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RESEARCH ARTICLE

HISTOPATHOLOGICAL EFFECTS OF DIBUTYL PHTHALATE ON *OREOCHROMIS MOSSAMBICUS* PETERS, 1852

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ABSTRACT

Dibutyl phthalate was investigated in the present study for histopathological studies in *Oreochromis mossambicus*. Fishes were exposed to 0.1% concentration of dibutyl phthalate and examined 96 hours after exposure. Histopathological changes like hypertrophy and hyperplasia, vacuolization in primary gill lamella; periportal inflammation; and vacuolization in renal tubular epithelia was observed in gill, liver and kidney respectively. The findings of the present study served as biomarkers for pollutant toxicity on fish health.

INTRODUCTION

Environmental problems of concern today are attributed to the production and release of toxic chemicals capable of interacting with the environment and disrupting the ecosystem (Abumourad *et al*., 2013). Heavy metal is distributed throughout the environment and it is derived from industrial processes, agricultural activities, burning of fossil fuels and weathering of geologic formation (WHO, 1989). Natural and anthropogenic sources also continuously release heavy metals into aquatic ecosystem. The heavy metals after reaching freshwaters cause serious problem due to their long persistence, bioaccumulation, biomagnification in the food chain, and toxicity to the organisms. Pollutants discharged into environment are in the form of pesticides, fertilizers, heavy metals, oxidants, etc. These pollutants or toxicants are released by refineries, tanneries, plating industries, agrochemical industries, textile industries, chemical manufacturing units etc. (Rosenblatt and Townsend, 1989) and disposal of these industrial effluents is a major problem (ATSDR, 1999). A plethora of chemicals enter animal feed, human food and water either as undesirable contaminants or as a part of the components of a diet. Phthalate esters and Phthalic Acid Esters (PAEs) are used as plasticizers to increase the flexibility and durability of various materials and primarily are used in manufacturing Poly Vinyl Chloride (PVC) polymers and plastisol (Staples, 1997). They are also used in personal care products, paints, adhesives, agricultural adjuvants, food products, building materials, medical devices, detergents and surfactants, toys, printing, pharmaceuticals, inks and coatings (Hauser and Calafat, 2005.). Though most phthalate esters have low acute toxicity, phthalate esters are potentially harmful when exposed to humans that can cause toxic and mutagenic effects (Zeng *et al*., 2009). Phthalates and their metabolites are excreted from human urine and waste water (such as water that washes off cosmetics, facial cream, lotion, shampoo) which reaches the environment via untreated sewage discharged into streams, rivers, lakes, oceans and other bodies of water (Shanker *et al*., 1985). Phthalates also reach the natural environment via pesticides, industrial lubricants and phthalate-containing garbage that humans throw away (Katherine *et al*., 2006). Dibutyl phthalate is one of the most common phthalates since it leaches from many products and has become a common contaminant in the environment (ATSDR, 2001). These chemicals can enter aquatic environment through waste disposal from manufacturing units, accidental spillage from industries, effluents from waste water treatment plants and consumer products. Fish, being dominant inhibitors of aquatic environment are considered as indicators for heavy metal pollution (Srivastav *et al*., 2013) because they concentrate toxins in their tissues directly from water and also through their diet. The tolerance of aquatic organisms to toxicants in domestic effluents may vary among species and

