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Short term exposure of pendimethalin induces biochemical and histological perturbations in liver, kidney and gill of freshwater fish

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ABSTRACT

Our study was designed to evaluate effects of an herbicide, pendimethalin on biochemical biomarkers and histopathological indices of the freshwater fish *Channa punctata* Bloch. Fish were acutely exposed (96 h) to sub-lethal concentrations (0.5 and 0.8 ppb of pendimethalin). Various oxidative stress indicators such as thiobarbituric acid reactive substances levels and protein carbonyl content, as well as antioxidant defenses parameters, such as glutathione-S-transferase (GST), catalase (CAT), reduced glutathione (GSH) and non-protein thiols (NP-SH) levels were studied, using the liver, kidney and gill tissues. Pendimethalin exposure increased lipid peroxidation and protein oxidation processes. There was significant inhibition in levels of GSH and NP-SH. The activity of antioxidant enzymes GST and CAT depleted in all the tissues in a dose dependent manner. The histopathological change in the gill showed necrosis and atrophy of primary and secondary gill lamellae. The tissue damages like degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, are some histopathological changes observed in the liver. The changes in histoarchitechture observed in the kidney included necrosis, cellular hypertrophy and granular cytoplasm. The present study demonstrates the disturbances in antioxidant armamentarium and importance of study in the potential risk assessment of herbicides on fish species.

1. Introduction

Human activity is greatly responsible for polluting aquatic ecosystem by addition of chemical substances especially pesticides may result in depletion of quality water along with a negative impact on the biotic communities. Herbicide application is a common practice in agriculture to control weeds to improve the crop yield. However, the aquatic environment may get exposed to unsystematic use of herbicides leading to various unwanted responses (Moraes et al., 2007). Herbicide pendimethalin contaminates water bodies mainly through runoff and spray drift (Danion et al., 2012). Only few studies on toxic effects of pendimethalin on non-target organisms such as fish are available (El-Sharkawy et al., 2011). It is a systemic toxicant rated as moderately to extremely toxic to fish and aquatic organisms, and which can give rise to long-lasting metabolites. The determination of toxicity is essential for assessing the sensitivity of animals to specific toxicants, for evaluating the degree of damage to specific organs, and for assessing the extent of ensuing behavioral, physiological, and biochemical disorders.

Oxidative stress results due to imbalances in the pro-oxidants to antioxidants ratios, which leads to the reactive oxygen species (ROS) generation. Environmental contaminants such as herbicides, heavy metals etc. are known to cause antioxidant defense systems modulation and oxidative damage in aquatic organisms via ROS production. Interaction of ROS with biological macromolecules, likely causes enzyme inactivation, oxidation of membrane lipids and protein, DNA damage, and even cell death (Banudevi et al., 2006). Toxic effects such as cell injury and cell death induced by pollutants such as herbicides may occur due to membrane damage (Glusczak et al., 2006). Fish are equipped with antioxidant defense system for counterbalancing ROS; these pathways include catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST), which constitutes the major enzymatic antioxidant systems while non-enzymatic antioxidants are dominated by reduced glutathione (GSH) and other non-protein thiols (NP-SH) (Monteiro et al., 2006; Modesto and Martinez, 2010). The protective and adaptive roles of GSH against oxidative stress-induced toxicity are well established in aquatic animals (Saera-Vila et al., 2009) and provide a first line of defense against ROS (Li et al., 2007).

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A study of the toxicological effect of pendimethalin on a freshwater fish, *Channa punctata* Bloch will address its plausible ecological significance. Therefore, this study was aimed to gather more data on the toxicity on the biochemical parameters and histopathological alterations in fish tissues exposed to herbicide pendimethalin through its effects on oxidative stress and antioxidant biomarkers. This study gave brief insight of possible ill effect of herbicide pendimethalin on the targeted tissue, which can be further correlated to its exposure on human beings.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), 5,50-dithiobis (2-nitrobenzoic acid) (DTNB), oxidized glutathione (GSSG), reduced glutathione (GSH), reduced NADP(H), thiobarbituric acid (TBA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 1-Amino-2-naphthol-4-sulphonic acid (ANSA), butylated hydroxyl toluene (BHT), 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine (DNPH), EDTA, orthophosphoric acid (OPA), perchloric acid (PCA), sulphosalicylic acid, sodium azide and trichloroacetic acid (TCA) were purchased from Merck Limited (Mumbai, India). Parijat agrochemicals (Delhi, India) provided pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) as a gift sample.

