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Oxidative effects of the acute exposure to a pesticide mixture of cypermethrin and chlorpyrifos on carp and zebrafish – A comparative study

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ABSTRACT

The use of commercial pesticides combinations increases the risk of intoxication in *non-target aquatic organisms*. Here, we investigate the potential of a commercial pesticide formulation containing (CYP) plus chlorpyrifos (CPF) to induce oxidative damage on two fish species (common carp and zebrafish). Carp and zebrafish were exposed for 96 h under laboratory conditions. Fish were divided in three different groups: CTL, 0.3 μg L^{-1} or 0.6 μg L^{-1} of CYP and 0.5 or 1 μg L^{-1} of CPF in commercial formulation. Both carp and zebrafish showed an increase in lipid peroxidation (LPO) and glutathione-S-transferase (GST) activity when compared to control group. Other oxidative parameters responded differently to exposure in carp and zebrafish. There were an increase in ascorbic acid (ASA) levels and decrease in catalase (CAT) activity and non-protein thiols (NPSH) levels in treated groups of carps. In the other hand, zebrafish showed significant decrease in ASA and increase in CAT activity and NPSH levels. Overall, we demonstrate noxious effects on redox parameters in two fish experimental models and different effects were observe in each fish species exposed to commercial pesticide formulation. This difference responses observed can be related with specific mechanisms of detoxification and antioxidant defense system of each species.

1. Introduction

Pest resistance to conventional pesticides has increased the industrial production or synthesis of new pesticides. The presence of pesticide mixtures in environments reflects the current interest of using active ingredients combinations to maximize production (Idris et al., 2012). These formulations can lead to contamination of aquatic environment and to intoxication of non-target organisms, such as fish (Laabs et al., 2002; Idris et al., 2012). The commercial mixture of Cypermethrin (CYP) and Chlorpyrifos (CPF) insecticides is frequently used in households and in farming activities.

CYP (alpha-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylic acid) is a II type pyrethroid insecticide considered moderately toxic (Singh et al., 2016). The pyrethroids are very toxic to fish due to the low carboxylesterases levels and consecutively low ability of fish to hydrolyze these compounds (Haya, 1989). CPF (0,0-didsethyl0–3,5,6-trichloro-2-pyridylthiophosphate) is an organophosphates (OPs) pesticide with a broad spectrum which inhibits acetylcholinesterase activity, responsible for controlling the nerve impulse in the cholinergic synapses (Narra et al., 2015). Both CYP and CPF per se may induce the reactive oxygen species (ROS) formation (Jin et al., 2011; Xing et al., 2012; Ural, 2013; Jin et al., 2015). However, irreversible inhibition of esterases by organophosphates reduces the cleavage of the ester bond of pyrethroids (Fulton et al., 2013). Thus, blockage of pyrethroids hydrolysis impairs pesticide metabolism and potentiates the effects of organophosphorus-pyrethroid (Idris et al., 2012).

Oxidative damage results from an imbalance between oxidants and antioxidant levels, which increases ROS generation. ROS react with biomolecules, such as lipids and proteins, affecting cell viability (Yu, 1994). However, fish have efficient antioxidant defenses, composed by different antioxidant enzymes (e.g., catalase (CAT) and glutathione S-transferase (GST)), as well as other low molecular weight scavengers, such as non-protein thiols (NPSH) and ascorbic acid (ASA) (Almroth et al., 2005; Menezes et al., 2012; Yonar, 2013).

Among freshwater fish, zebrafish (*Danio rerio*) and common carp

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(*Cyprinus carpio*) are species widely used as experimental models in aquatic ecotoxicology (Menezes et al., 2012; Nunes et al., 2017). These species are easily raised in laboratory and are recommended for assessing the effects of pollutants in aquatic ecosystems (US EPA, 2000). Since pesticides affect non-target organisms in aquatic environment, we investigated the oxidative profile of two fish species (zebrafish and carp) after exposure to a sublethal concentration of a commercial formulation containing CYP and CPF.

2. Materials and methods

2.1. Fish

Two freshwater fish species (juvenile common carp and adult zebrafish) of both sexes were used in this study. Carp (weight, 16.0 ± 1.0 g; length, 8.0 ± 1.0 cm) were obtained from a fish farm (RS, Brazil) and zebrafish of short fin wild-type (weight, 0.5 ± 0.1 g; length, 3.0 ± 1.0 cm) were obtained from a commercial supplier (Hobby Aquários, RS, Brazil). Each specie was acclimated separately in laboratory conditions, maintained in aquaria (40 L) filled with non-chlorinated water treated with AquaSafe™ (Tetra, VA, USA), containing at a density of three animals per liter for 15 days. Fish were kept in continuously aerated water in a static system with a natural photoperiod (12 h light/12 h dark). Water parameters were measured every day and set as follows: temperature 23.5 ± 2.0 °C; pH 6.7 ± 0.5 units; dissolved oxygen 7.4 ± 1.0 mg/L; non-ionized ammonia 0.80 ± 0.05 $\mu g\,L^{-1}$; and nitrite 0.086 ± 0.01 mg/L. During acclimation, both species were fed twice daily. Carp were fed with commercial fish pellets (Supra, Brazil) and zebrafish were fed with alcon BASIC™ Flakes (Alcon, Brazil).

