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Effects of Cypermethrin on Antioxidant Enzyme Activities and Lipid Peroxidation in Liver and Kidney of the Freshwater Fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.)

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Superoxide dismutase (SOD; EC 1.15.1.1.), catalase (CAT; EC 1.11.1.16) and glutathione peroxidase (GPx; EC 1.11.1.9) that are among the biologic antioxidants related to the metabolism of oxygen reduction have been studied in fish because they are an important enzymatic chain in toxicity experiments (Winston 1991). SOD accelerates the dismutation of toxic superoxide radicals which occur during oxidative energy production to molecular oxygen and H_2O. GPx is responsible for the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) with H_2O_2, while CAT is responsible for the catabolism of H_2O_2.

Cypermethrin is widely used to protect the cotton fields against harmful species of Lepidoptera and it is one of the most toxic insecticides (Croft 1990). Pyrethroid insecticides show their toxic effects by inhibiting impulse transmission (Casida et al. 1983). Most studies carried out with cypermethrin deal with its acute toxicity rather than its biochemical effects (Reddy and Yellema 1991). Therefore, it is essential to understand whether it causes oxidative stress in animals, particularly in fish and also it is essential to find out the response metabolism for the antioxidant.

The aim of this study is to comparatively investigate the effects of cypermethrin on the activities of SOD, GPx and CAT, and the content of malondialdehyde (MDA) in liver and kidney tissues of *Oreochromis niloti*cus and *Cyprinus carpio* (L.). This study also aims to investigate the relationship between antioxidant enzyme activities and lipid peroxidation against oxidative stress in insecticide and species point of view.

MATERIALS AND METHODS

Male *Oreochromis niloticus* (Perciformes: Cichlidae) (131.40±30.91 g, 21.22±2.82 cm) and *Cyprinus carpio* (L.) (Cypriniformes: Cyprinidae) (105.14±41.41 g, 19.8±2.73 cm) were obtained from the culture pools in the University of Çukurova and transferred to the experimental room where temperature was kept 20±2 °C with (12:12 L:D). The tap water used throughout the experiments had a pH value of 7.6, an alkalinity of 326 ppm $CaCO_3$ and an oxygen concentration of 7.02 mg/L. The fish were allowed to acclimatise for two weeks to these conditions.

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Commercial formulation of cypermethrin (± α-cyano-3-phenoxybenzyl (±)cis, trans–3-, 2,2-dichlorovinyl) -2,2-dimethylcyclopropane carboxylate, Imperator, Hoechst, 250 g/L) were used in the experiments. 3μg/L which is 1/20 of LC_{50} value of cypermethrin (Reddy and Philip 1990) was used as a sublethal concentration for the both fish species.

Experiments were carried out in glass aquariums sized 40x100x40 cm using tap water for all experiments. For each species, 4 aquaria were used, 2 for cypermethrin exposure and 2 for controls and put in each 10 fish. Aquaria were refreshed every two days during the 10-day experimental period.

At the end of the experimental period, liver and kidney tissues were dissected and put in petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept –85 °C until the analysis. The tissues were homogenized in 1.15% KCl solution (1:10 w/vol) using a glass-teflon homogenizer (Heidolph S0110R2R0) and then centrifuged at 9000 g for 30 min (Eppendorph Centrifuge 5403). All these processes were carried out at 4 °C. Supernatants obtained were used to measure antioxidant enzyme activities and MDA levels using a spectrophotometer (Shimadzu-UV 260).

Superoxide radicals, 2-[4-Iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT) which occur as a result of reactions between xanthin and xanthin oxidase form the red colored formazon and these are used in determination of SOD activity. If there is SOD activity in the medium, superoxide radicals are removed, and therefore formation of formazon is inhibited. SOD activity is measured at 505 nm and is calculated with % inhibition of formazon formation (McCord and Fridovich 1969).

In the determination of GPx activity, t-butil hydroperoxide is used. GSSG occurs in the medium reduced to GSH by GPx and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The activity of GPx is measured at 340 nm by calculating the difference in absorbance values during the oxidation of NADPH to $NADP^+$ (Beutler 1984).

CAT activity is measured at 230 nm because H_2O_2 absorbs light in this wavelength and rate of the decomposition of H_2O_2 is used to calculate CAT activity (Beutler 1984).

