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Original Article

Oxidative damage and changes in Glutathione S-transferase activity in juvenile African catfish, *Clarias gariepinus* exposed to cypermethrin and chlorpyrifos

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ABSTRACT: Cypermethrin and chlorpyrifos are broad-spectrum insecticides routinely used as household and agricultural insecticides. Since aquatic environments serve as sinks for numerous environmental pollutants, the effects of these substances on the resident aquatic organisms can be quite serious. In this study, we investigated the effects of exposure of African catfish to cypermethrin and chlorpyrifos on oxidative damage and the activity of glutathione S-transferase (GST). Juvenile African catfish were exposed to 1.25 μg/L cypermethrin, 1.25 μg/L chlorpyrifos, 2.5 μg/L cypermethrin or 2.5 μg/L chlorpyrifos for 96 h. Control fish were maintained in borehole water. At the end of the 96 h exposure, tissue lipid peroxidation (LPO), protein carbonylation and GST activities were determined. Contaminant exposure resulted in a significant increase ($p < 0.05$) in the levels of LPO and protein carbonylation and the activity of GST in the gills, liver and muscle of exposed fish compared to the controls. Oxidative damage was more serious in the groups exposed to chlorpyrifos compared to those exposed to cypermethrin, thus implying that chlorpyrifos is more toxic to these fish than cypermethrin. The results of this study indicate that the pollution of aquatic ecosystems with cypermethrin and chlorpyrifos may cause oxidation of biomolecules (lipids and proteins) that are involved in essential physiological and biochemical activities in animals.

KEYWORDS: Insecticides; oxidative stress; African catfish; lipid peroxidation; protein carbonylation; GST activity.

INTRODUCTION

The remarkable increase in the rate of use of insecticides either for household or agricultural purposes has resulted in higher concentrations of these substances in a variety of environments (Grube *et al.*, 2011; Polidoro *et al.*, 2008). Since aquatic ecosystems are usually the reservoirs for these contaminants (Forstner and Prosi, 1979), their levels in streams and rivers may be high enough to pose serious health risks to humans when contaminated water is consumed (Thurman *et al.*, 1991). The toxicity of common insecticides to aquatic life has been widely studied, primarily using mortality as the endpoint (Kruetz *et al.*, 2008; Vidyarani *et al.*, 2010). Most insecticides exert their toxic action on organisms through the excessive generation of free radicals, overwhelming the scavenging activity of antioxidant enzymes and thus resulting in oxidative stress (Almeida *et al.*, 1997; Valko *et al.*, 2005). Indicators of oxidative stress such as the quantification of plasma thiols, protein carbonyl content, peroxidation of lipids and the activities and expression of antioxidant enzymes are reliable methods for the detection of non-lethal effects of most contaminants; they are very

sensitive for detection of low concentrations of pollutants (Dalle-Donne *et al.* 2003; Lopez-Barea and Gomez-Ariza, 2006; Hwang and Kim, 2007).

Cypermethrin is a synthetic pyrethroid that is widely used as a household and agricultural insecticide while chlorpyrifos is an organophosphate insecticide that is routinely applied to agricultural fields (Chowdhury *et al.*, 2013). Fish have proven to be a useful model system for the investigation of the toxicity of a wide variety of pollutants in vertebrates. In this study, we have therefore compared the effects of two different classes of insecticides; pyrethroid (cypermethrin) and organophosphate (chlorpyrifos) on juveniles of African catfish, *Clarias gariepinus*, using various biomarkers of oxidative stress. The results may be useful in the assessment of potential harm of these chemicals to aquatic life and the data on the levels of their toxicities could help to make some useful decisions on their controlled usage.

MATERIALS AND METHODS

Experimental organisms and contaminant exposure

Juvenile catfish (N=75) of average length 10 ± 2.5 cm were collected from a commercial fish hatchery located in Abere, Osun State, Nigeria (07˚43'55N; 004˚31'07E). They were then transported in plastic containers to the Research Laboratory of the Department of Biological Sciences of the Osun State University, Osogbo, Nigeria. Fish were acclimatized to laboratory conditions for seven days prior to commencement of experiments. During acclimatization, water was exchanged every 24 h and fish were fed with commercial fish pellets twice daily. At the end of the seven-day acclimatization period, fish were divided into five groups of about fifteen fish per group; control, 1.25 *µ*g/l cypermethrin, 1.25 *µ*g/ chlorpyrifos, 2.5 *µ*g/l cypermethrin and 2.5 *µ*g/l chlorpyrifos. Experiments were conducted in 7.5 litre plastic buckets and contaminant exposure lasted for 96 h. Three separate buckets containing 5 fish each were used for each treatment.

