Ecohydrology & Hydrobiology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ecohydrology & Hydrobiology

journal homepage: www.elsevier.com/locate/ecohyd

Original Research Article

Sublethal concentration of bisphenol A induces hematological and biochemical responses in an Indian major carp *Labeo rohita*

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ARTICLE INFO

Article history:
Received 27 December 2016
Accepted 26 June 2017
Available online xxx

Keywords:
Bisphenol A
Hematological
Biochemical
Sublethal
Labeo rohita

ABSTRACT

Bisphenol A (BPA) an organic compound which is widely used in the production of synthetic polymers has been detected in surface water, sediments and biota and emerged as a ubiquitous contaminant in the aquatic environment. In the present study, *Labeo rohita* was exposed to sublethal concentration of BPA and hematological and biochemical responses were studied. The median lethal concentration of BPA to *L. rohita* was evaluated by probit analysis method and the value was calculated as 7.3 mg/L for 24 h. Fish were exposed to a sublethal concentration of 0.73 mg/L (1/10th of the 24 h LC50 value) of BPA for 35 days and hemato-biochemical and marker enzyme assays was performed at the end of 7, 14, 21, 28 and 35 days. The results revealed that there was a significant ($P < 0.05$) decrease in hematological (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin) and biochemical (protein) parameters in the BPA treated fish compared to control groups. White blood cells, glucose, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase parameters were enhanced in experimental fish relative to control groups. However, the response of red blood cells, mean corpuscular hemoglobin concentration and gill Na^+/K^+-ATPase activity were found to be biphasic. In conclusion, the present investigation showed that analysis of hematological and biochemical parameters can be used as biomarkers for monitoring of BPA in the aquatic ecosystem.

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1. Introduction

Endocrine disrupting chemicals (EDCs) are widely used in agricultural, food packaging, industrial, personal care products, etc., and grouped as major environmental contaminants (Kavlock et al., 1996; Ottinger et al., 2009, 2013). The presence of EDCs in the aquatic environment may potentially cause hazards to aquatic organisms (Rotchell and Ostrander, 2003; Sumpter and Johnson, 2005). Bisphenol A (BPA), an endocrine disruptor is widely used in many industries such as production of automotive lenses, building materials, compact disks, drinking water pipe linings, dyes, electrical and electronic components, glazing thermal paper, paints, protective coatings, and as liners in plastic containers for food and beverages (Staples et al., 1998; Vandenberg et al., 2009; Kamaraj et al., 2013; Naderi et al., 2014). The global market demand for BPA may go beyond 10 million tonnes by 2020 (Vandenberg et al., 2013; Grand View Research, 2014). Due to its wide use BPA is released in to the environment through sewage treatment process, wastewater treatment plants, landfill leachate, etc. (Wintgens et al., 2003; Crain et al., 2007). As a

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http://dx.doi.org/10.1016/j.ecohyd.2017.06.003
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Please cite this article in press as: Krishnapriya, K., et al., Sublethal concentration of bisphenol A induces hematological and biochemical responses in an Indian major carp *Labeo rohita*. Ecohydrol. Hydrobiol. (2017), http://dx.doi.org/10.1016/j.ecohyd.2017.06.003

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result BPA has been found in surface water, soil, sediments and biota (Staples et al., 1998; Flint et al., 2012; Lu et al., 2015). Although the half-life of BPA is 0.5–6 days, several papers report the BPA occurrence in aquatic ecosystem including surface water, estuaries and streams (Klecka et al., 2009; De Kermoysan et al., 2013) and also in the fish samples (Liu et al., 2011; Miège et al., 2012).

For example BPA has been detected up to 880 ng/L in Mondego River, Portugal (Ribeiro et al., 2009), 3.2 μg/mL in Lake Superior (Environmental Health Division, 2014), 0.105 ± 0.204 μg/L in River Elbe in Germany (Heemken et al., 2001) and up to 0.33 μg/L in Dutch surface waters (Belfroid et al., 2002; De Kermoysan et al., 2013). Likewise, BPA level has been detected up to 17 mg/L in landfill leachate and pulp mill effluents (Flint et al., 2012), 43 g/kg dw in river (Flint et al., 2012), up to 191 g/kg dw in marine sediments (Koh et al., 2006) and 100 μg/kg dw in soils (Canesi and Fabbri, 2015). Based on the different concentrations of BPA in various environmental matrixes the determination of the environmentally relevant concentration of BPA is very difficult (Canesi and Fabbri, 2015).