MDA occurs in lipid peroxidation and this is measured after incubating at 95 °C with thiobarbituric acid in aerobic condition (pH 3.4). The pink colour formed in these reactions is measured in the spectrophotometer at 532 nm to measure MDA levels (Ohkawa et al. 1979).

Total protein content in the tissues were determined by Lowry et al. method using bovine serum albumin as standard (Lowry et al. 1951).

Statistical analyses of data were done using Kruskall-Wallis one-way Anova test. For this SPSS statistical package program was used.

RESULTS AND DISCUSSION

The activities of antioxidant enzymes in the liver and kidney of control *O. niloticus* and *C. carpio* were determined and given in Tables 1 and 2.

Table 1. Effects of 3 μg/L concentration of cypermethrin on the activities of SOD (U/mg protein), GPx (U/mg protein), CAT (U/mg protein) and the levels of MDA (nmol/mg protein) in the liver of *O. niloticus* and *C. carpio*.

	FISH	SOD	GPx	CAT	MDA
CONTROL	*O. niloticus* *C. carpio*	8.19±1.32 5.67±0.50 NS	0.16± 0.01 0.59± 0.03 ***	129.90±10.13 085.19±2.06 **	4.58±0.50 6.62±0.55 NS
EXPOSURE	*O. niloticus* *C. carpio*	11.96±0.81 09.96±0.62 *	0.28±0.02 0.43±0.03 ***	196.30±16.56 177.40±9.10 NS	14.56±0.70 24.62±3.58 ***
P VALUE	*O. niloticus* *C. carpio*	< 0.05 <0.001	<0.001 <0.01	<0.01 <0.001	<0.001 <0.001

Mean values and associated standard errors are given in this table. Statistical comparisons were done between control and exposure data from the same species (given as P value) and between exposure and control data of the fishes (given as asterisk). * : P<0.05, **: P<0.01, ***: p<0.001. NS: not significant (P>0.05).

Table 2. Effects of 3 μg/L concentration of cypermethrin on the activities of SOD, GPx, CAT and the levels of MDA in the kidney of *O. niloticus* and *C. carpio*.

	FISH	SOD	GPx	CAT	MDA
CONTROL	*O. niloticus* *C. carpio*	2.58±0.14 0.16±0.02 ***	0.05±0.08 0.25±0.07 ***	145.10±7.65 005.59±0.20 ***	1.72±0.39 2.49±0.11 **
EXPOSURE	*O. niloticus* *C. carpio*	8.17±0.14 01.23±1.86 ***	0.92±0.41 0.41±0.01 **	38.61±4.56 59.24±2.56 **	10.13±1.84 20.91±1.99 ***
P VALUE	*O. niloticus* *C. carpio*	< 0.001 <0.001	<0.001 <0.001	<0.001 <0.001	<0.001 <0.001

Mean values and associated standard errors are given in this table. Statistical comparisons were done between control and exposure data from the same species (given as P value) and between exposure and control data of the fishes (given as asterisk). * : P<0.05, **: P<0.01, ***: p<0.001. NS: not significant (P>0.05).

SOD activity and MDA levels in the liver did not show any significant difference (P>0.05) between the fishes, although GPx activity in the liver of *C. carpio* was 268.75% times higher than *O. niloticus*. Oppositely, CAT activity in *C. carpio* was 52.48% lower than *O. niloticus* (Table 1). In the kidney, activities of SOD and CAT in *O. niloticus* were higher than *C. carpio*, 1512.50% and 496.70%

in *C. carpio*. Nevertheless, in both species antioxidant enzyme activities and MDA levels were higher in the liver than in the kidney.

After the pesticide exposure, the activities of antioxidant enzymes and MDA levels were also given in Tables 1 and 2. In both fish species exposed to cypermethrin, enzyme activities and MDA levels in the liver were increased significantly ($P<0.001$) when compared to control values, except GPx activity in *C. carpio*. However, in the kidney of both fish species, levels of all the parameters increased significantly ($P<0.001$), except CAT activity in *O. niloticus*.

The alteration in the parameters between control values and values after the exposure showed differences between the two fish species. The increase in SOD activity in the liver of *C. carpio* after the exposure was 75.66%, while it was 46.03% in *O. niloticus* (Figure 1). Similarly, the increase in CAT activity in *C. carpio* was 108.24%, while it was 51.12% in *O. niloticus*. MDA levels increased 217.90% in *O. niloticus* and 271.90% in *C. carpio* when compared to their control values. However, GPx activity in fishes exposed to cypermethrin showed a 75.00% increase in *O. niloticus* and an 37.20% decrease in *C. carpio*.