Tissue preparation for biochemical measurements

At the end of the 96 h exposure, six fish were randomly selected from each group and were euthanized in an overdose of benzocaine, carefully dissected, and the liver, muscle and gill samples were further processed. Dissected tissue samples were homogenized in ice-cold 0.05 M potassium phosphate buffer (pH 7.4). The homogenate was preserved in -20 ˚C for later biochemical analyses.

Protein carbonylation assay

The protein carbonyl content was quantified as described by Resnick and Packer (1994). Two samples of the homogenates from each tissue were placed in two different glass tubes. To one tube 10 mM DNPH in 2.5 M HCl was added, while to the other tube 2.5 M HCl was added. Tubes were left for 1 h of incubation at room temperature in the dark. Then, 20% TCA (w/v) was added in both tubes, left in ice for 15 min and centrifuged at 6000 ×g to collect the protein precipitates. The precipitates were dissolved in 6 M guanidine hydrochloride and were left for 10 min at 37 ˚C. Absorbance was read at 390 nm using a UV/Vis spectrophotometer (Jenway, Model 6400), and converted to concentration using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}. Results were expressed as *µ*mol carbonyl per mg protein.

Lipid peroxidation (LPO) assay

Lipid peroxidation was measured spectrophotometrically by the method reported by Dorts *et al.* (2009) with minor modifications. LPO results in formation of malondialdehyde, which reacts with thiobarbituric acid to form a colored product, thiobarbituric acid reactive substance (TBARS), which is then quantified spectrophotometrically. Briefly, the tissue homogenates were added 1:1 (v/v) to 5% trichloroacetic acid and then incubated for 15 min on ice. The resulting solution was then mixed in a ratio of 2:1 with 0.67% thiobarbituric acid and centrifuged at 2200 g for 10 min at 4 ˚C. The supernatant was then boiled for 10 min and allowed to cool to room temperature. Absorbance was then measured at 535 nm using a UV/Vis spectrophotometer (Jenway, Model 6400). The amount of LPO was expressed as µM TBARS/mg protein in the tissue using a molar extinction coefficient of 156,000 $M^{-1}cm^{-1}$.

Glutathione S-transferase (GST) activity assay

The glutathione S-transferase activities in the gills, liver and muscle were determined following the method of Habig *et al.* (1974), using 1-chloro 2, 4 dinitrobenzene (CDNB) as substrate. The assay was performed in a test tube by adding 1.7 ml of phosphate buffer to 0.1 ml of 30 mM CDNB. The mixture was allowed to incubate for 5 min at 37 ˚C, after which 0.1 ml of the homogenate was added, and absorbance was read after min at 340 nm. The specific activity of GST was expressed as *µ*mol of GSH-CDNB conjugate formed/min/mg protein, using the molar extinction coefficient of 9.6 $M^{-1}cm^{-1}$.

Protein estimation

The protein content of aliquots of the tissue extracts was determined by a Bradford assay (Bradford, 1976) using bovine serum albumin as a standard.

Statistics

The data were checked for normality using the Shapiro-Wilk test. Since there was no significant deviation from normal, parametric tests were used to analyze the data. We used a two-way mixed model analysis of variance, factors being the treatment group and experimental buckets (since multiple buckets were used per group). Because the experimental bucket effect was not significant, this factor was eliminated from the model. The tissue LPO level, protein carbonyl