Generally, BPA is readily biodegradable and in surface waters and sediments microorganisms can easily degrade BPA (Klecka et al., 2001; West et al., 2001). However, discharge of BPA from various industrial processes may affect the aquatic organisms (Mihaich et al., 2009). Among the aquatic organisms fish species are most susceptible to EDCs (Eggen et al., 2003; Qin et al., 2013). To monitor the environmental quality and the health of the organisms in aquatic ecosystem biomarkers are widely used. The commonly used biomarkers are hematological and biochemical parameters (Ramesh et al., 2014a,b).

Hematological parameters such as hemoglobin (Hb), hematocrit (Hct), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) can be used as bioindicators in fish and target organs of toxicity following exposure to xenobiotics such as pesticides and metals (Singh and Srivastava, 2010; Ramesh et al., 2014a,b). Likewise, changes in biochemical parameters such as glucose and protein levels are widely used to monitor the health condition of the fish in aquatic environment and are also used to understand the physiological changes in aquatic organisms under stress (Saravanan et al., 2011).

Aquatic pollutants may alter the enzyme activities of fish and hence the responses of enzyme activities can be used to demonstrate the health of fish (De la Torre et al., 2000; Barnhoorn and van Vuren, 2004). Transaminases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) play a vital role in protein and glucose metabolism and the changes in these enzyme activities can be used to detect tissue damage caused by pollutants in aquatic organisms (Nemcsok et al., 1981; Webb et al., 2005; Malarvizhi et al., 2012). In addition AST and ALT are widely used to assess of liver function. Likewise, lactate dehydrogenase (LDH) activity can be used as a sensitive enzymatic biomarker in cellular level and anaerobic capacity of tissue (Diamantino et al., 2001; Rendonvon Osten et al., 2005). Moreover, alterations in gill Na^{+}/K^{+}-ATPase activity in fish are widely used as a sensitive indicator of environmental contaminants (Saravanan et al., 2011).

The toxicity of BPA on growth (Liu et al., 2011), reproduction (Liu et al., 2014), gene expression (Gentilcore et al., 2013), behavior (Faheem and Lone, 2013), oxidative stress (Hulak et al., 2013) and toxicogenomic and phenotypic analyses (Lam et al., 2011) of fish has been well documented. To the best of our knowledge the toxicity of BPA on freshwater fish species particularly in Indian major carps is scanty. Further a high concentration of BPA has been detected in sea water, sediment, river water and soil of Tamil Nadu, India recently (Kamaraj et al., 2013). Likewise BPA has also been detected in freshwater such as Kaveri, Vellar and Tamiraparani of Tamil Nadu, India (Selvaraj et al., 2014). In the present study, we attempted to evaluate the toxicity of BPA at sublethal concentration in an Indian major carp *Labeo rohita*. The carp *L. rohita* is commonly found in rivers and freshwater lakes of South India and also a cultivable fish species.

2. Materials and methods

2.1. Experimental animal

Specimens of *L. rohita* with an average weight of 6.0 ± 0.5 g and length of 7.5 ± 0.5 cm were obtained from Aliyar Fish Farm, Aliyar, Tamil Nadu in India. Fish were safely transported to the laboratory and stocked in a large cement tank (1000 L capacity) (Ramesh et al., 2014a,b). Dechlorinated tap water was used throughout the study period, with the following hydrological features such as; temperature 26.2 ± 1.5 °C, pH 7.1 ± 0.05, salinity 0.27 ± 0.7 ppt, dissolved oxygen 6.6 ± 0.04 mg/L and total hardness 17.1 ± 0.8 mg/L. Fish were acclimatized to laboratory condition for a period of 20 days and fed daily with rice bran and ground nut oil cake. The water in the aquarium was renewed daily by removing three-fourth of the water daily. Feeding was suspended 24 h before the start of the experiment. During the acclimatization period the mortality rate was less than 5%.

2.2. Determination of median lethal concentration of BPA (24 h)

A stock solution of BPA was prepared by dissolving 1 g in appropriate quantity of dimethyl sulphoxide (DMSO) and made up to 1000 mL using tap water. To determine the median lethal concentration of BPA for 24 h, different concentrations (2, 4, 6, 8 and 10 mg/L) of BPA were prepared from the stock and added in five circular plastic tubs, filled with 10 L of water. Ten fish were introduced into each tub for a period of 24 h prior to the experiment. Three replicates were maintained for each concentration. Mortality/survival of the test organisms was recorded at the end of 24 h and the dead fish were removed immediately. The median lethal concentration (LC50) was calculated by probit analysis method of Finney (1978). In the present study the 24 h LC50 was calculated as 7.3 mg/L. The experiments were conducted in 12:12 natural photoperiod.

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