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Short Communication

Effect of Di-n-butyl Pthalate on Oxidative Stress Parameters in Liver and Gills of *Labeo rohita*

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ABSTRACT

Phthalates are plasticizers, released from different sources, are ubiquitous in the aquatic environment. In the present study, freshwater fish (*Labeo rohita*) juveniles were exposed to low concentrations (10µg/l and 100µg/l) of di-n-butyl phthalate (DBP) for 7 days to evaluate oxidative stress in liver and gills by analyzing activity of glutathione-S-transferase (GST), catalase (CAT), glutathione (GSH) and by measuring lipid peroxidation (LPO). Catalase activity increased significantly in liver and gills of fish exposed to 10µg/l of DBP. Level of GSH and LPO increased significantly in gills while a non-significant decrease was recorded for GSH and GST in liver. These results showed that exposure to low concentrations of di-n-butyl phthalate are capable of inducing oxidative stress in vital organs of fish that may cause detrimental effects on the fish health.

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IJ, ZS and MA performed the experiments and analyze data under supervision of KPL and MF. MF wrote the manuscript.

Key words
Oxidative stress, Dibutyl phthalate, *Labeo rohita.*

Phthalates are esters of phthalic acid and are involved in the production of polyvinyl chloride (PVC), polyvinyl acetates, polyurethanes (Xu *et al*., 2013). Some phthalates, like diethyl hexyl phthalate (DEHP) and benzyl butyl phthalate (BBP), are used for adding flexibility to the plastics, packaging of food material and manufacturing of medical tubing while diethyl phthalate (DEP) and dibutyl phthalate (DBP) are also used as solvents in insecticides and cosmetics and in personal care product (Ferguson *et al*., 2011). Due to their high use in various products, phthalates are produced in high amount and are ubiquitous in our environment.

Oxidative stress can be defined as the imbalance between production of reactive oxygen species (ROS; free radical) and antioxidant defense. Oxidative stress results in deregulation of cellular functions and lead to pathological conditions (Bandyopadhyay *et al*., 1999).

ROS readily react with proteins, nucleic acid, carbohydrate and lipids present in cellular components and result in their impaired structure and function. Unsaturated fatty acids present in the cell membranes are attacked by ROS and results in lipid peroxidation. Estimation of LPO with thiobarbituric reactive oxygen species (TBARS) is a reliable and most widely used method (de Zwart *et al*., 1999). Antioxidant enzymes *i.e.* catalase, SOD, GST and free radical scavengers like GSH are responsible for the protection of cell and tissues from the toxic effects of these ROS (Banerjee *et al*., 1999; Valko *et al*., 2007). Therefore, antioxidant parameters and oxidative stress indices are considered potential biomarkers and are frequently used as screening tools to assess the impacts of environmental stress in biological system.

The Present study was designed to investigate whether a repeated 7-day exposure to low and realistic concentrations of DBP produces any signs of intoxication in fish. *Labeo rohita* is a commercially important fish of Pakistan and toxicity evaluation of DBP using this species is a first step to understand complete mechanism of action of DBP in *L. rohita* and will be helpful in future environmental monitoring and human health.

Materials and methods

Healthy *L. rohita* juveniles (56.48g±5.2 BW; 16.7±0.63 cm TL) were purchased from Manawan Fisheries Teaching and Research Institute Lahore. After acclimatization for 15 days, 30 fish were equally divided into six aquariums filled with total 60 L of water. Aquariums were randomly assigned control and treatment groups in replicates. Fish were given commercial carp feed from Oryza Organics twice a day during acclimatization and experimental period. Fish were healthy and pathogen free.

Fish were exposed to 10 µg/l and 100 µg/l of di-n-butyl phthalate (DBP), prepared in dimethyl sulfoxide and diluted in aquarium water, for 7 days. Up to 60% of water was changed daily during the experiment and fresh

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toxicant was added daily. After 7 days, fish were collected and anesthetized using clove oil (5 ppm in water). Gills and liver were taken out, homogenized in phosphate buffer (0.1 M, pH=7.4) using glass homogenizer and centrifuged at 13000 rpm (30 min at 4°C) to get post mitochondrial supernatant (PMS). A portion of homogenate was used for estimation of lipid peroxidation according to Wright *et al*. (1981). Glutathione was quantified by method of Jollow *et al*. (1974) as described by Faheem and Lone (2017). Catalase (CAT) activity was measured according to Claiborne (1985) and glutathione-S-transferase (GST) activity according to Habig *et al*. (1974). Protein content was estimated by using Bradford reagent as described by He (2011) with bovine serum albumin as standard. Data was analyzed using Garphpad Prism 7. One way analysis of varience (ANOVA) followed by Tukey's Post Hoc test was used to determine difference between means. Data was expressed as mean±S.E.M.