The activities of SOD and GPx and the content of MDA in the kidney increased significantly ($P<0.001$) after the exposure (Figure 2), although CAT activity showed differences between the two fish species; it increased 959.75% in *C. carpio*, while it decreased 275.81% in *O. niloticus*.

The increases in the activities of SOD and GPx and MDA levels in the kidney of *O. niloticus* were higher than the increases in the liver. Similar results were also found for *C. carpio* as the increases in the SOD and CAT activities and MDA levels were higher in the kidney when compared to the increases in the liver.

Comparison between the control fishes showed that SOD activity and MDA levels in the liver were not significantly different, while GPx and CAT activities was significantly different between the species. Similar differences were also found on the antioxidant enzyme activities in the kidney of the fishes. There are several studies that compare antioxidant enzyme activities in different species. For example, Mazeaud et al. (1979) compared *C. carpio* and human metabolism and found that *C. carpio* had 67% SOD activity, 8%CAT activity and 4000% GPx activity compared to that of human's. Tappel et al. (1982) compared GPx activity in *Salmo gairdneri*, *Salvelinus fontinalis*, *Lepomis macrochirus*, *C. carpio* with humans and found that except *C. carpio*, all fishes showed lower GPx activity than that of mammals.

GPx activity in the liver is normally higher than that of in the kidney (Thomas and Murthy 1975). This was also true for the present study as GPx activity in the liver of both fish species were found to be higher than in the kidney, while the activities of the other enzymes were similar in both tissues of fish. Tappel et al. (1982) indicated that the liver can be accepted as the source of GPx, and therefore, a higher level is found in this organ compared to the other organs.

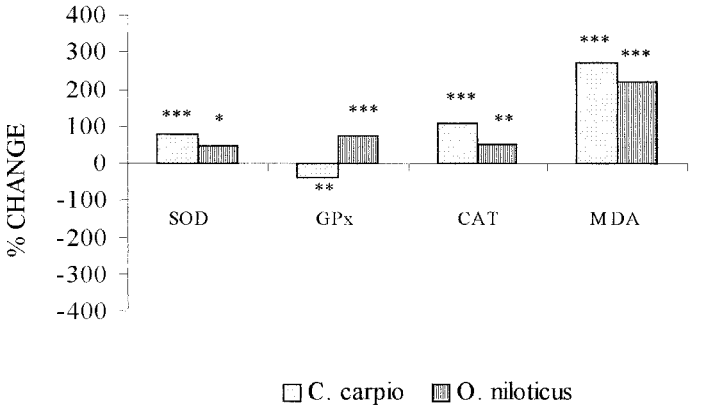

Figure 1. The effects of 3 µg/L cypermethrin on the SOD, GPx, CAT activities and MDA levels in liver tissues of *O. niloticus* and *C. carpio*. Values are averages of 10 individual. * P<0.05, ** P<0.01, *** P<0.001.

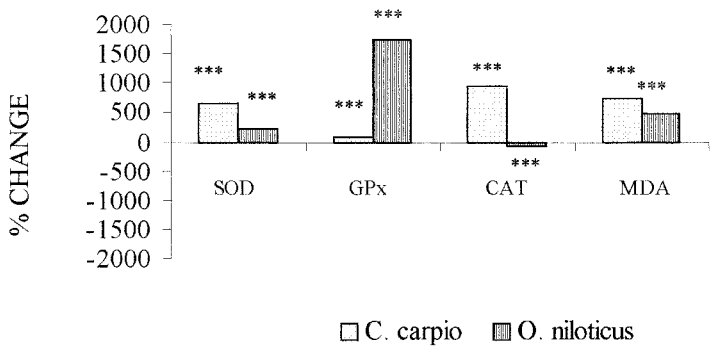

Figure 2. The effects of 3 µg/L cypermethrin on the SOD, GPx, CAT activities and MDA levelsin liver tissues of *O. niloticus* and *C. carpio*. Values are averages of 10 individual. * P<0.05, ** P<0.01, *** P<0.001.

