Punjab University Journal of Zoology

35(1): 25-29 (2020)
https://dx.doi.org/10.17582/journal.pujz/2020.35.1.25.29

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Research Article

Changes in Glutathione S-transferase Activity and Total Protein Contents of *Labeo rohita*

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Article History
Received: July 26, 2018
Revised: January 24, 2020
Accepted: February 05, 2020
Published: April 30, 2020

Authors' Contributions
QS executed the research work. SA supervised and guided in planning the research. HN and LS helped in statistical analysis. KA was member of supervisory committee and facilitated the author in conducting the research in his laboratory.

Keywords
Fish, Chronic exposure, Antioxidant enzyme, Pesticide

Abstract | This work was performed to evaluate glutathione S-transferase (GST) activity and total protein contents (TPCs) in tissues viz. brain, gills, kidney, heart, muscle and liver of *Labeo rohita* kept under sub-lethal dose (4.13 μgL^{-1}) of chlorpyrifos. Fish was kept under chlorpyrifos stress for two months and samples were collected on weekly basis. It was noted that GST level varied significantly with duration. The GST level was raised in first 28 days after that it was dropped off up to 56-day. The trend of GST in fish tissues was observed as muscle<heart<brain<kidney<gills< liver. However, TPCs in selected tissues of stressed fish was lower significantly as compared to control.

Novelty Statement | Increased use of pesticides is not only hazardous to target animals but also to non-target aquatic organisms like fish. Pesticides can alter the biochemical parameters such as glutathione S-transferase in fish. The evaluation of GST is a useful bio-marker for assessing the environmental stress due to these pesticides.

To cite this article: Siddique, Q., Abdullah, S., Naz, H., Abbas, K. and Shafique, L. 2020. Changes in glutathione s-transferase activity and total protein contents of *Labeo rohita*. *Punjab Univ. J. Zool.*, **35**(1): 25-29 (2020). https://dx.doi.org/10.17582/journal.pujz/2020.35.1.25.28

Introduction

The widespread use of insecticides in agriculture leads to the contamination of environment (Yonar and Sakin, 2011). These insecticides contaminate the aquatic bodies either via direct spraying on target species or surface runoff. When these insecticides reached to water bodies caused detrimental effects on non-target organisms especially to aquatic animals including fish which have high economic value for humans (Yonar *et al*., 2012; Saravanan *et al*., 2011).

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Organophosphate pesticide such as chlorpyrifos (CPF) is widely used to kill the various agricultural pests and domestic species (Shittu *et al*., 2012). Toxicity associated with chlorpyrifos is an alarming threat not only to aquatic animals but also to human health (Xing *et al*., 2012). Chlorpyrifos induces toxicity by changing the physiological and antioxidant activities of fish (Tripathi and Shasmal, 2010). It also directly affects the nervous system by inhibiting the acetylcholine esterase (AChE) activity and can also amass in tissues of aquatic individuals (Oruc, 2010).

Pesticides cause oxidative stress by stimulating the production of reactive oxygen species (ROS) which contain oxygen like OH^-, H_2O_2 and O^{-2} radicals which

inhibit the activities of antioxidants in fish (Kumar *et al.*, 2011). To minimize the ROS toxicity, organisms have antioxidant defense mechanism which contains superoxide dismutase, glutathione peroxidase, catalase and glutathione S-transferase (Monteiro *et al.*, 2006).

Glutathione S-transferase (GST) belongs to a phase II family responsible for detoxification of toxicants such as pesticides and polyaromatic hydrocarbons by the conjugation of glutathione (Strange *et al.*, 2000; Richardson *et al.*, 2009). By keeping in view above mentioned toxicity of chlorpyrifos, this research was carried out to check the total protein contents and glutathione S-transferase of *Labeo rohita* under sub-lethal effects of chlorpyrifos.

