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Evaluation of Detoxification Enzyme Levels in Egyptian Catfish, *Clarias lazera,* Exposed to Dimethoate

R. R. Hamed,[1] Sh. E. Elawa,[2] N. M. Farid,[1] F. Sh. Ataya[1]

[1] Molecular Biology Department, Genetic Engineering Division, National Research Centre, El-Tahrir St. Dokki, Cairo, Egypt
[2] Faculty of Science, El Mansura University, Egypt

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Living organisms, including fish, possess a pre-existing defence mechanism capable to eliminate toxic chemicals either by enzymatic degradation, conjugation or excretion. Fish are sensitive indicators of residues in water in which the amounts of pesticides are too small to be reliably analysed chemically. Laboratory model ecosystems of microsomes are now well-established tools for investigating the environmental toxicology of pesticides. The xenobiotic metabolizing enzymes have been used as biomarkers for chemical induced cytotoxicity including carcinogenesis in mammals and aquatic communities (Hendrich and Pilot 1987; Bailey et al. 1992; Naqvi and Vaishnavi 1993; Kirby et al. 1995). Among these enzymes are glutathione peroxidase (E.C. 1.11.1.19, GSH PX). It catalyses the reduction of hydroperoxidase by reduced glutathione. Glutathione reductase (E.C. 1.6.4.2), catalyses the reduction of oxidized glutathione using NADPH as a hydrogen doner. Glutathione-S-transferases (E.C.2.5.1.18) are a group of enzymes that catalyse the chemical conjugation of reduced glutathione, to a variety of electrophilic compounds (Lauren et al. 1989).

In Egypt, both marine and fresh water fish are of considerable importance as a source of meat. El Elaimy et al. (1990) studied the effect of insecticides on the enzymes acetylcholinestrase, adenosine triphosphate carboxylestrase, glutamate pyruvate transaminase and glutamate oxalate transaminase in Nile water fish *Tilapia nilotica, T. zilla, Clarais lazera* and *Chrichrysis auratis. Clarias Lazera* is one of the most important fresh water fish in Egypt and Africa , well marketable , becoming important in aquaculture. Total production in 1996 from the river Nile only contributes about 17.5% of the total Nile catch in Egypt (Gafrd, 1997). In this study, the relation between the detoxyfying enzymes and Nile fish *Clarias lazera* to resist the insectiside (dimethoate) was examined.

MATERIALS AND METHODS

Reduced glutathione (GSH), H_2O_2, L-oxidized glutathione (GSSG), NADPH and glutathione reductase (Type III from baker's yeast. E.C.1.6.4.2, G-4751, 200 unit /mg) were perchused from Sigma. The organophosphorus insecticide

Correspondence to: R. R. Hamed

dimethoate[O,O-Dimethyl-S(N-methyl carbamoyl methyl) phosphorodithioate] and all other chemicals were of the highest purity commercialy avaliable.

The Nile fish *Clarias lazera* (130-l60 g each) were captured from Abu Rawash Farm, Giza Egypt. The fish were maintained for 7 days in previously aerated tap water in glass tanks (5 fish /25 L tank) for adaptation and clearance of pollutants from fish. Continous air flow and feeding with artificial dry food were provided. The unexposed fish served as the control samples. The fish were divided into groups (10 fish each) and subjected to insectiside treatment. The fish were exposed to ¼, ½ and LC_{50} (LC_{50} for dimethoate for 96 equal 45 mg/L). After this period the treated fish was killed by cervical dislocation, their livers, kidneys and gills were removed and stored at -20°C. Organ homogenates (20% w/v) were prepared using either 0.1 M potassium phosphate buffer, pH 6.5 for determination of the glutathione-S-transferase (GST) activity or 50 mM potassium phosphate buffer , pH 7.0 containing 1 mM EDTA for glutathione peroxidase (GP) and glutathione reductase (GR) enzymes. The homogenates were then centrifuged at 10,000xg for 30 min and the supernatants were filtered through a plug of glass wool to remove lipids.