The present study showed that exposure of *C. carpio* to cypermethrin caused a decrease in liver GPx activity. This result may be due to the occurrence of endogen $O^{\cdot}_2$ related to the increase in SOD activity in pesticide exposed fish. This study also showed that there were increases in GPx activity in the liver and kidneys of *O. niloticus* and in the kidney of *C. carpio*. It is known that high GPx activity increases the defense capacity for peroxide toxicity (Hasspieler et al. 1994). There is evidence that the relationship between lipid peroxidation and antioxidant enzyme activity in different fish species may also be different (Radi et

al. 1985; Filho et al. 1993). Amstad et al. (1994) indicated that the cells have high GPx/SOD rate; therefore they are protected against antioxidant originated toxicity and deviation from this rate may cause adverse effects in defense mechanisms of the cells.

Opposite of GPx, CAT breaks down H_2O_2 without needing the oxidation of other substrates such as thiol. Ames (1983) indicated that *I. punctatus* has a good defense mechanism for H_2O_2 peroxidation because it can increase the hepatic CAT activity when needed. Differences between species in CAT activity that provides a defense mechanism against oxidative stress may also affect the sensitivity of species on DNA damage caused by oxidants. Nevertheless, catalase is generally localized in peroxisomes (Geller and Winge 1984), and therefore, its role in the other parts of the cell is limited in peroxide metabolism which occurs through the redox cycle of xenobiotic. In the present study, cypermethrin caused an increase in GPx activity while it caused a decrease in CAT activity in the kidney of *O. niloticus* and in the liver of *C. carpio* or vice versa. It has been indicated that GPx and CAT works together in the elimination of H_2O_2 (Halliwell and Gutteridge 1995). GPx is found mainly in the cystosol and also in the matrix of mitochondria. Glutathione also shows similar distribution. Some H_2O_2 produced by urat oxidase and glicolate oxidase in the peroxisomes is disposed of by CAT, though some other H_2O_2 produced by mitochondria, endoplasmic reticulum or cytosolic enzymes such as SOD is eliminated by the peroxidase. Detoxification capacity of glutathione systems in other tissues depends on the activities of peroxidase, glutathione reductase and pentose-phosphate pathway enzymes. There are some situations in which catalase is inhibited, while glutathione reductase and glutathione peroxidase are unaffacted. In this situation, glutathione system can not cope with the extra load caused by the inhibition of catalase activity.

Highest increases in this study were in GPx activity in *O. niloticus* and CAT activity in *C. carpio*. Rodriguez-Ariza et al. (1993) also showed that highest increase in enzyme activity was found for GPx in *Mugil* sp. which lived in contaminated waters. Antioxidant enzymes behave as a defense mechanisms against oxidizing environment and help for adaptation to new conditions. It is known that GPx and GST work together to prevent lipid peroxidation in fish (Steadman et al. 1991). Winston and Di Giulio (1991) also agreed that the increase in GPx activity gives a good protection for lipid peroxidation.

The antioxidant enzyme activities in the liver of control fishes were higher than found in the kidney. Interesintly, the increases in antioxidant enzyme activities in the kidney were higher after the exposure. This may show that kidney has an important role in the detoxification of cypermethrin or its metabolite. Akhtar et al. (1994) indicated that deltamethrin is detoxified in the liver, while its metabolites are detoxified in the kidney. Because cypermethrin shows lipophilic characters, it accumulates mostly in fat, skin, liver and kidney tissues (WHO 1989). The main route for the detoxification of cypermethrin is hydroxylation, and during its detoxification, 4-hydroxyphenoxy compounds occur; these are eliminated as glucuronide conjugates through the ballast (Edwards and Millburn 1985). The increases in the activities of antioxidant enzymes in the kidney suggest that cypermethrin and its metabolites may be detoxified in this tissue.

MDA production in cypermethrin exposed fishes were higher than their control values, and this suggests that there is a relationship between cypermethrin toxicity and lipid peroxidation. High lipid peroxidation may be due to oxidation of molecular oxygen to produce superoxid radicals. This reaction is also the source of H_2O_2, which causes the production of MDA by initiating the peroxidation of unsaturated fatty acids in the membrane.

In this study, the differences in the sensitivity of two freshwater fish species to oxidative stress were determined. The alterations in antioxidant enzyme activities and MDA levels indicated that the fishes were under the oxidative stress after cypermethrin exposure. Therefore, it is thought that cypermethrin behaves as the producer of free radicals in fish metabolism. Additionally, results showed that the kidney was primary organ in the detoxification of cypermethrin.

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