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BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF DELTAMETHRIN EXPOSURE ON THE GILLS OF *CARASSIUS AURATUS GIBELIO (Pisces Cyprinidae)*

EFECTE BIOCHIMICE SI HISTOLOGICE ALE EXPUNERII LA DELTAMETRIN ASUPRA BRANHIILOR DE *CARASSIUS AURATUS GIBELIO*

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This study investigated the alterations in the activities of several antioxidant enzymes in the gills of the freshwater fish Carassius auratus gibelio exposed to deltamethrin. To get this goal, groups of 10 individuals were exposed for one, two, three, seven and fourteen days to sublethal concentration of deltamethrin (2 μg/L). Another group was used as control. The activities of catalase, gluthatione peroxidase and gluthatione reductase were significantly decreased, while the glutathione-S-transferase was up-regulated. All fish, exposed to 2μglL deltamethrin revealed gills morphological alterations after 48h of exposure which were accentuated after 14 days. In the gills hyperemia, fusion of secondary lamellae, epithelial layer rupture and chloride cells proliferation were observed. These results suggest that an immediate adaptive response to the oxidative stress appeared, demonstrating alterations in the antoxidant defense mechanism in the gills of deltamethrin intoxicated fish.

***Key words**: deltamethrin, gill, enzymatic antioxidant system, oxidative stress*

Introduction

The pyrethroid class of pesticides, including deltamethrin, are widely used as insecticides because of their short biodegradation period, and lack of the accumulation tendency in organisms (Laskowski, 2002).

The toxicity induced by deltamethrin in fish was previously noticed (Murty, 1986). The biochemical and histopathological effects on fish, at low and high concentrations of deltamethrin were studied (Koprucu et al., 2006; Velisek et al., 2006; Yildirim et al., 2006). The development of oxidative stress in different fish tissue

following deltamethrin exposure has been suggested as a main cause of toxicity (Marks
et al., 1996, Sayeed et al., 2003; Abdollahi et al., 2004).

The aim of this paper was to investigate both the consequences of deltamethrin exposure (2μg/L) in the gills of the freshwater fish, *Carassius auratus gibelio,* on the level of antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GST) and glutathione reductase (GR) and the structural changes induced by the pollutant.

Materials and Methods

Fish maintenance and treatments

A total of 100 specimens of the freshwater goldfish *Carassius auratus gibelio,* with a mean ± SE weigh of 200±20g and a mean ± SE length of 20 ± 2 cm, were acquired from The Nucet Fishery Research Station, Romania. After the period of acclimatization (15 days), the fish were randomly divided into two groups: the control one (10 individuals), and the deltamethrin treated ones, containing the other 90 fish. The pesticide was added only in the first day of experiment, in a sublethal concentration of 2 μg/L and during experiment, the fish were not fed. After one, two, three, seven and fourteen days, respectively, groups of ten fish were killed by cervical dislocation and the gills were dissected. The control fish were killed at the zero moment. The tissues were immediately frozen in liquid nitrogen, and stored at –80°C until analysis was done.

Enzyme activity assays

The CAT (EC 1.11.1.6) activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm, according to the method of Aebi (Aebi, 1974). It was calculated in terms of k/mg protein, where k is the first order rate constant. The total GPX (EC 1.11.1.9) activity was assayed by Beutler method (Beutler, 1984). One unit was expressed as 1 μmol of NADPH consumed per minute. GST (EC 2.5.1.18) was assayed spectrophotometrically at 340 nm by measuring the rate of CDNB conjugation with GSH, according to the method of Habig. (Habig et al., 1974). One unit of GST activity was defined as the formation of 1 μmol of conjugated product per minute. GR (EC 1.6.4.2) was determinated according to the method of Golberg and Spooner(1983). One unit of GR activity was calculated as 1 μmol of NADPH consumed per minute. The protein content was determined using the method of Lowry et al. (1951) using bovine serum albumine as standard.