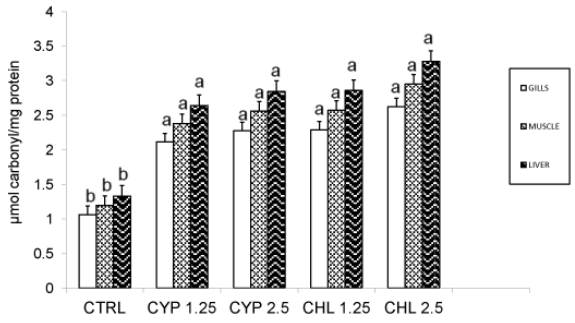

Figure 1: The protein carbonyl contents in the different tissues of *C. gariepinus* exposed to different treatments of insecticides; control (CTRL), 1.25 µg/L cypermethrin (CYP 1.25), 2.5 µg/L cypermethrin (CYP 2.5), 1.25 µg/L chlorpyrifos (CHL 1.25) and 2.5 µg/L chlorpyrifos (CHL 2.5). Each bar is mean ± standard error (n = 6). For each tissue, bars with different letters are significantly different.

contents and GST activity data were analyzed with one-way analysis of variance, in order to detect the differences among the means of the different treatment groups. This was followed by Tukey's multiple comparison tests whenever there was a significant difference. All statistics were performed using JMP version 9.0 software (SAS Inc. 2010). For reporting purposes, data were expressed as mean ± standard error and statistical significance was assumed at P ≤ 0.05.

RESULTS

Protein carbonylation assay

The protein carbonyl contents differed significantly among the groups for the gills ($F_{4, 15}$ = 4.7032, p = 0.0207), liver ($F_{4, 15}$ = 7.1018, p = 0.0057) and muscle ($F_{4, 15}$ = 5.2463, p = 0.0146) samples. The exposure to either cypermethrin or chlorpyrifos insecticides resulted in increased carbonylation of protein molecules in the gills, liver and the muscle, compared to the control, with the effects more pronounced in the liver samples. The levels of protein carbonylation did not differ significantly with respect to the type of the insecticides (i.e. whether cypermethrin or chlorpyrifos) or the concentrations of the insecticides the fish were exposed to (Figure 1).

Lipid peroxidation assay

There was a significant difference in the gills ($F_{4, 15}$ = 6.3180, p = 0.0134) and liver ($F_{4, 15}$ = 6.4792, p = 0.0127) LPO levels while the LPO levels in the muscle did not differ significantly ($F_{4, 15}$ = 4.7032, p = 0.0207) among the groups. Exposure of fish to the insecticides caused an increase in the tissue LPO levels. The LPO levels were highest in the liver, followed by the gills and lowest values were observed in the muscle. The groups that were exposed to chlorpyrifos had higher levels of tissue LPO compared to those that were exposed to cypermethrin. Also, the LPO levels were higher in tissues from fish that were exposed to a higher concentration of the insecticides (Figure 2).

Glutathione S-Transferase (GST) activity assay

The GST activity levels differed significantly among the groups for the gills ($F_{4, 15}$ = 8.0042, p = 0.0008), liver ($F_{4, 15}$ = 16.1816, p < 0.0001) and the muscle ($F_{4, 15}$ = 15.3608, p < 0.0001). The GST activity levels seemed not to be different with respect to the type of the insecticides, although there was an indication that activity levels are higher in the groups that were exposed to chlorpyrifos. For each tissue, the GST activity levels did not differ in relation to the concentration of the insecticides to which the fish were exposed to (Figure 3).

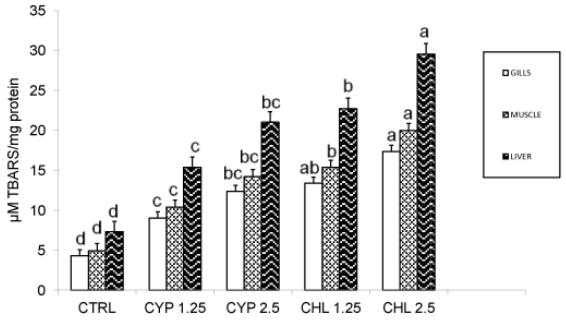

Figure 2: The lipid peroxidation levels in the different tissues of *C. gariepinus* exposed to different treatments of insecticides; control (CTRL), 1.25 µg/L cypermethrin (CYP 1.25), 2.5 µg/L cypermethrin (CYP 2.5), 1.25 µg/L chlorpyrifos (CHL 1.25) and 2.5 µg/L chlorpyrifos (CHL 2.5). Each bar is mean ± standard error (n = 6). For each tissue, bars with different letters are significantly different.