The activity of GST was determined spectrophotometrically by following the formation of GSH conjugate with 1-chloro-2,4 dinitrobenzene (CDNB) at 340 nm using extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ (Habig et al. 1974). The reaction mixture contained in 1 mL volume : 0.1 M potassium phosphate buffer, pH 6.5, 1mM GSH, 1mM CDNB in ethanol and the enzyme solution. One unit of transferase activity is defined as the amount of enzyme which catalyse the formation of 1 µmole of thioether per min. The activity of GR was determined spectrophotometrically at 25°C following the decrease in absorbance at 340nm according to the method described by Zanetti (1979). The reaction mixture contained in 1 mL volume: 50 mM potassium phosphate buffer, pH 7.0, 1mM EDTA, 0.1 mM NADPH, 0.5 mM GSSG and the enzyme solution. The activity of GP was determined spectrophotometrically at 25°C according to the method described by Weinhold et al. (1990) in which the GSSG produced due to peroxidase activity is coupled to the reaction catalyzed by GR. The reaction mixture contained in 1mL volume: 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.75 mM H_2O_2, 1 mM GSH, 0.2 mM NADPH, 1.6 IU/ml GR and enzyme solution. One unit of GR or GP activity is defined as the amount of enzyme which oxidize 1 nmol of NADPH/min under the assay conditions. The controls containing buffer instead of the substrate CDNB for GST, NADPH or GSSG for GR and NADPH or GSH for GP were routinely included and treated under the same conditions of the enzyme assay. Protein was determined by coomassie brilliant blue G 250 using bovine serum albumin as standard (Bradford 1976). The total glutathione was measured calorimetrically according to the method of Saville (1958). Tissue was homogenized in 0.1 M potassium phosphate buffer, pH 7.0 and the tissue extract was mixed with equal volume of 13% TCA. The precipitated proteins was removed by centrifugation at 2,000xg for 10 min and the supernatant was used in the assay for determination of total glutathione.

RESULTS AND DISCUSSION

The effect of insecticide dimethoate at ¼, ½ and LC_{50} (LC_{50} for Dimethoate for 96 hr equal 45 mg/L) on glutathione and the enzymes GST, GP and GR were examined in the gills, liver and kidney of the fish *C. lazera.*

The highest level of glutathione was in the kidney tissues compared to liver and gills (3.2 and 5.5 fold respectively, P > 0.001). The level of glutathione in liver, kidney and gills increased in the fish treated with ¼ LC_{50}. The increase in the liver is statistically significant (P>0.00l), while the increases in the gills and kidney are statistically insignificant (P>0.001). At higher dimethoate concentration the accumulation of glutathione decreased (Table 1). The protein extracted from the three organs was insignificantly affected by treatment of the fish with dimethoate.

Table 1. Effect of dimethoate on glutathione and protein of *C. lazera* organs.

Organ	Sample	GSH (mg/g tissue)	GSH %	Protein (mg / g tissue)
Liver	Control	118.30 ± 16.3		88.96 ± 7.05
	1	353.8 ± 50.6	299.0	90.44 ± 9.9
	2	253.56 ± 43.7	214.3	82.78 ± 5.97
	3	197.80 ± 37.0	167.2	89.88 ± 8.33
Kidney	Control	388.20 ± 39.8		45.72 ± 4.09
	1	473.98 ± 39.8	122.2	52.2 ± 7.82
	2	328.61 ± 60.4	84.6	47.16 ± 5.02
	3	268.12 ± 17.7	69.0	51.22 ± 3.22
Gills	Control	70.60 ± 9.9		8.88 ± 0.45
	1	75.14 ± 14.00	106.4	11.3 ± 3.52
	2	46.0 ± 3.88	65.2	6.63 ± 0.49
	3	38.26 ± 8.12	45.2	12.13 ± 2.64

1, 2, 3 refered to fish treated with ¼ , ½ and LC_{50} respectively. Each figure represents the mean ± SE and n = 7.

Numerous differences related to glutathione and its metabolizing enzymes were observed in the tissues of *C. lazera.* The GSH concentration was significantly low when compared to the values reported for fish species e.g. 991± 83, 534 ± 46 and 380 ± 80 nmole/g liver tissue for channel catfish, brown bulhead and pike respectively (Koss et al. 1991; Hasspieler et al. 1994). Generally the values reported for fish liver were several fold lower than those of mammals (Hunaiti and Abu Khalaf 1986; Gallagher and DiGiulio 1992). Also the value of 2.28 ± 0.24 nmole/Kg kidney tissues was reported for the rainbow trout *Salmo gairdneri* (Nimmo 1986). This value is several fold higher than that of C. *lazera.*

Table 2. Effect of dimethoate on glutathione - S - transferase, glutathione reductase and glutathione peroxidase levels of *C. lazera* liver.

Sample	GST			GR			GP		
	Specific activity (unit / mg protein)	%	Activity (unit /g tissue)	Specific activity (unit / mg protein)	%	Activity (unit / g tissue)	Specific activity (unit / mg protein)	%	Activity (unit / g tissue)
Control	0.22 ± 0.02	100	19.8 ± 2.7	0.57 ± 0.1	100	49.8 ± 7.4	2.3 ± 0.3	100	210 ± 29.0
1	0.21 ± 0.03	95	18.2 ± 1.4	0.81 ± 0.11	142	69.3 ± 5.2	2.7 ± 0.4	117	236 ± 22.0
2	0.14 ± 0.03	64	11.7 ± 2.7	1.04 ± 0.34	183	84.4 ± 25.5	2.4 ± 0.1	104	202 ± 24.0
3	0.12 ± 0.01	55	10.7 ± 1.3	0.81 ± 0.1	142	71.7 ± 0.2	2.3 ± 0.2	100	202 ± 21.0

1,2,3 refered to fish treated with ¼, ½ and LC_{50} respectively. Each figure represents the mean ± SE and n = 7.