Materials and Methods

Labeo rohita was chosen for this experiment. Fish were obtained from Fish Seed Hatchery, Faisalabad and shifted into cemented tank at Fisheries Research Farm, UAF for acclimatization. The tests were carried out in glass aquaria (70-L) each having a group of fish (n=10). The technical grade insecticide chlorpyrifos was used as a test chemical. The LC_{50} (96 h) value as 16.53 μgL^{-1} of chlorpyrifos for *L. rohita* was estimated by Illyas (2015). On the base of this LC_{50} value fish were kept under sub-lethal dose (4.13μgL^{-1}) of chlorpyrifos for two months (Figure 1). The tests were conducted with triplet at stable pH (7.25), total hardness (245 mgL^{-1}) and temperature (27 °C). Fish was sampled on weekly basis and sacrificed to get the tissues viz. brain, gills, kidney, heart, muscle and liver.

Tissue homogenate

To prepare tissues homogenate, each organ viz. brain, gills, kidney, heart, muscle and liver were isolated. Each organ was homogenate for 12 minutes in phosphate buffer of pH 6.5 mixed in the ratio of 1:4 (w/v). The homogenate was filtered and obtained filtrate was centrifuged in refrigerated centrifugal machine at 4 °C and 10,000 rpm for 10 minutes. Supernatant was separated for GST estimation.

