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Proportioning of Biomarkers (GSH, GST, Ache, Catalase) Indicator of Pollution at *Gambusia affinis* (Teleostei Fish) Exposed to Cadmium

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Abstract: In this experimental study we were interested in cadmium, a heavy metal frequently met in the watery ecosystems located in residential and industrialized areas. This research aims at evaluating the impact of cadmium, diluted with various amounts (0.1, 1 and 5 μg L^{-1}) in the water of various batches of *Gambusia affinis*, by the proportioning of certain biomarkers indicator of pollution: the GSH, the GST, the catalase (on the level of the liver) and the acetylcholinesterase (on the level of the brain). The lethal concentrations (100% of mortalities) are equal to 10 cadmium μg L^{-1}. The results of proportionings show that the contents of the biomarkers vary according to the amount and from the exposure time to cadmium. The exposure of fish to 5 cadmium μg L^{-1} causes an activation of the system of detoxification which is translated, as from the 7th day of exposure, by the reduction in the rate of GSH and the increase in the activity of the GST. The activity of the hepatic GST presents a significant and very significant difference in fish exposed during 15 days to the respective amounts of 1 μg L^{-1} and 5 cadmium μg L^{-1}. With regard to the catalase of the falls of the content are raised at the 7th day of exposure at the unit of the batches treated with cadmium. As for the acetylcholinesterase it is in fish exposed to 5 μg L^{-1} during more than 15 days that a significant difference is observed.

Key words: GSH, GST, Catalase, acetylcholinesterase, *Gambusia affinis*, cadmium

INTRODUCTION

Contrary to many metals (copper, zinc, iron...); cadmium does not have any known metabolic role and does not seem biologically essential or beneficial with the metabolism of the alive beings (Chiffoleau *et al*., 1999). But it is, on the other hand, xenobiotic placed on the black list of the majority of the International Conventions of pollution because of its cytotoxicity, its genotoxicity, its potential of bio-accumulation and its persistence (Taylor, 1983). Cadmium reaches the aquatic environments by the atmospheric way or scrubbing of the grounds and forward thrusts anthropic (Miramand *et al*., 2000). Many studies concerning the contamination of coastal fish by heavy metals and in particular cadmium, were focused on the bio-accumulation of this metal in the bodies of the fish (Smith *et al*., 1976; Cattani *et al*., 1996; Miramand *et al*., 2000; Al-Yousuf *et al*., 2000; Tayal *et al*., 2000; Pigeot, 2001; Scott *et al*., 2003; Tophon *et al*., 2003).

As regards monitoring of the ecosystems it is more relevant to prevent the effects of pollutants on the health of the organizations and the ecosystems rather than to note them a posteriori. So the biological analyses, which integrate the interactions between all the pollutant present and the organizations, make it possible to provide a more realistic diagnosis of the impact of pollution on the organizations which populate the ecosystems. The biomarkers are thus of the interest to be able to quickly detect interactions pollutants/organisms after exposure and in certain cases, to predict potential toxic effects. In this context, we chose the observation of the impact of a chemical aggression induced by cadmium in a predatory fish of larvae of mosquitos "*Gambusia affinis*", by the proportioning of reduced Glutathion, of Glutathion-S-transferase, the Catalase and the acetyl cholinesterase.

MATERIALS AND METHODS

Biological material: *Gambusia affinis* are a Teleosteen fish extremely robust and rustic which adapts well to all kinds of qualities of water, of fresh waters with brackish water. The fish used in this experimentation are fished in the Kherraza wadi; they are placed in aquariums (of a capacity from 40-75 L) equipped with a filter, an air pump and a diffuser.

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Experimental protocol: The choice of the cadmium amounts used in this experiment is based on data of acute toxicity reported in the literature (0.5 μg L^{-1} with 500 mg L^{-1}). Under our conditions of exposure (pH = 8. 86; Temperature = 25°C), the lethal amount (100% of mortality) is of 10 μg L^{-1} and the sub lethal amounts are of 0.1, 1 and 5 μg L^{-1}. The fish used are all of sex female, mature weighting on average 3.5±0.75 g. They are distributed, at a rate of 60 specimens per batches, in 5 aquariums, of which one will be used as witness and will thus not be treated. After 48 h of acclimatization, half of the water contained in each aquarium is renewed and cadmium is introduced with the amounts making it possible to obtain the final concentrations of 0.1, 1 and 5 μg L^{-1}. The durations to xenobiotic are of 7, 15, 21 and 30 day.

