred to Fisheries Research Farm, University of Agriculture, Faisalabad. After that juveniles of experimental fish were kept in cemented tank and adapted to laboratory conditions for 2-week. The experiment was carried out in triplicates in aquarium, which is made up of glass and have a capacity of 100 L water. The 10 juveniles were placed in each aquarium. The LC_{50} value (96h) of CPF for *L. rohita* was calculated as 16.53 mgL^{-1} (Illyas, 2015). *L. rohita* were exposed to different sub-lethal (1/3rd, 1/5th and 1/6th

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of LC_{50}) concentration of technical grade CPF for 2-month and sampling was done after 7 days. The physico-chemical parameters of water such as total hardness (250 mgL^{-1}), temperature (30ºC), and pH (7.00) were kept constant throughout the experimental period. However, CO_2, Na, Ca, K, Mg, NH_3 and electrical conductivity (EC) were also measured (APHA, 2005). Air pump with capillary system is used to supply continuous air to all the test and control mediums. After each sampling, GST activity and total protein contents were measured in different tissues viz., cardiac, hepatic, renal, gills, brain, and muscle of both control and treated fish. The GST activity was calculated by observing obsorbance of the conjugated molecules of GSH with 1-chloro, 2, 4-dinitrobenzene (CDNB) at 340 nm (Mannervik, 1985). One unit of enzyme make conjugated molecule with GSH with 10.0 nmol of CDNB per minute at 25ºC. Total protein content was measured by Biuret method (Gornall *et al*., 1994).

The obtained data was analyzed by suitable methods of statistics (Steel *et al*., 1997). ANOVA was applied to compare the variables of both treated and control fish.

Results

The present study showed that selected tissues of CPF treated *L. rohita* had increased GST activity compared to control group. The GST activity in tissues of treated fish followed the order: hepatic>gills>renal>brain>cardiac>muscle. During the first 28-days GST activity was enhanced and then declined up to 56-day (Fig. 1). Comparison among different concentrations showed that 1/3rd of LC_{50} had greater impact on GST activity followed by 1/5th and 1/6th.

Results showed that total protein contents of kidney, heart, gills, liver, brain and muscle of *L. rohita* was considerably decreased after exposure to sub-lethal concentrations (1/3rd, 1/5th and 1/6th of LC_{50}) of CPF in contrast to untreated group. Total protein contents in tissues of *L. rohita* followed the order: muscle>hepatic>brain>gills>renal>cardiac (Fig. 2).

Discussion

CPF has broadly and successfully been used all over the world. In fish, oxidative stress has been caused by pesticide exposure and other pollutants (Taju *et al*., 2014; Sinhorin *et al*., 2014). In fishes, oxidative stress takes place by two ways, firstly due to unnecessary buildup of the ROS. Secondly, when the critical ratio between anti-oxidants and oxidants is disturbed, due to decline in the ration of antioxidants. Both of these mechanisms ultimately cause damage (Scandalios, 2005). In aquatic species, ROS causes alterations in antioxidant enzyme systems which play important role in scavenging of these free radicals (Livingstone, 2001). GSTs play protective role against these free radicals (Blanchette *et al*., 2007; Frova, 2006). Various studies reported the mechanism of enzyme action

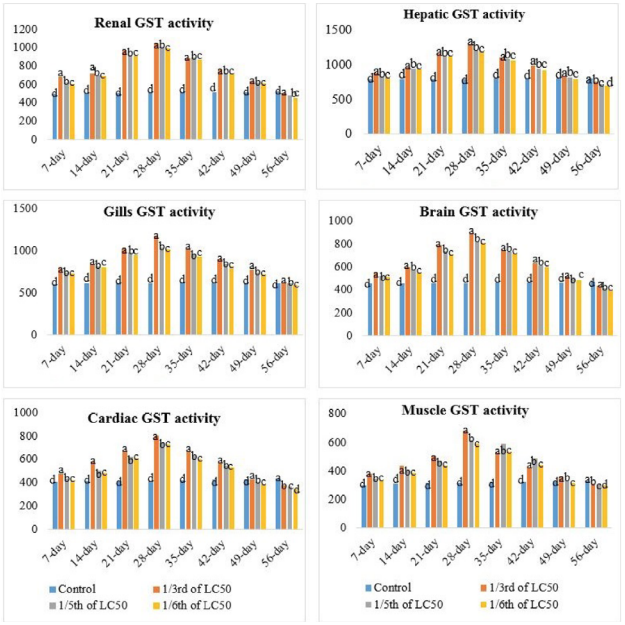

Fig. 1. GST activities in gills, heart, cardiac, renal, brain and muscle of *Labeo rohita* under sub-lethal exposure of chlorpyrifos for different time intervals.