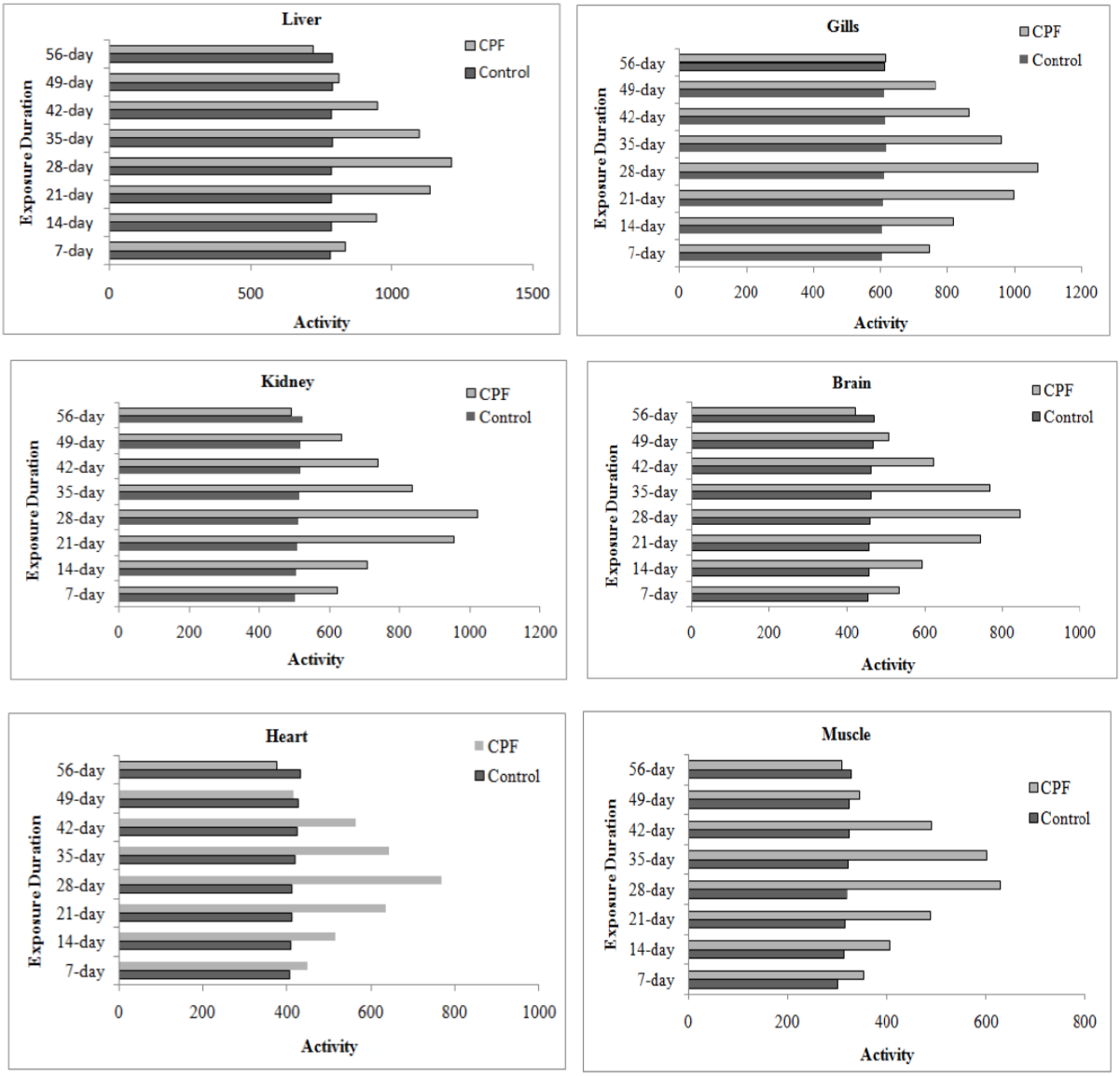

Figure 1: Effect of chlorpyrifos on GST activity of *L. rohita*.

Glutathione S-transferase (GST)

Activity of GST was measured by spectrophotometer at A_{340nm} by adopting the procedure of Mannervik (1985).

Total protein contents (TPCs)

According to Gornall et al. (1949) protocol the Biuret method was applied to check the total protein contents of samples.

Analyses of data

Data was analyzed by appropriate methods of Statistics (Steel et al., 1997). Analysis of variance was applied to compare difference between treatments by using Statistix version 8.1.

Results and Discussion

Estimation of GST

It was noted that the GST level in all observed tissues of chlorpyrifos stressed fish was significantly increased in first 28-day after that it was dropped off up to 56-day in comparison to control. The trend of GST activity in tissues of fish was observed as muscle<heart<brain<kidney<gills<liver. Similarly, Naz et al. (2019) noted the increased GST level in all tissues of *L. rohita* under endosulfan+chlorpyrifos mixture. Abdullah et al. (2018) also studied the higher level of GST in gills, liver, muscle and kidney of *Channa striata* under endosulfan+deltamethrin expousure. Batool et al. (2018) also reported the increased GST activity in hepatic tissues of *Wallago attu* under sub-lethal dose of toxicants. Sub-lethal dose of malathione stimulated the GST level in liver, kidney and gills of rohu (Karmakar et al., 2016). Nile tilapia showed increase in liver GST activity under sub-lethal stress of chlorpyrifos (Hamed, 2015). Cypermethrin and chlorpyrifos treated African catfish showed increased GST activity in liver, muscle and gills (Adeyemi et al., 2014). Huculeci et al. (2009) documented the malathion caused modulation GST level in kidney and gills of *Carassius auratus gibelio*. Alterations in gills, muscle and liver GST of rainbow trout due to diazinon and methyl parathion was noted by Isik and Celik (2008).