Proportioning of proteins and measurements of the enzymatic activities: After anesthesia with ether, according to the method of Vivien (1941), the hepatopancreas and the brain are taken, weighed using a balance of precision (Sartorius of precision 1/10 mg) and are maintained at low temperature using ice. The hepatopancreases and the brains of each batch are pooled at a rate of 100 mg then deposited in tubes or they will be used for the preparation of the homogenate. This last is centrifuged with 5000 tours min^{-1} during 15 min, the supernatant obtained is recovered to be used for proportionings of total proteins (according to the method of Bradford, 1976), the reduced glutathion (Weckbeker and Cory, 1988) and the activities of the GST (Habig *et al*., 1965), of the catalase (Aebi, 1983) and of the acetylcholinesterase (Ellman *et al*., 1961).

Statistical analysis: The statistical analysis of the results was realized using a software of data analysis and processing (Minitab 4.1, *Eds*, 2004). The results are expressed in averages at least the standard deviation and were compared by the application of the test T of Student.

RESULTS

The contents of hepatic GSH vary according to the amount and from the exposure time (Fig. 1): the contents of GSH of fish exposed to cadmium present significant differences at the amounts of 0.1, 1 and 5 μg L^{-1} for respective exposure times, of 30, 15 and 7 days (T vs 0.1 μg L^{-1}, $p<0.05$ at 30 days; T vs 1 μg L^{-1}, $p<0.05$ at 15 days; T vs 5 μg L^{-1}, $p<0.05$ at 7 days). However, very significant differences are noted as from 21 days in exposure to the amount of 5 μg L^{-1} (T vs 5 μg L^{-1}, $p<0.01$ at 21 and 30 days).

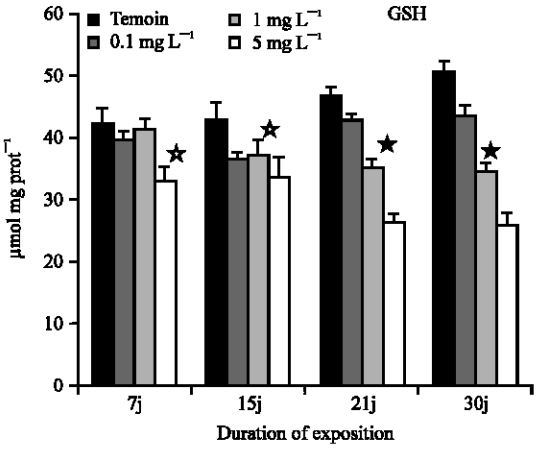

Fig. 1: Variation of the content of hepatic GSH in μmol mg^{-1} protein at *Gambusia affinis* exposed to cadmium (☆$p<0.05$ vs T et ★$p<0.01$ n = 15)

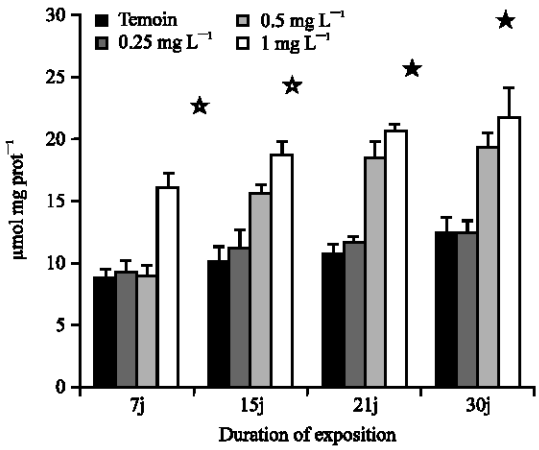

Fig. 2: Activity of the hepatic GST at *Gambusia affinis* exposed to cadmium (☆$p<0.05$ et ★$p<0.01$ vs T. n = 15)

