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Propiconazole induced toxicological alterations in brain of freshwater fish *Channa punctata* Bloch

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ABSTRACT

Fungicides are a class of pesticides which are used indiscriminately in large amounts and pose a serious threat to the environment. Propiconazole (PCZ) is a systemic foliar fungicide with a broad range of activity. The potential of this fungicide to induce toxicity has not been fully explored. The present study was designed to investigate the dose dependent neurotoxic effect of propiconazole (PCZ), with *Channa punctata* Bloch as a model organism. Effect of PCZ on the brain specific enzyme activity such as acetylcholinesterase (AChE), monoamine oxidase (MAO) and Na^+-K^+-ATPase was determined in the fish brain tissue exposed to sub-lethal concentrations (0.5 and 5 ppm) for 96 h. Also, levels of oxidative stress reflected by various enzymatic and non-enzymatic antioxidants were measured. Neurotransmitter (epinephrine) level was also assessed. PCZ exposure induced oxidative stress as reflected by the significant increase in fish brain lipid peroxidation and protein carbonyl content with decrease in reduced glutathione levels, as well as the significant inhibition of glutathione dependent metabolizing enzymes and CAT activities. In addition, AChE, MAO and Na^+-K^+-ATPase activities were significantly lowered along with reduction in epinephrine levels in PCZ exposed fishes than those of the control in a dose dependent manner. Also, histopathological alterations were observed in fish brain of the treated fishes. The results point towards the potential neurotoxicity in the fish caused by PCZ exposure but the application of these findings will need more detailed study before they can be established as special biomarkers for toxicity monitoring the aquatic environment.

1. Introduction

Chemicals originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface runoff or leaching and frequently accumulate in soft-bottom sediments and aquatic fauna (Rodrigues et al., 2013). Environmental stressors can alter the physiological and toxicological parameters in fish, including morphological indices, antioxidant responses and energy metabolic parameters (Pandey et al., 2003; Parvez et al., 2006a). Exposure to pollutants like pesticides can have both acute as well as chronic neurotoxic effects on organisms (Costa et al., 2008). Fungicides has the potential for damaging central nervous system affecting a wide array of functions associated with it. Nervous system controls a wide array of behavioral and physiological activities in fishes with neurotransmitters playing an important role in this regulation (Rahman and Thomas, 2012).

Propiconazole (PCZ) is a triazole fungicide implicated in ecotoxicology of pesticides. It is used on grasses grown for seed, mushrooms, corn, wild rice, peanuts, almonds, sorghum, oats, pecans, apricots, peaches, nectarines, plums and prunes. It works against several fungal species such as *Erysiphe graminis*, *Leptosphaerian odorum*, *Puccinia* spp., and *Septoria* spp. (Thomson, 1997). Because of its peculiarities of non-target fungicide, many aquatic organisms (e.g. fish) can be affected (Sumpter, 2008). Fungicides are potentially capable of generating oxidative stress in non-target organisms. Bioaccumulation of toxic substances triggers redox reactions generating free radicals, especially free oxygen radicals, but also other reactive oxygen species (ROS) are produced, that induce biochemical alterations in fish tissues (Parvez and Raisuddin, 2006a). To counteract the toxic effects of ROS, aerobic organisms use both enzymatic and non-enzymatic antioxidants to scavenge the free radicals. However, when ROS generation exceeds the capacity of the cellular antioxidants, it will cause oxidative stress and oxidative damage (Shao et al., 2012). Neurons are relatively sensitive to ROS and neurodegenerative disorders have been linked to damage caused by ROS (Li et al., 2010a). As an organ in which homeostasis must be strictly maintained, brain

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tissue contains large amounts of polyunsaturated fatty acids rendering it particularly vulnerable to free radical attacks. In central nervous system, acetyl cholinesterase (AChE) and monoamine oxidase (MAO) play an important role in the neurotransmitter release, synaptic plasticity, and the regulation of neuronal electrical activity (Li et al., 2015). Additionally, Na^+-K^+-ATPase is a ubiquitous membrane-bound enzyme which concentrates in the membranes of nerve endings and controls the ionic environment essential for neuronal activity in the central nervous system (Sridevi et al., 2007). Not enough data exists on the effect on nervous system in fish exposed to this fungicide. In the present study, biochemical parameters of whole brain tissue were analyzed to determine the dose dependent effects of PCZ on *Channa punctata* Bloch, and to assess PCZ-induced brain specific biochemical, enzymatic, non-enzymatic biomarkers along with histopathological alterations based on measurement of oxidative stress parameters.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide (ATC), benzylamine hydrochloride (BAHC), bovine serum albumin (BSA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), oxidized glutathione (GSSG), reduced glutathione (GSH), reduced NADP(H) and 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), epinephrine, ethylenediaminetetraacetic acid (EDTA), orthophosphoric acid (OPA), perchloric acid (PCA), sulphosalicylic acid, sodium azide and trichloroacetic acid (TCA), xylene, were purchased from Merck Limited (Mumbai, India). PCZ was provided by Parijat Agrochemicals (Delhi, India) as a gift sample. PCZ was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution at a concentration of 100 mg/mL.