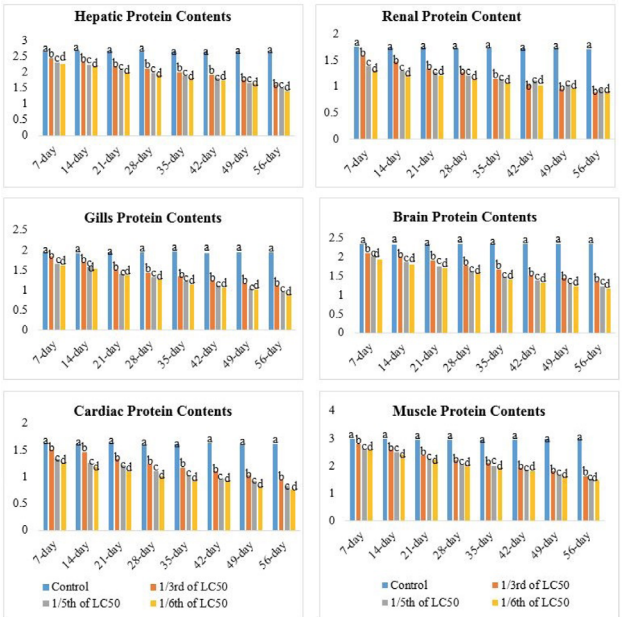

Fig. 2. Total protein contents in gills, cardiac, liver, renal, brain and muscle of *Labeo rohita* under sub-lethal exposure of chlorpyrifos for different time intervals.

and detoxification of free radicals in fish (Mortensen and Arukwe, 2007; Monferran *et al*., 2008).

In this study the GST activity was stimulated in various tissues of CPF treated fish when compared to that of control group. GST enzyme is concerned with the detoxification of various xenobiotics, hence the activation of GST enzyme has been considered as a pollutant indicator. It has been reported by various studies that hepatic tissue showed enzymatic induction under pesticide exposure in fish. Therefore, GSTs are vital in detoxification and elimination of electropositive compounds from the body (Peebua *et al*., 2007; Rao, 2006).

Hepatic and renal tissues showed greater response towards pesticide toxicity because they are vital organs in detoxification and elimination pathways (Abdollahi *et al*., 2004). The greater sensitivity of these tissues is vital for controlling the balance between oxidants and antioxidants under pesticide exposure (Sharma *et al*., 2005; Mates, 2000). CPF is processed by microsomal enzymes having oxidative activity at active oxons which ultimately causes oxidative damage (Albores *et al*., 2001; Tang *et al*., 2001). According to Keramati *et al*. (2010) exposure of CPF and methyl parathione altered the GST activity in all the chosen tissues of *Oreochromis niloticus*. Similarly, *O. niloticus* showed enhanced GST activity under CPF exposure (Egaas *et al*., 1999).

L. rohita exposed to CPF showed significantly higher GST activity in all selected tissues when compare with control. Yonar (2013) reported that treatment with OP pesticide (malathion) showed variations in GST activity in gills, hepatic and renal tissues of carp. Sharbidre *et al*. (2011) described alterations in GST activity in gills, hepatic and muscle tissues of *P. reticulate* when exposed to OP pesticide (methyl-parathion and CPF) concentrations ($1/4^{th}$, $1/8^{th}$ and $1/10^{th}$ of LC_{50}).

Xing *et al*. (2012) observed significant changes in renal and brain GST activity of *Cyprinus carpio* under the long term administration of CPF and atrazine. Oruc (2010) reported that sub-lethal concentrations (5, 10 and 15 ppb) of CPF showed significant increase in GST activity after 30 days in *O. niloticus*. Similarly, Karmakar *et al*. (2016) investigated the enhanced GST activity in gills, hepatic and renal tissues of *L. rohita* under sub-lethal (18.12 mgL^{-1} to 105.2 mgL^{-1}) exposure of malathion.

In current study, protein contents in different tissues of *L. rohita* declined under sub-lethal concentrations ($1/3^{rd}$, $1/5^{th}$, $1/6^{th}$) of CPF. CPF is found to be more toxic for fish (Ali *et al*., 2009). In the presence of OP pesticide protein content is reduced either due to the inhibition of protein synthesis or increased degradation/oxidation of proteins by ROS (Tilak *et al*., 2005; Tripathi and Shasmal, 2010). Glucose is synthesized by the metabolic consumption of keto acids which ultimately result in the depletion of protein contents (Vutukuru, 2005; Venktramana *et al*., 2006; Muley *et al*., 2007; Kumari, 2007; Chezhian *et al*., 2010) and related alterations were also investigated in *C. punctatus* under exposure of technical grade malathion (Agrhari *et al*., 2006).

Conclusion

It is concluded that chlorpyrifos significantly enhance the GST activity and reduce the protein contents in different tissues of fish. Furthermore, these parameters of fish can be used as a valuable biomarker of insecticides toxicity in water bodies.

Statement of conflict of interest

The authors have declared no conflict of interest.

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