Histological analysis

In order to perform light microscopy observations, gill fragments were fixed in Bouin fixative reagent for three hours long, dehydrated and paraffin embeded. Then

the 8 μm thick slices were dyed in Hematoxylin-Mayer-Eozine and examined in Olympus microscope.

Statistical analysis

All values were expressed as means ± SE. The differences between control and deltamethrin-treated experimental groups were compared by Student's t test using standard social science statistical packages. The results were considered significant only if the p value was less than 0.05.

Results and Discussions

Figure 1 presents the effects of deltamethrin on the CAT (a) and GPX (b) specific activities, which reduce the levels of peroxides and thus protect the cells from peroxidative damage. Both gills enzymes were down-regulated after exposure. Beginning with the second day of deltamethrin exposure, a significant decrease in the CAT activity was noticed. The higher decrease in CAT specific activity, by 75,5%, was recorded after the second day of treatment. The modifications in CAT activities were not time-dependent (Figure 1 a).

An alteration of GPX specific activity was registrated beginning the second day of deltamethrin exposure, when it has been decreased by 34,4% and remained the same for the all period of administration (Figure 1 b).

The gill GR specific activity also decreased, starting with the second day of exposure. The most significant effect was seen in the 14th day of treatment, when the GR activity was only 14.2% of the control one (Figure 2 a).

Excepting the 14th day, an up-regulation of GST activity occured. The greatest GST activity, increased by 5-fold, was noticed after the third day, wheareas after 14 days, this activity returned to the control one. (Figure 2 b).

It was demonstrated that, the pyrethroid metabolism generates reactive oxygen species in intoxicated fish (Sayeed et al., 2003).

Our results have shown a decrease in gill antioxidant enzymes CAT, GPX and GR after deltamethrin exposure, which according to George et al. (2000) could generate an altered physiological condition of the animal, and ultimately, death if essential tissues are affected. The increase in the GST activity, noticed after 7 days of exposure, could suggest the role of this enzyme in deltamethrin detoxification. The alterations of the antioxidant enzyme activities are probably, responsible for the gills severe damage noticed in this experiment.

The respiratory lamella is lined by an epithelium that is two squamous cell layers thick. Internal to the epithelium is the lamellar blood sinus, lined and spanned by pillar cells of contractile function. A marginal blood channel, linned by endotehelium occurs within the apex of the lamella. A thick stratified epithelium lines

the filament between gill lamellae. In the interlamellar epithelium, there are scattered cells of two special types: chloride and mucous cells (Mallat, 1985).

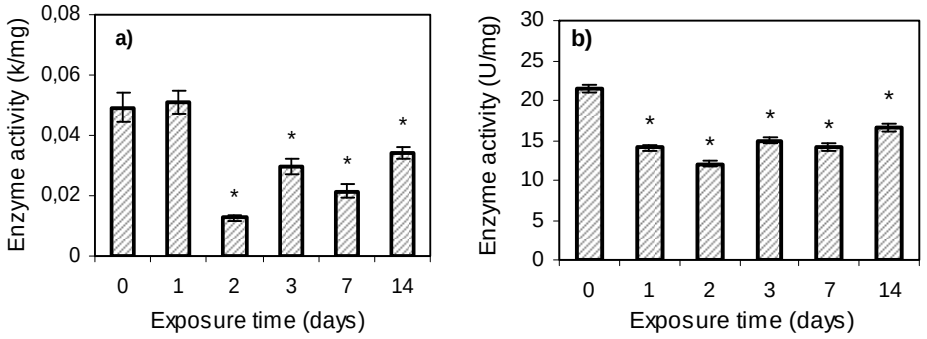

Figure 1. Effects of deltamethrin exposure on CAT (a) and GPX (b) activities in gills of *Carassius auratus gibelio*
**p < 0.05 vs. control*