Results and discussion

Anthropogenic pollutants enter into aquatic ecosystem from land based sources (Tornero and Hanke, 2016). These pollutants have adverse effects on animal's physiological system and also disturb their normal functions (Rhind, 2009). Pthalates are largely used in the production of plastics and a large amount of pthalates are released into aquatic ecosystem every year because of anthropometric activities that result in detrimental effects on fish health.

Free radicals can damage cell membrane by attacking the polyunsaturated fatty acid and form lipid peroxides thus leads to lipid peroxidation (LPO). Increase in lipid peroxidation after exposure to environmental contaminants has been reported in many studies (Qu *et al*., 2015; Faheem and Lone, 2017; Karataş 2018). Flounder exposed to low concentrations of DEP had increased level of LPO in liver and kidney tissues (Kang *et al*., 2010). Similarly, carp exposed to various concentrations of DEP resulted in increased hepatic lipid peroxidation (Zhang, 2014). In current study with *L. rohita,* the measured levels of lipid peroxidation expressed as concentration of thiobarbituric acid reactive species (TBARS) in liver and gill tissue are shown in Figure 1A. The 7-day exposure to DBP resulted

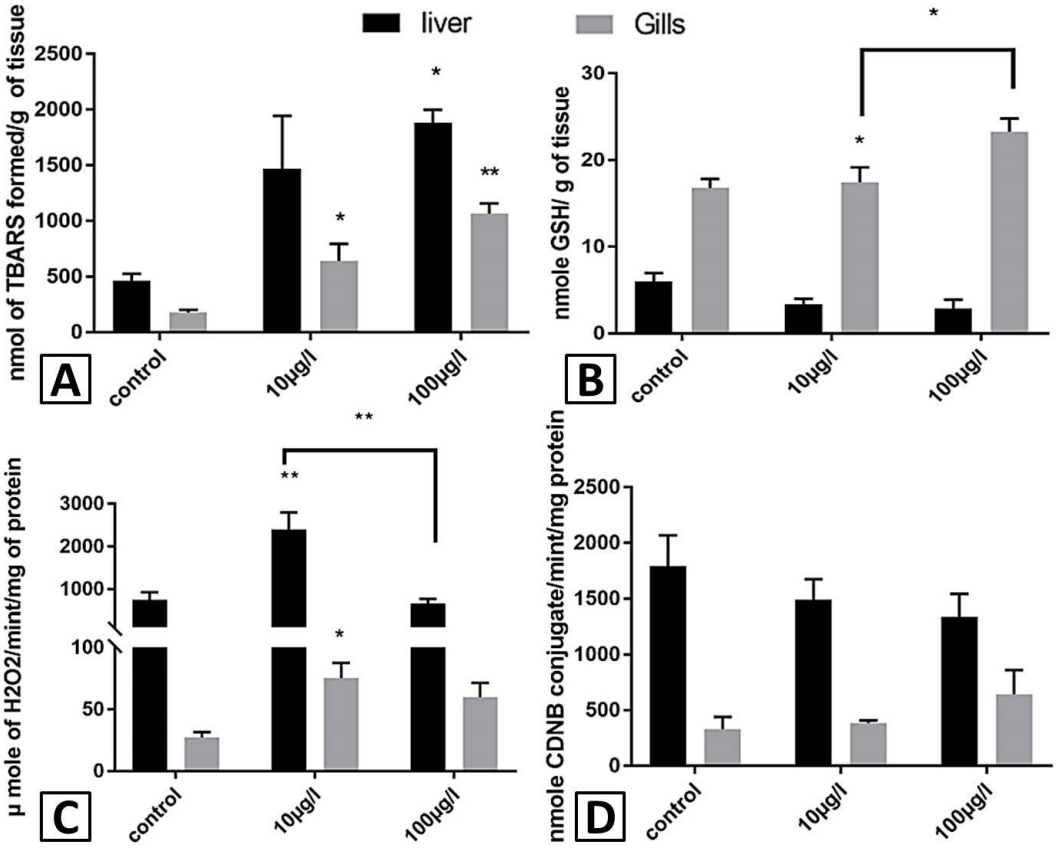

Fig. 1. Effect of different concentrations (10 µg/l and 100 µg/l) of di-n-butyl phthalate administered for 7 days on lipid peroxidation (A), glutathione level (B), catalase activity (C) and glutathione-S-transferase (D) of liver and gills of *Labeo rohita* (n=5). The values are mean ± S.E.M.

in increased lipid peroxidation, especially in animals exposed to 100 µg/l (462.822 nmol of TBARS formed/g of tissue vs. 1883.72 nmol of TBARS formed/g of tissue in liver; 177.835 nmol of TBARS formed/g of tissue vs. 1065.17nmol of TBARS formed/g of tissue in gills) (Fig. 1A).

