Egypt. J. Aquat. Biol. & Fish., Vol. 10, No.4: 181 –193 (2006) ISSN 1110 – 6131

BIOCHEMICAL RESPONSES IN *OREOCHROMIS NILOTICUS* AFTER EXPOSURE TO SUBLETHAL CONCENTRATIONS OF DIFFERENT POLLUTANTS

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Keywords: biochemical responses, *O.niloticus*, malathion, lannate, phenol, AChE, GST

ABSTRACT

Fish responses have been used as biomarker of aquatic pollution. The objective of this study was to determine some biochemical responses in *Oreochromis niloticus* after exposure to sublethal concentrations of three different pollutants . In the present study ,malathion (1.75mg/L), (organophosphorus insecticide) ,lannate (0.65 mg/L) (carbamate insecticide)) and phenol (9 mg/L) (phenolic compound) were selected as model compounds of three different pollutants. Studies were carried out to determine the effect of these compounds in acetylcholinesterase enzyme(AchE) as a biomarker of effect and glutathione S – transferase (GST) enzyme as a biomarker of defense in brain , serum and liver of *O . niloticus* after three weeks of exposure. The results indicated that : 1-the activity of acetylcholinesterase enzyme decreased significantly in brain ,serum and liver of *O. niloticus* exposed to malathion and lannate after 1, 2 and 3 weeks when compared with the control group, phenol treated group has low effect. 2- the activity of glutathione S- transferase decreased significantly in brain and serum of *O. niloticus* exposed to malathion and lannate , but in phenol treated group the activity of glutathione S – transferase increased significantly in brain and serum. 3-the activity of glutathione S- transe ferase increased significantly in liver of *O. niloticus* exposed to phenol more than malathion and lannate.

INTRODUCTION

As a result of human developmental activities, a number of environmental pollutants such as pesticides, heavy metals and aromatic compounds entered the water courses through air, river discharge and land drainage. The behavior of aquatic organisms largely depends on the

physical and chemical state in which they are present and the prevailing environmental condition.

In recent years a number of biomarkers have been suggested to environmental biomonitoring programs for assessment of stress caused by pollution , especially in aquatic organisms. These biomarkers include: 1-biomarkers of exposure (defense) , which are only indicative for an exposure to an environmental stress, and 2- biomarkers of effect (defect),which are indicative both for exposure and occurrence of adverse (e.g toxic) effects on the (bioindicator) organisms studied (Sanders and Martin,1993). The aim of programs based on biomarkers was the establishment of early warning systems allowing the detection of environmental stress before irreversible damage occurs.

The impact of aquatic pollution on human and animal life has become a matter of great concern. Fish responses have been used as biomarkers of aquatic pollution (Berntessen *et al*., 2003; Ek *et al*., 2005) .

The responses of various Xenobiotic metabolizing enzymes in fish are rapidly evolving as important biomarkers for monitoring unacceptable levels of environmental contamination .

Organophosphorus and carbamate compounds are very widely used insecticides. They are toxic because they inhibit acetylcholinesterase (AChE), the enzyme that is responsible for hydrolyzing and so deactivating acetylcholine in the nervous system. AChE – inhibiting pesticides can contaminate surface waters through unintentional drift of aerial spraying for agricultural use, through watershed drainage or accidental spillage and even through intentional application (Ibrahim *et al.,* 1998) .

Phenol is a common toxic component of various industrial waste waters. It has antiseptic properties and is a major intermediate in the manufacturing of dyes, medical products, resins and other industrial compounds (Assem *et al*., 1992) . Phenolic compounds can affect fish adversely by direct toxicity to fish and their consumers by lowering the amount of available oxygen by tainting the fish flesh and on enzyme system by uncoupling oxidative phosphorylation and tissues damage (Mohamed *et al* ., 1997) .

In Egypt, effluent of oil refiners contains phenol and the receiving waters are subjected to low level chronic phenol pollution .

Xenobiotic metabolism in fish is often referred to a biotransformation reaction or detoxification reaction and the pathway involved is usually divided into phase I and phase II reactions. In phase I

reaction a function group of xenobiotic oxidized or hydrolyzed by mixed function oxidase system (MFO) is involved in oxidation by a variety of isozymes of cytochrome P450. Phase II reaction biosynthetic reaction in phase I derived metabolite is covalently linked to endogenous molecules such as glutathione, which is catalyzed by glutathione S transferase enzymes.