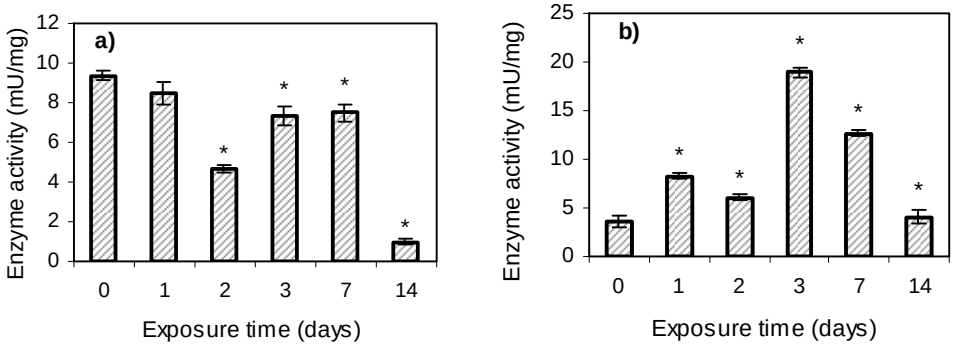

Figure 2. Effects of deltamethrin exposure on GR (a) and GST (b) activities in gills of *Carassius auratus gibelio*
**p < 0.05 vs. control*

Hyperemia was one of the pollutant effect. It is the condition in which blood congests in the gill, due to the presence of metabolites and an overall change. When the tissue increases its activity, there is a well characterized fall in the partial pressure of oxygen and pH, an increase in the partial pressure of carbon dioxide, and a rise in temperature and the concentration of potassium ions. The blood vessels near the injury site dilate, the permeability of the capillary walls increases, which produces an

exudation of the fluid which leads to a congestion of the blood cells in these vessels. The blood-derived exudate can enter nearby epithelia (Roberts, 1978, Robbins-Cotran, 1979)

The chloride cell occupies only a small fraction of the total surface area of all epithelial cells exposed to the environment. The chloride cells are concentrated in the interlamellar regions and at the junctions between the filament and lamella and only sparsely distributed on the lamellae. Under conditions of proliferation, the lamellae may be inundated with chloride cells and consequently impede the diffusion of respiratory gases. Chloride cells proliferation was noticed after 14 days of exposure (Figure 3). This structural change seems to reflect some role for chloride cells in the toxicant extrusion or neutralization. (Karnaky, 1980, Crespo etal., 1981, Mallat et al., 1985, Oronsaye-Brafield, 1984).

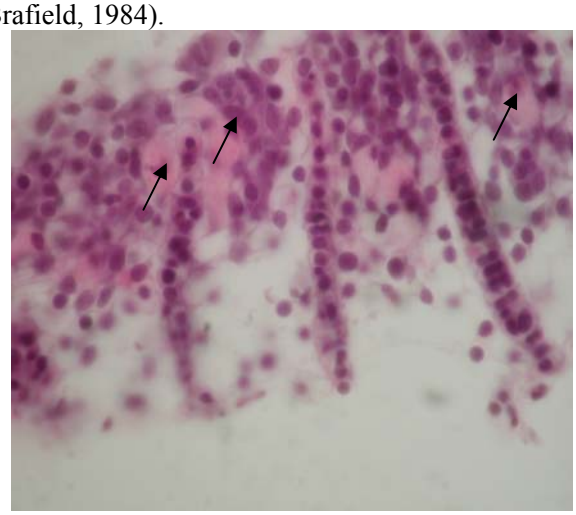

Figure 3. Chloride cells proliferation (H-E x 100).

Lamellar fusion observed after 48 hours exposure to the pollutant (Figure 4 A) could be protective in that it diminishes the amount of vulnerable gill surface. Branchial responses that serve to slow entry of toxicant have the undesirable side effect of threatening to suffocate the fish (Skidmore, 1964, Burton et al., 1972).