2.2. Fish

The investigations were carried out in a freshwater fish, *C. punctata* Bloch, procured from pollution-free water sources (fish hatcheries, which were free from any kind of pollutant exposure). The morphometric parameters of fish included weight 80–100 g. Fish were maintained following standard fish maintenance procedure (APHA 1998) in glass aquaria (22′ × 18′ × 18′) containing 40-L dechlorinated water. Fishes were acclimatized for 15 days before use. Aquarium water was kept aerated and its temperature was maintained at ambient laboratory temperature (25 ±2 °C). Fish were transferred to a fresh volume of water every 24 h to minimize contamination from metabolic wastes.

2.3. Experimental design

2.3.1. Exposure

The acclimatized fishes were divided into three groups ($n = 10$) and three nominal exposure conditions were tested, namely: (i) control (ii) 0.5 ppb of pendimethalin and (iii) 0.8 ppb of pendimethalin. The dose of pendimethalin was based on previously published reports (Danion et al., 2014; Tabassum et al., 2015). During the 96 h of the exposure period, the fishes were exposed to a diluted pendimethalin concentration. Throughout the experiment, the fishes were maintained in the same tanks and were fed once a day. At the end of the exposure the fishes were sacrificed for conducting biochemical and histopathological studies. No mortality was observed either in control animals or in any of the treatment groups.

2.3.2. Preparation of post-mitochondrial supernatant (PMS)

After decapitation, fish were weighed, tissues (liver, kidney, and gill) properly excised, which, were promptly weighed and washed in ice-cold saline (0.85% NaCl). Gill filaments were carefully dissected free from the arches. The tissues were cleaned, thoroughly minced and homogenized 10% (w/v) to prepare PMS using the method of Ashafaq et al. (2014). The supernatant was centrifuged in a refrigerated centrifuge at 10,500 × *g* for 30 min at 4 °C to obtain the PMS, which was used for the estimation of thiol profile.

2.4. Determination of lipid peroxidation (LPO)

LPO was measured using the procedure of Tabassum et al. (2007). The absorbance was measured at 535 nm. The rate of LPO was expressed as μmol TBARS formed/h/g tissue based on the molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.5. Determination of protein carbonyl (PC) content

PC content was quantified by the procedure of Parvez and Raisuddin (2005). The results were expressed as nmol DNPH incorporated/mg protein based on the molar extinction coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

2.6. Estimation of thiol profile

GSH content was estimated according to the method of Parvez and Raisuddin (2006a). The GSH concentration was calculated as μmol GSH/g tissue using a molar extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$. Non-protein bound thiol (NP-SH) groups in the PMS were determined using the method of Parvez et al. (2003). The values are expressed as $\mu M\ g^{-1}$ of wet tissue.

2.7. Glutathione-S-transferase activity

The method of Waseem and Parvez (2013) with some modification was used to measure the glutathione-S-transferase (GST) activity. The enzyme activity was calculated as μmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6 \times 10^3\ M^{-1}\ cm^{-1}$ at 340 nm.

2.8. Catalase (CAT) activity

CAT activity was assayed by the method of Chaudhary et al. (2015). CAT activity was calculated in terms of μmol H_2O_2 consumed/min/mg protein using a molar extinction coefficient of $39.6\ M^{-1}\ cm^{-1}$.

2.9. Histopathology

Liver, kidney and gill arches were excised from different fish groups and processed as described by Khan and Parvez (2015). Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution (75 ml saturated picric acid, 25 ml formaldehyde (37–40%) and 5 ml glacial acetic acid) for 48 h. Subsequently liver and kidney tissues were processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills were processed by double embedding technique. Sections were cut at 4–6 μm thickness with the help of 820-Spencer rotatory microtome, stained with hematoxylin–eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in *Canada balsam*. The photographs at 200× magnification were taken with computer aided microscope (Intel play Qx3, Intel Corporation, China).

2.10. Protein estimation

The protein content was determined in liver, kidney and gill tissue supernatant by the method of Bradford (1976) using BSA as a standard.