The activity of the hepatic GST depends on the cadmium amount to which the fish were exposed and of the exposure time (Fig. 2): this activity of the GST shows a significant difference in fish exposed to the intermediate amount (cadmium 1 μg L^{-1}) as from 15 days (T vs 1 μg L^{-1}, $p<0.05$ at 15, 21 and 30 days of exposure). It is, in addition, with the amount of 5 cadmium μg L^{-1} which the activity of the hepatic GST is significant at 7 days of treatment ((T vs 5 μg L^{-1}, $p<0.05$ at 7 days) and very significant after 15 days of exposure (T vs 5 μg L^{-1}, $p<0.01$ at 15, 21 and 30 days).

The activity of the hepatic catalase does not show great variations at the gambusies of the various batches (Fig. 3). The statistical analysis of the data shows that

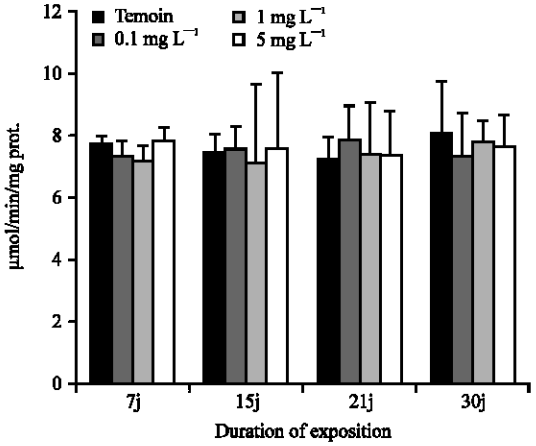

Fig. 3: Activity of the hepatic catalase at *Gambusia affinis* exposed to cadmium (n = 15)

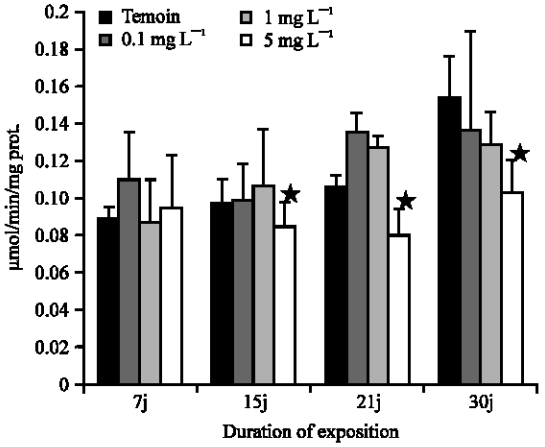

Fig. 4: Activity of Acetylcholinesterase at *Gambusia affinis* exposed to cadmium (★p<0.01 vs T n = 15)

there is not significant difference between the values of the pilot batch and those of the batches exposed to the various cadmium amounts.

The activity of Acetylcholinesterase depends on the amount and the exposure time to cadmium (Fig. 4): The statistical analysis of the data reveals the absence of significant differences between the values of the activity of the AchE at the pilot batches and those of the batches exposed to 0.1 and 1 µg L^{-1}. It is however in fish exposed to 5 µg L^{-1} during more than 15 days that a significant difference (T vs 5 µg L^{-1}, $p<0.01$ at 15, 21 and 30 days) is observed.

DISCUSSION

The results of the proportioning of the content of GSH and the activity of the GST at the batches of fish *G affinis* treated with cadmium reveal variations according to the amount and of the exposure time to metal. We observe at the exposed gambusies with the strong cadmium amount a significant increase in the activity of the GST whereas the concentration in GSH decreases significantly compared to the witness. The reduction in the GSH can be explained by the fact why in front of this type of toxicity, the liver does not manage to eliminate concentration such an increased from toxic metabolites (Kojima *et al.*, 2000); As for the increase in the activity of the GST, it corresponds to the increase in the capacity of detoxication of the liver for stage to the invasion of the organization by cadmium. Mumtaz *et al.* (1994), announced an increase in the hepatic and cervical GST following a cadmium injection to guinea-pigs.