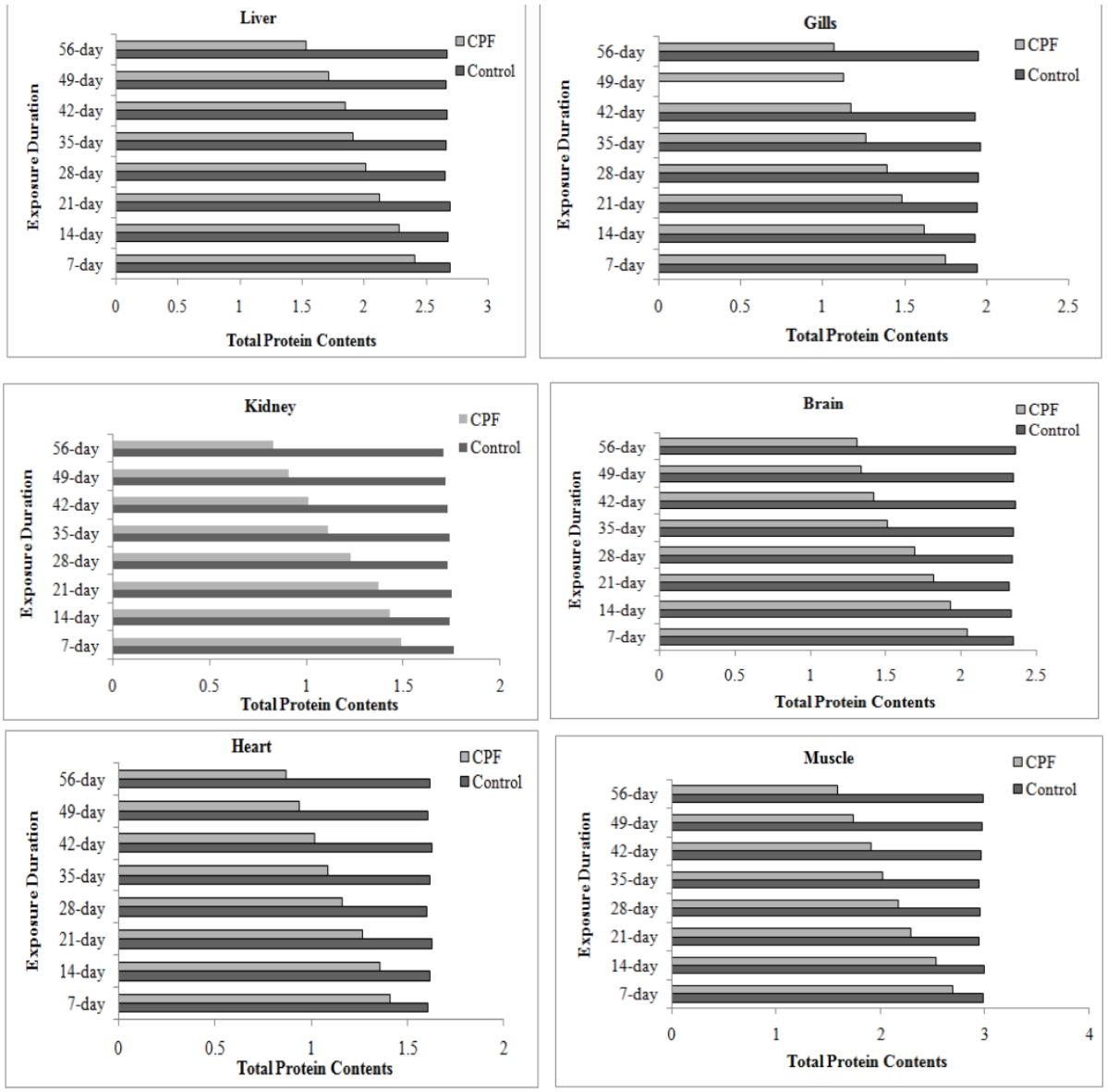

Figure 2: Effect of chlorpyrifos on total protein contents of *L. rohita*.

Estimation of TPCs

The TPCs in all organs of chlorpyrifos stressed *L. rohita* were significantly reduced in relation to control (Figure 2). Comparison among tissues for TPCs in fish showed the following trend: muscle>liver>brain>gills>kidney>heart. It was also noted that TPCs were significantly reduced with increasing time of exposure. Similarly, Batool et al. (2018) also noted the lower TPCs in liver of *Wallago attu* due to sub-lethal dose of toxicants. Phenthoate exposure caused reduction in TPCs of liver, brain and gills of *L. rohita* (Somaiah et al., 2014). Some authors said that the decreased TPCs may be due to metabolic consumption of keto acids in the production of glucose or for the ionic and osmatic regulation (Chezhian et al., 2010; Kumari, 2007; Muley et al., 2007). Venktramana et al., 2006; Vutukuru, 2005; Tilak et al. (2003) also confirmed the decline in TPCs. Agrhari et al. (2006) examined the similar results in *C. punctatus*.

Conclusion

In conclusion GST activity and TPCs are good biomarkers for evaluation of pesticides toxicity in aquatic animals. Fish is a suitable indicator for bio-monitoring the aquatic pollution.

Conflict of interest

No conflicts of interest.

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