Table 3. Effect of dimethoate on glutathione - S - transferase, glutathione reductase and glutathione peroxidase levels of *C. lazera* kidney.

Sample	GST			GR			GP	
	Specific activity (unit / mg protein)	%	Activity (unit / g tissue)	Specific activity (unit / mg protein)	%	Activity (unit / g tissue)	Specific activity (unit / mg protein)	%	Activity (unit / g tissue)
Control	0.11 ± 0.02	100	5.1 ± 0.34	2.34 ± 0.42	100	104.83 ± 14.0	49.8 ± 9.7	100	2260 ± 446
1	0.05 ± 0.01	45	2.8 ± 0.45	2.5 ± 0.18	73	128.01 ± 12.9	22.5 ± 3.7	45	1104 ± 115
2	0.04 ± 0.01	36	1.7 ± 0.38	2.43 ± 0.19	71	113.7 ± 12.52	17.0 ± 4.0	34	809 ± 177
3	0.04 ± 0.01	36	1.9 ± 0.28	1.5 ± 0.23	44	71.55 ± 2.10	28.0 ± 4.1	56	1530 ± 418

1,2,3 refered to fish treated with ¼, ½ and LC_{50} respectively. Each figure represents the mean ± SE and n = 7.

The GST activity (units /g tissue) and specific activity (units / mg protein) decreased with increasing the concentration of dimethoate in both the liver and kidney (Table 2,3). The decreases at ½ and LC_{50} are statistically highly significant ($P > 0.0001$). The contrary of this behaviour was observed in the gills where highly significant increase was recorded at ½ and LC_{50}. GR and GP levels are higher in the kidney compared to liver with 2.1 fold and 10.8 fold increase respectively. Since liver is the major site for detoxification of exogenous and endogenous electrophilic compounds, it is therefore important that the tissue possesses adequate amount of the detoxifying enzymes GST and its substrate, GSH, likewise gills and kidneys are placed in anatomical positions where they can be exposed to toxic electrophiles and therefore they possess their own GST which might play an essential role in the detoxification mechanism in these tissues (Table 2, 3).

The levels of GP and CR in liver tissues were slightly affected by treatment with dimethoate (Table 2). The changes observed in the mean activity are statistically insignificant ($P > 0.1$). At ¼ and ½ LC_{50} GR specific activity of the kidney was not affected while a highly significant decrease was observed in GP. The decrease in the level at LC_{50} of either GP or GR is statistically insignificant. GP and GR activities were not detected in the gills under the experimental conditions.

The kidney of *C. lazera* had an active glutathione redox cycle compared to liver. In fish treated with dimethoate the peroxidatic activity (GSH --> GSSG) was significantly decreased. Also GST was significantly reduced, while the GR was not affected. The later enzyme is primary responsible for maintenance of the intracellular concentration of the GSH, (Kurata et al. 1993). This could be the reason for keeping the level of GSH in the kidney not affected. On the contrary of this behaviour, the redox cycle in the gills are almost absent, and GST increase in the fish treated with dimethoate (Table 4). These directly affected the GSH level, where it is significantly decreased.

Table 4. Effect of dimethoate on glutathione-S-transferase levels on *C. lazera* gills.

Sample	Specific activity (unit / mg protein)	GST %	Activity (unit / g tissue)
Control	0.02 ± 0.004	100	0.18 ± 0.04
1	0.06 ± 0.004	300	0.13 ± 0.03
2	0.08 ± 0.003	400	0.49 ± 0.12
3	0.01 ± 0.003	50	0.11 ± 0.03

1,2.3 refered to fish treated with ¼, ½ and LC_{50} respectively. Each figure represents the mean ± SE and n=7.

GSH conjugation, catalyzed by GST, is an important phase II reaction. Phase II reactions are catalyzed by the conjugation enzymes joining the phase I metabolites to an endogenous conjugating agent forming more water soluble derivatives that are eliminated from the body. Elevated expression of glutathione-S-transferase has a protective effect against environmental carcinogens (Gulick and Fahl, 1995). Hence, decrease in GST activity could result in enhanced toxicity of environmental chemicals or endogenous compounds and the possibility that some forms of neoplasms may be induced in fish. Fish live in an environment where they are exposed to various amounts of toxic chemicals. The answer to whether the inhibition of GST is good as an early monitoring for pollution depends on the chemical environment of the fish.

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