DISCUSSION

The increasing and continual usage of agrochemicals has resulted in the contamination of aquatic ecosystems with these chemicals, thus exposing the organisms living in contaminated sites to physiological and biochemical stress. In this study, exposure of *C. gariepinus* to either cypermethrin or chlorpyrifos resulted in oxidative damage to important biomolecules such as lipids and proteins in the liver, gills and muscle. There are reports of certain insecticides and pesticides resulting in excessive production of reactive oxygen species which may in turn oxidize important biomolecules, thus causing protein carbonylation and lipid

peroxidation (Ribera *et al*., 1990; Shacter *et al.* 1994). The levels of oxidative damage in *C. gariepinus* followed a tissue-specific pattern. The extent of protein carbonylation and lipid peroxidation was highest in the liver, followed by the muscle and then the gills. This result is consistent with the results of a similar study in which the deltamethrin-induced oxidative damage was higher in the liver of a freshwater fish, *Channa punctuata* compared to other tissues (Kaur *et al*., 2011). This high tendency of oxidative damage in the liver may be due to the role of hepatocytes in numerous redox cycling mechanisms that could result in excessive production of reactive oxygen species, thus causing oxidative stress (Klein, 1992; Reinke, 1995).

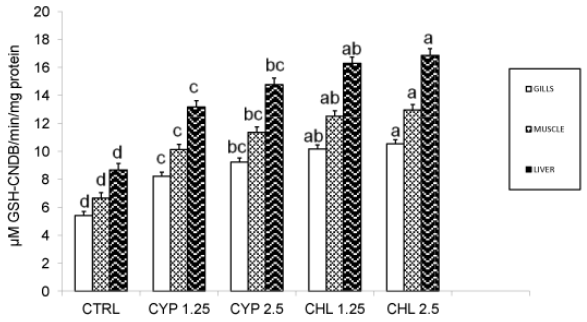

Figure 3: The levels of glutathione S-transferase activity in the different tissues of *C. gariepinus* exposed to different treatments of insecticides; control (CTRL), 1.25 *µ*g/L cypermethrin (CYP 1.25), 2.5 *µ*g/L cypermethrin (CYP 2.5), 1.25 *µ*g/L chlorpyrifos (CHL 1.25) and 2.5 *µ*g/L chlorpyrifos (CHL 2.5). Each bar is mean ± standard error (n = 6). For each tissue, bars with different letters are significantly different.

The changes in the activities of important antioxidant molecules have been widely used as biomarkers of oxidative stress in fish (Adeyemi and Klerks, 2013; Bagnyukova *et al.* 2007; van der Oost *et al.* 1998). Usually, in response to oxidative stress, living cells increase the transcription and activity of enzymes such as superoxide dismutase, catalase, glutathione S-transferase, peroxidases that scavenge reactive oxygen species. In this study, the glutathione S-transferase activity increased significantly in fish that were exposed to either of the two insecticides with respect to the activity levels in the control. Again, the GST activity levels followed a tissue-specific pattern in which the activity levels were significantly higher in the liver of exposed fish compared to the gills and the muscle. The higher GST activity in the liver of exposed fish is in agreement with the findings of Lopes *et al.* (2001), which reported a high GST activity in the liver of Iberian endemic minnows (*Leuciscus alburnoides*) populations inhabiting sites polluted with heavy metals. The high hepatic GST activity is an indication that the liver may be playing significant role in oxidative stress in aquatic organisms that have been exposed to environmental stressors.

The results of this study suggest that exposure to the organophosphate insecticide, chlorpyrifos caused more oxidative damage in *C. gariepinus* compared to the extent of oxidative damage caused by the pyrethroid insecticide; cypermethrin. This was evidenced by the higher tissue lipid peroxidation and glutathione S-transferase activity levels in the group that were exposed to chlorpyrifos. In conclusion, we found that exposure of juveniles of *C. gariepinus* to two commonly used insecticides caused oxidative damage. However, because chlorpyrifos has a shorter half-life compared to cypermethrin in the aquatic environment; 0.52 day for chlorpyrifos and1.16 day for cypermethrin (Medina *et al*., 2004; Zhang *et al*., 2012), it may be more environmentally friendly to use more of chlorpyrifos than cypermethrin.

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