GSH is one of the important tripeptide and undergo scavenging of reactive oxygen species (Srikanth *et al*., 2013). In present study, the reduced GSH content increased significantly in the gills (16.80 nmol GSH/g of tissue vs. 17.44 nmol GSH/g of tissue) while a non-significant decrease was observed in liver (6.01 nmol GSH/g of tissue vs. 3.34, 2.84 nmol GSH/g of tissue) following the 7-day exposure to DBP (Fig. 1B). Zhang (2014) reported that high concentrations of DEP resulted in significant decrease in GSH content in liver of carp. Nile tilapia exposed to DBP at concentration of 10 mg L^{-1} for 24 and 96 h, resulted in decreased content of gills GSH (Erkmen *et al*., 2015). This decrease may be due to the fact that fish were exposed to very high concentration of DBP which cause such decrease in level of GSH in gills, while low concentration as used in present study can increase the GSH content to cope with the damage caused by toxicant.

Catalase (CAT) is an antioxidant enzyme that can detoxify H_2O_2 into oxygen and water and is first line of defense against oxidative stress (Velisek *et al*., 2011). In current study, a significant increase was observed in catalase activity in fish liver (784.397 µmole H_2O_2/mint/mg of protein vs. 2398.85 µmole H_2O_2/mint/mg of protein) and gills (27.533 µmole H_2O_2/mint/mg of protein vs. 75.190 µmole H_2O_2/mint/mg of protein) in group exposed to 10 µg/l DBP after 7days. A non-significant decrease compared to control was observed for liver (784.397 µmole H_2O_2/mint/mg of protein vs. 662.254 µmole H_2O_2/mint/mg of protein) in group exposed to 100µg/l DBP (Fig. 1C). CAT activity increased to protect cell from damage as catalase is the first line of defense against oxidative stress (Harman, 2006). Mankidy *et al*. (2013) reported that fertilized eggs of fathead minnows exposed to 1mg/l DBP resulted in 14 fold more expression of CAT mRNA compared to the control. Similar increase in catalase activity was recorded in various fish species exposed to different toxicants (Stara *et al*., 2012; Abdel-Tawwab and Hamed, 2018). Zhao *et al*. (2014) reported that exposure of DBP to common carp caused decreased in catalase activity. This decrease may be due to the fact that fish were exposed to high concentration of DBP which cause decrease in activity whereas in our study very low concentrations were used.

GST enzyme can metabolize the harmful compounds by adding thiol group and making them more water soluble (Ashor *et al*., 2016). In our study, GST activity was not significantly changed compared to the respective negative control fish. However a non-significant decrease was observed in liver (1791.84 nmol CDNB conjugate/min/mg protein, 1490.07 nmol CDNB conjugate/min/mg protein vs. 1339.28nmol CDNB conjugate/min/mg protein). In case of gills, most of the measured values were within the limits of the control values (330.909 nmol CDNB conjugate/min/mg protein, 385.017 nmol CDNB conjugate/min/mg protein vs. 642.687nmol CDNB conjugate/min/mg protein) (Fig. 1D). Decreased GST activity after exposure of DBP and other related phthalates was reported in various studies (Kang *et al*., 2010; Zhang, 2014). On the other hand, Mankidy *et al*. (2013) reported that exposure of 1mg/l of DBP had no effect on mRNA level of GST.

Conclusion

Present study showed that exposure to low concentrations of DBP caused oxidative stress by altering activity of the antioxidant enzymes and enhancing lipid peroxidation in gills and liver tissue considering that these tissues are the main target of action of anthropogenic chemicals. Endpoints measured in the present study may accurately reflect the various imbalances that occur in a body following exposure of dibutyl phthalate and will be helpful in environmental monitoring using *L. rohita* and related species.

Statement of conflict of interest

The authors declare no conflict of interest.

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