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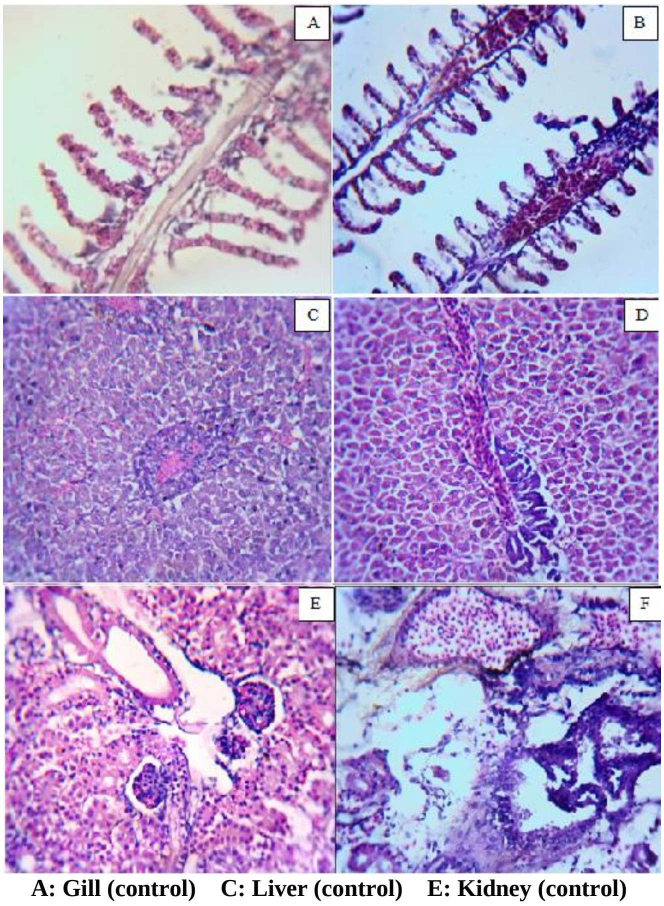
their integrative effects may lead to reproductive failure or reduction in number of fish species (Minier *et al*., 2006). The response to chemical stress can be used as biomarkers of environmental conditions. Biomarkers are early responses or measurable biological event due to exposure to pollutants after acute or chronic exposure and the morphological findings has been largely considered in biomonitoring studies (Oliveria-Ribeiro *et al*., 2005; Miranda *et al*., 2008). Histopathological events are considered fast and efficient for detection of acute and chronic adverse effects in fish and may express the health condition of exposed contaminants (Myers and Fournie, 2002; Ayas *et al*., 2007; Liebel *et al*., 2013). The Mozambique Tilapia, *Oreochromis mossambicus*, one of the most popular fresh water fish consumed in several countries (Alwan *et al*., 2009) has been described by many researchers as a suitable bioassay organism (Nussey, 1998; Barnhoorn, 2001) extensively used in biological and behavioural research studies (Skelton, 1993) due to its availability and adaptability nature and well-known for its ability to tolerate many types of environmental stressors (Stickney, 1986). Further, it acts as a good biological model for toxicological studies due to diverse characteristics, great resistance to diseases and good tolerance to a wide variety of environmental conditions (Fontainhas-Fernandes, 1998). Histopathology is often the reliable method of assessing both short and long term effects (Hinton and Lauren, 1990; Teh *et al*., 1997). Furthermore, histopathological studies reflect the present health of fish by examining the specific target organs (gill, liver, kidney and intestine) which are responsible for vital function like respiration, metabolism, excretion and absorption of organism (Au *et al*., 1999; Camergo and Martinez, 2007; Vasanthi *et al*., 2013). Keeping in view of the above mentioned factors, the present study was assessed to characterize the histopathological effects of dibutyl phthalate on gill, liver and kidney of *Oreochromis mossambicus*.

MATERIALS AND METHODS

Oreochromis mossambicus obtained from Selaiyur lake, East Tambaram, Chennai, Tamil Nadu, India was utilized as the test organism in the present study since it has a well-documented general biology, easy culturing and year round reproduction. The experimental fish were acclimatized in glass aquaria (150L) filled with dechlorinated water under laboratory conditions for a period of twenty days. The fish were fed daily with commercially balanced fish food. The fishes were maintained on a photoperiod with 12 light/12 dark cycle. Fishes were exposed to 0.1% concentration of dibutyl phthalate for a period of 96 hours. Fishes maintained in pesticide free water served as control. After treatment, both the experimental and control fishes were sacrificed and gill, liver and kidney were removed and dropped in aqueous Bouins fluid. After fixation for 24 to 30 hours, the tissues of each organ were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in paraffin. Sections of 4-6μm were prepared from paraffin blocks by using a rotary microtome. These sections were then stained with haematoxylin-eosin, examined for histopathological lesions and photographed using a photomicroscope (100x).

RESULTS

The histopathology studies showed that the gill of control fish was found to be normal (Figure 1A) but the treated gill showed sanguineous congestion which had tightness and stuffiness of the gill accompanied by hypertrophy and hyperplasia of gill epithelium (Figure 1B). The blood vessels in the gill were found to be ruptured. The histopathologic alterations observed included alterations in the structure of epithelium in the secondary lamella. Presence of hyperplasia and vacuolization of epithelium in primary gill lamella was witnessed. Further, proliferation of epithelial cells in primary lamellae induced the lamellae fusion and consequently decreased the surface area for gas exchange, which was noted by the anxiety and restlessness of the treated fish. No changes were observed in the control liver (Figure 1C) but periportal inflammation (Figure 1D) was noted in the liver of the treated fish which led to necrosis of the liver tissues which might have probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. No abnormalities were seen in kidney of control group (Figure 1E), whereas in treated fish, the kidney showed congestion, cloudy swelling and focal vacuolations in the renal tubular epithelia. Interstitial aggregations of round cells were observed (Figure 1F).