The results obtained confirm the known capacities of cadmium to generate cellular effects on various metabolic levels. This metal thanks to its oxidizing capacity and its capacity to be interfered with the groupings intracellular thiols would generate, according to certain authors, the formation of aberrant mixed complexes proteins-proteins or proteins-glutathion (Singhal *et al.*, 1987; Stohs and Bagchi, 1995).

The toxicity of cadmium reported at many watery organizations relates especially to many physiological parameters which depend on the species tested and the experimental conditions (Chiffoleau *et al.*, 1999). Cadmium can reduce the survival of organizations such as the amphipode *Gammarus fossarum* and even result in their death when they are exposed to a cadmium concentration of 1 mg L^{-1} (Abel and Barlocher, 1988). Cadmium can also stimulate the metamorphosis of larvae of the polychète euryhalin *Capitella* sp. with external cadmium concentrations from 1-2 mg L^{-1} (Pechenik *et al.*, 2001). In the sea urchins, cadmium, present in the solid sediment with concentrations higher or equal to 2 g L^{-1}, can decrease the success of the embryogenesis of *Paracentrotus lividus* (Amiard-Triquet, 1998), this metal can also weaken the reproductive success of *Strongylocentrotus intermedius* to concentrations ranging between 0.05 and 0.1 mg L^{-1} through a reduction in the quality of the gamètes (Au *et al.*, 2001). Cadmium can also result in the death of various organizations; Ramachandran *et al.* (1997) DL50 pay to 48 H of 0.312 µg ml-1 in larvae of sea urchin *Diadema setosum* (malformations) and of 0.078 µg mL^{-1} in crab larvae *Scylla seratta* (mortalities). Moreover, an important mortality (40%) was observed in larvae of copepode *Tigriopus brevicornis* after 8 days of exposure to polluted sediments (Amiard-Triquet, 1998).

With regard to the activity of the catalase, our results do not show large variations at the gambusies of the various batches. At *Carassius auratus* exposed to 20 cadmium mg L^{-1} during 15 days, Zikic *et al.* (2001)

brings back an increase in the activity of two enzymes (SOD and CAT) to the level of the erythrocytes and a tissue deterioration of fish. At Epinoches adults males and females, the exposure to copper during 21 days generated a reduction in the activity of the CAT as from the 8eme day of exposure (INERIS, 2003). It is interesting to note that the transitory response of antioxidants defenses precedes accumulation by metal in the liver which is visible only as from 7 days with the strong amount. The temporal shift between the antioxidants answers and the accumulation of metal goes in the direction of an implication of these first in the regulation/detoxification of this metal and its oxidizing impact, before it does not accumulate. It is probable that other mechanisms of defence, like the metallothioneines, are brought into play.

The activity of the acetylcholinesterase (AchE) measured at the gambusies exposed to cadmium does not show significant differences between the pilot batches and the batches exposed to 0.1 and 1 μg L^{-1}; It is only in fish exposed to 5 μg L^{-1} during more than 15 days that a significant difference is observed. Devi and Fingerman (1995) report that the exposure of the fish *Procanbarus clarkii* to cadmium and lead causes an inhibition of the activity of the AchE. According to Caragorgeorgiou *et al.* (2004), important inhibitions of the AchE activity are observed in rats males having received cadmium; these inhibitions could moderate the cholinergic mechanisms of the SNC and involve a neuronal exitability. According to certain authors, the measurement of the activity of the AchE was especially used as biomarker for the other xenobiotics ones such as the pyrethroides (used in the domestic products of the fight against the insects) and incidentally for heavy metals (Amiard, 1990; Fulton and Key, 2001).

This research is a contribution to the description of the answers molecular and cellular adaptive of an organization not noted *Gambusia affinis* following radicalizing attacks induced by an exposure to sub lethales concentrations of cadmium. In prospects, it would be interesting to study the evolution of a battery of biomarkers such as: The Superoxyde Dismutase (SOD) key enzyme implied in the mechanism of the dismutation of the anions superoxyde (O^2), very active free radical, the EROD which reflects the activity of the P450 cytochrome, the metallothioneine for the measurement of the contamination by heavy metals.

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