2.2. Experimental design

Fish were exposed singly for 96 h to the same commercial pesticide (acaricide), Colosso FC30 (manufactured by Ourofino, Brazil) containing CYP (15%) and CPF (25%) of active ingredient. Briefly, each fish species were acclimated during 15 days and after divided in three experimental groups (n = 15 per group): (1) control (untreated, CTL), (2) 0.3 $\mu g\,L^{-1}$, and (3) 0.6 $\mu g\,L^{-1}$ of commercial pesticide. The nominal concentrations of treated groups containing: CYP (0.3 and 0.6 $\mu g\,L^{-1}$) and CPF (0.5 and 1 $\mu g\,L^{-1}$) of commercial formulation. These sublethal concentrations were based on usually detected levels of CYP and CPF found on literature ranging 0.01–9.8 $\mu g\,L^{-1}$ in water column (Laabs et al., 2002; Xing et al., 2012). The concentrations used in the present study were also based in studies that shows the 96 h $LC_{(50)}$ of *C. carpio* exposed to CYP (29.1 $\mu g\,L^{-1}$) (Dobsíková et al., 2006) and CPF (565 $\mu g\,L^{-1}$) (Xing et al., 2010). Fish were not fed during exposure and the solution was renewed daily to maintain a similar concentration. All protocols used were previously approved by Institutional Animal Ethics Committee and followed International guidelines for ethical animal use. Protocol number: 17705116-2016.

2.3. Biochemical assays

2.3.1. Sample preparation

After the exposure, fish were anesthetized with 0.25 $g\,L^{-1}$ tricaine (Wilson et al., 2009) and euthanized by punching the spinal cord behind the opercula. The whole-body was homogenized with 150 mM saline solution (0.5 mL g^{-1}), packed in Teflon tubes and kept at −80 °C for posteriors assays, according to İspir et al. (2017).

2.3.2. Determination of lipid peroxidation (LPO)

LPO was estimated by malondialdehyde (MDA) reaction with 2-thio-barbituric acid (TBA) (Draper and Hadley, 1990). Briefly, whole-body homogenates (200 µL) were mixed to 10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) in a final volume of 1.0 mL. The reaction mixture was placed in a micro-centrifuge tube and incubated for 30 min at 95 °C and optical density was measured in a spectrophotometer at 532 nm. TBARS levels were expressed as nmol MDA/mg protein.

2.3.3. Non-protein thiols and ascorbic acid levels

Non-protein thiols (NPSH) and ascorbic acid (ASA) levels were determined in whole-body homogenates following Ellman (1959) and Roe (1954) respectively. The whole-body homogenates were homogenized again with 1.5 mL of 50 mM Tris-HCl (pH 7.5) followed by centrifugation at 3.000 ×*g* for 10 min. Supernatants (1.0 mL) were mixed (1:1) with 10% trichloroacetic acid and then centrifuged. To determine NPSH, 400 mL of supernatant was added in 0.5 mM phosphate buffer (pH 6.8) and 10 mM 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB). The color reaction was measured at 412 nm. NPSH levels were expressed as µmol NPSH/g of whole-body. For ASA determination, 300 µL of supernatants were mixed to 2,4-dinitrophenylhydrazine (4.5 mg mL^{-1}), 0.6 mg mL^{-1} thiourea, $CuSO_4$ (0.075 mg mL^{-1}), and trichloroacetic acid 13.3%; and incubated for 3 h at 37 °C. Afterwards, H_2SO_4 65% (v/v) was added and ascorbic acid levels were expressed as µg ascorbic acid/g of whole-body.

2.3.4. Glutathione S-transferase (GST) activity

The whole-body were homogenized once in a Potter-Elvejhem glass/Teflon homogenizer with 20 mM potassium phosphate buffer, pH 7.5 (1:20 dilution) and centrifuged at 10.000 ×*g* for 10 min at 4 °C. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Habig et al., 1974). The levels of S-2, 4-dinitrophenyl glutathione were monitored at 340 nm. The molar extinction coefficient used for CDNB was 9.6 m M cm^1 and enzyme activity was expressed as µmol GS-DNB/min/mg protein.

2.3.5. Catalase activity

Catalase activity was performed by ultraviolet spectrophotometry (Nelson and Kiesow, 1972). The assay mixture consisted of 2.0 mL potassium phosphate buffer (50 mM, pH 7.0), 0.05 mL H_2O_2 (0.3 M) and 0.05 mL homogenate. Absorbance changes were measured by spectrophotometry at 240 nm for 60 s. Catalase activity was expressed in µmol/min/mg protein.

2.3.6. Protein determination

Protein was determined by the Comassie blue method using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm (Bradford, 1976).

2.4. Statistical analysis

Homogeneity of variances was tested with Levene's test. Comparisons among treatments were performed using one-way ANOVA followed by Tukey's test and expressed as mean ± standard error. Differences were considered significant at $p < 0.05$.

3. Results

Carps exposed 0.6 $\mu g\,L^{-1}$ of commercial pesticide formulation showed increased LPO in relation to control (Fig. 1A). LPO increased in both concentrations tested in zebrafish (0.3 $\mu g\,L^{-1}$ and 0.6 $\mu g\,L^{-1}$) when compared to control (Fig. 1B). Conversely, NPSH and ASA levels responded differently between the species. Carps showed a decrease in NPSH levels in both concentrations of exposure (Fig. 2A), while zebrafish showed NPSH levels increased in a concentration-dependent manner when compared to control (Fig. 2B). Although ASA levels did not change in carps exposed to commercial pesticide (Fig. 3A), zebrafish showed a *concentration-dependent* decrease in ASA levels when compared to control (Fig. 3B). A significant increase in GST activity was observed in zebrafish and carps after the exposure. Carps showed an