A: Gill (control) C: Liver (control) E: Kidney (control)
B: Gill (treated) D: Liver (treated) F: Kidney (treated)

Figure 1. Histopathological effects of dibutyl phthalate on the gill, liver and kidney of *Oreochromis mossambicus*

DISCUSSION

Fishes are sensitive to contaminants of the water, and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasi *et al*., 1989). Fishes are largely being used for the assessment of the quality of the aquatic environment and also as bioindicators of environmental pollution (Lopes *et al*., 2001; Dautremepuits *et* *al*., 2004). Phthalates such as dibutyl phthalate, di (2-ethylhexyl) phthalate and diisononyl phthalate altered liver and kidney function causing a variety of alterations in kidney function in animals including renal cysts, reduction in creatinine clearance and transitional cell carcinoma (Woodward, 1990). Zeid *et al*. (2014) stated that the histopathological changes in gills, livers and kidneys may be attributed to the generation of oxidative stress and consequent lipid peroxidation occurred by dibutyl phthalate in many fish species. *Cyprinus carpio* gill and liver tissues when exposed to sub-lethal concentration of dibutyl phthalate for 96 hours showed irregular mRNA level changes in liver. Gill showed epithelial lifting, hyperplasia, fusion of secondary lamellae, passive hyperemia and hydropic degeneration, cellular damage and lesions (Agus *et al*., 2015). Histopathological changes were observed in *Oreochromis mossambicus* when exposed to chlorpyrifos, where liver tissues showed swollen hepatocytes with granular cytoplasm and necrosis. Biliary hyperplasia was observed at certain regions of the hepatic tissue which might indicate the regenerating hepatic cells to withstand the toxic stress condition. Whereas, the changes observed in gills were hyperemia, and edema with prominent clubbing, separation of primary gill lamellae, hemorrhage in the blood vessels outside the secondary gill lamellae, hyperemia of the gill filaments that engorged with blood vessels and hyperplasia in secondary gill lamellae, which led to fusion of adjacent primary and secondary gill filaments (Kunjamma *et al*., 2008). Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale *et al*., 2011). Histological changes not only give an early indication of water pollution hazard, but also provide useful data on the nature and degree of damage to cells and tissues (Shaikh *et al*., 2010). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Canton, 1991; Thophon *et al*., 2003) and field studies (Hinton *et al*., 1992; Schwaiger *et al*., 1997; Teh *et al*., 1997). One of the advantages of using histopathological biomarkers in environmental monitoring is that they allow examining specific target organs, including gills, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta *et al*., 2003), and serve as warning signs of damage to animal health (Hinton and Lauren, 1990; Camergo and Martinez, 2007).

Gills are frequently used in the assessment of impact of aquatic pollutants in freshwater habitats (Fernandes *et al*., 2007; Jimenez-Tenorio *et al*., 2007). Gills are the first organs which come in contact with environmental pollutants. The gills of aquatic organism represent primary target of disturbance by pollutants as they are in direct contact with the external medium to perform gaseous exchange and ionic regulations. This organ has linking sites to promote its regular functions, and these sites link to toxic substances with differentiated charges, triggering mechanical responses and toxic effects to the organism (Jadhav *et al*., 2007). The gills have the thinnest epithelium of all the organs and thereby metals can penetrate through the thin epithelia cells (Bebianno *et al*., 2004). Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and absorption and secondly, their detoxification system is not as robust as that of liver. Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid (Athikesavan *et al*., 2006; Fernandes *et al*., 2007). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic processes. Thus, any harm in the gills leads to impairment of vital functions revealing respiratory distress, impaired osmoregulation and retention of toxic wastes. The kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The selection of liver cells as appropriate targets is due to their cytological sensitivity as biomarkers of organic contaminants and environmental pollution as liver plays an important role in vital functions in basic metabolism; major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo-Fernandes *et al*., 2006). Liver is the main organ responsible for detoxification of harmful substances. Toxicants reach the liver through circulation. Histologically, the liver is made of roughly hexagonal hepatic lobules that consist of cords of polygonal hepatic cells called hepatocytes concentrating towards the central vein. The rapid opercula movement, erratic swimming and loss of balance observed in this study suggested possible liver disorder. Further, the inability of fish to regenerate new liver cells may also have led to necrotic effect on hepatocytes. Kidneys excrete nitrogen containing waste products from the metabolism *viz*., ammonia, urea and creatinine. Kidney of fishes receives much of the largest proportion of postprandial blood and therefore renal lesion might be expected to be good indicators of environmental pollution (Ortiz *et al*., 2003) and the exposure to chemical contaminants induces a number of lesions and injuries to kidneys (Oliveria-Ribeiro *et al*., 2002). The findings of the present study indicated histological changes observed in gills, liver and kidney of *Oreochromis mossambicus* when exposed to dibutyl phthalate which could serve as biomarkers for pollutant toxicity on fish health. Further, the present study indicated occurrence of long term health consequences in fishes due to anthropogenic activities which affects the human population on consuming fishes since they serve as a rich source of proteins. Therefore, measures have to be taken to protect the aquatic bodies in order to eliminate or minimize the constant sources of pollution.

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