2.2. Fish

The selected fish species for present study was *C. punctata* Bloch. Randomly selected fish were allowed to acclimatize for at least 2 weeks in glass aquaria supplied with 60 L of dechlorinated water, pH 7.6, temperature $25 \pm 2\,^{\circ}C$ and oxygen concentration $5.23 \pm 0.49\,mg\,L^{-1}$. Fish were maintained following standard fish maintenance procedure during acclimatization and exposure (APHA, 2005). To minimize the metabolic waste contamination the water was replaced every 24 h.

2.3. Pesticide tested

Sub-lethal concentrations of active ingredient tested were 0 (control), 0.5 and 5 ppm for PCZ. Groups of twelve fish were exposed for 96 h in aerated glass aquaria with 60 L of test medium. The nominal concentration of PCZ were estimated on previous reported data (Li et al., 2010a, 2013). Specimens were fed only once a day during the assay and test media was not renewed. Termination of the assay was followed by extraction of brain tissue of the sacrificed fishes.

2.4. Sample preparation of homogenate and post-mitochondrial supernatant (PMS)

In accordance to method by Parvez and Raisuddin (2006b), extracted brain tissues were weighed and kept in cold 0.85% NaCl. 10% homogenate of cleaned tissues was prepared in (w/v) in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%) which was used to prepare PMS.

2.5. Determination of lipid peroxidation (LPO)

LPO was measured after incubation at 95 °C with TBARS in aerobic conditions in terms of μmol TBARS formed/h/g tissue based on the molar extinction coefficient of $1.56 \times 10^5\,M^{-1}\,cm^{-1}$. The method of Parvez and Raisuddin (2006c) was followed.

2.6. Protein carbonyl (PC) content estimation

PC content was represented as nmol DNPH incorporated/mg protein based on the molar extinction coefficient of $2.1 \times 10^4\,M^{-1}\,cm^{-1}$ in the tested tissue sample with the protocol adapted from Parvez and Raisuddin (2005).

2.7. Reduced glutathione (GSH) measurements

GSH levels in brain tissue were calculated as μmol GSH/g tissue using a molar extinction coefficient of $1.36 \times 10^4\,M^{-1}\,cm^{-1}$ and assayed by the colorimetric determination method of Parvez and Raisuddin (2006b).

2.8. Glutathione-S-transferase (GST) assay

GST was assayed according to the method of Waseem and Parvez (2013). The enzyme activity was measured by following the change in absorbance at 340 nm of the substrate, CDNB conjugate using a molar extinction coefficient of $9.6 \times 10^3\,M^{-1}\,cm^{-1}$ at 340 nm.

2.9. Catalase (CAT) activity

CAT activity was determined in terms of μmol (hydrogen peroxide) H_2O_2 used/min/mg protein using a molar extinction coefficient of $39.6\,M^{-1}\,cm^{-1}$ according to the method of Chaudhary et al. (2015).

2.10. Estimation of acetylcholinesterase (AChE) activity

The method of Naseem and Parvez (2014) was employed for determination of AChE activity in fish brain tissue and was represented as nmol ATC hydrolysed/min/mg protein using a molar extinction coefficient of $1.36 \times 10^4\,M^{-1}\,cm^{-1}$.

2.11. Analysis of Na^+, K^+-ATPase activity

Method reported by Parvez et al. (2006b) was followed to measure Na^+, K^+-ATPase activity in terms of inorganic phosphate (Pi) released as μg Pi liberated/min/mg protein.

2.12. Monoamine oxidase (MAO) assay

Changes in MAO activity was assessed by measuring the aldehyde formed by the method of Ashafaq et al. (2014). The results have been expressed as μmol BAHC hydrolysed/min/mg protein using a molar extinction coefficient of $7.6925\,M^{-1}\,cm^{-1}$.

2.13. HPLC analysis of epinephrine

Epinephrine level was measured from fish brain tissue which was homogenized in ice cold (1:5, w/v) 0.05 M perchloric acid. After centrifugation at a speed of 9000 *g* for 4 min at 4 °C, an aliquot of the supernatant was injected into the HPLC system. The HPLC system that was used for epinephrine measurements comprised a