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

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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

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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.