The branchial epithelium rupture (Figure 4 A), noticed after 48 hours of exposure, could develop via autolysis, induced by the cells own enzymes, following the toxicant induced disruption of cell processes or rapid lysis caused by the direct lytic action of the toxicant on cell constituents (Abel, 1976). Rarely, lamellar cells hypertrophy and basal cells pycnosis were noticed. Generally our results are similar to those obtained by Yildrim et al., 2006 in *Oreochromis niloticus.*

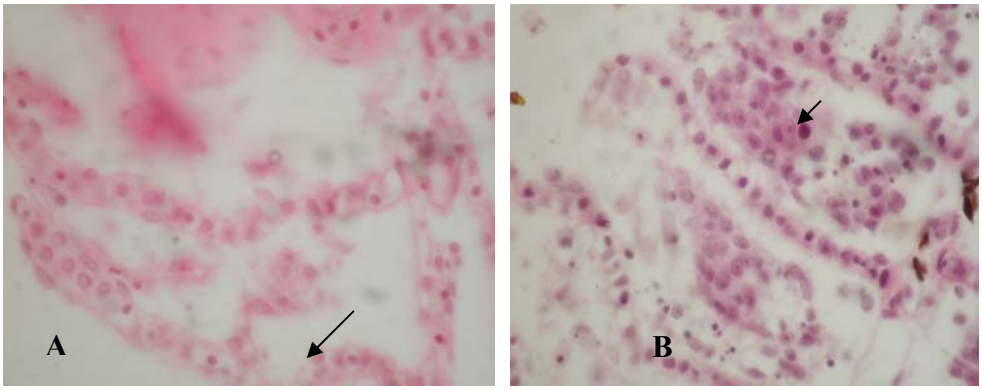

Figure 4. Histological changes in gill structure after 48 of Deltamethrin exposure (H-E) A Epithelial rupture and lamellar cells hypertrophy (x 100); B Nuclear pycnosis in the undifferentiated basal cells, (x 40)

Conclusions

This study showed both morphological and antioxidant system alterations in the in gills of *Carassius auratus gibelio* exposed to deltamethrin. The activities of CAT and GPX, the most sensitive enzymes to ROS production in aquatic organism, were decreased after pesticide administration. One the other side, the up-regulation in GST activity suggested the implication of this enzyme in deltamethrin detoxification. The results of this study suggested that deltamethrin exposure induced oxidative stress and modulated CAT, GPX, GR and GST activities in the gills of *Carassius auratus gibelio.* Epithelial rupture, chloride cells proliferation, lamellar cells hypertrophy, secondary lamellae fusion occurred. The various parameters studied in this investigation could be used as biomarkers of exposure to deltamethrin.

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EFECTE BIOCHIMICE SI HISTOLOGICE ALE EXPUNERII LA DELTAMETRIN ASUPRA BRANHIILOR DE *CARASSIUS AURATUS GIBELIO*

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Acest studiu a investigat modificăr ale activităţilor mai multor enzime antioxidante induse în branhiile speciei dulcicole Carassius auratus gibelio expuse la deltametrin.Pentru a realiza acest obiecti, loturi de câte 10 indivizi au fost expuse timp de una, două, trei, şapte şi paisprezece zile la o concentraţie subletală de deltametrin (2 μg/L). Un alt lot a fost utilizat drept martor. Activităţile specifice ale catalazei, glutation peroxidazei şi glutation reductazei au scăzut semificativ, în timp ce cea a glutation-S-transferazei a crescut. Peştii expuşila o concentraţie de 2μg/L au prezentat alterări morfologice la nivelul branhiilor după 48 h, care s-au accentuat după 14 zile. S-au înregistrat hiperemie, fuziunea lamelelor secundare, rupture stratului epithelial şi proliferarea celulelor clorogene. .Aceste rezultate sugerează un răspuns adaptativ imediat la stres oxidativ şi demonstrează alterări la nivelul sistemelor antioxidante în branhiile peştilor intoxicaţi cu deltametrin.

Key words: deltametrin, branhie, sistem enzimatic antioxidant, stres oxidativ