Phase II enzymes were measured by catalytic activity of glutathione –S transferase (GST) (Behrens and Segner , 2001) .

Inhibition of acetylcholinesterase activities (neurotoxicity) and induction of glutathione –S- transferase activities (antioxidant defense) in fish are used as indicator of environmental pollution . So the aim of this study was to evaluate brain , serum and liver acetylcholinesterase activities as a biomarker of the effect and glutathione –S transferase activities as a biomarker of defense in *Oreochromis niloticus* after exposure to sublethal concentrations of malathion, lannate and phenol for a period of three weeks

MATERIALS AND METHODS

Pollutants

1- Malathion: is an organophosphorus insecticide manufactured by El-Nasr pharmaceutical chemicals company as a stock solution (57%) with M. Wt of 330.
2- Lannate : It is an carbamate insecticide with M. Wt. of 160 and available in 9o % powder and soluble in water manufactured by Dupont Co.
3- Phenol : Technical grade of phenol C6H5OH, M. wt. 94.11 manufactured by El.Nasr pharmaceutical chemical company

Experimental animals

Healthy living specimens of *Oreochromis niloticus* were collected from a fish farm at EL Kanater –AL Khairya. Fish were examined for any pathological symptoms. They were transported to the laboratory conditions for a period of 15 days for acclimation in well aerated dechlorinated tap water. The water in each aquarium was continuously aerated by an air compressor. They were transferred to experimental glass aquaria each of 100 L .Water temperature was recorded daily and water analyses were carried out each week to determine pH , dissolved oxygen , ammonia, nitrate and nitrite according to APHA (1992). Fish were fed on artificial pellets of 30% protein once per day .

Experimental design

Experiments were carried out on *Oreochromis niloticus* weighing 70 ± 12.0 g with total length 17.3 ± 2.0 cm .

Based on the results of Mohamed *et al*. (1997) Gad ,(1999)the sublethal concentrations (1/4 LC_{50}) of malathion , lannate and phenol were 1.75 , 0.65 and 9 mg/L respectively.

In the present experiment fish were divided into four groups, in three replicates each group was 24 fish as follows :

Group I : Exposed to 1.75 mg/L of malathion .
Group II : Exposed to 0.65 mg/ L lannate .
Group III : Exposed to 9 mg/ L phenol .
Group IV: Served as control.

The water in each aquarium was renewed every four days during the experimental period (three weeks) and freshly prepared solution was added to bring the concentrations of the pollutant to the requisite levels . Six fish from each of the four groups were taken out for dissection on 1^{st}., 2^{nd} and 3 weeks of exposure.

Blood samples were taken by severance of the caudal peduncle of fish and collected in small vials, left to clot and then centrifuged at 3000 r. p. m for 10 minute to obtain serum for enzyme determination.

Brains and livers tissues were isolated immediately .The tissues were homogenated in cold 0.15 M Kcl solution using homogenizer. Tissue homogenates were centrifuged at 15, 000 r .p .m for 10 min and used for the enzyme assays .

The activities of acetylcholinesterase was measured using commercial kit produced by Bohringer Mannhim based on Ellmann *et al* . (1961) and the activities of glutathione –S –transferase was measured using Vassey and Boyer (1984) . The enzymes activities were expressed as U mol /min/ mg protein .

Statistical analysis

The significance between control and experimental results was analyzed statistically by using the Student' s t – test (Snedecor , 1962) .

RESULT

The activity of acetylcholinesterase was measured after 1,2 ,and 3 weeks of exposure to malathion , lannate and phenol . The results are reported in Table (2).

Table (2) shows that the activities of AChE in brain , serum and liver of *O. niloticus* exposed to malathion and lannate were decreased

significantly after 1, 2 and 3 weeks of exposure ($P < 0.01$) when compared with the control group. However, in phenol treated groups the activities of AChE were not significantly changed.

In general, most polluted fish had inhibited acetylcholinesterase activities when compared with the control group and the greatest inhibition was observed in malathion and lannate exposed fish and significant inhibition in phenol exposed fish after 3 weeks.

Table (3) reveals that the activity of glutathione - S transferase in brain and serum of *O . niloticus* was significantly decreased (0.44 ± 0.12 , 0.49 ± 0.01 and 0.047 ± 0.002 ,0.050 ± 0.002 U mol / min / mg protein) in malathin and lannate exposed fish after 3 weeks of exposure as compared with the control group (0.60 ± 0.13 and 0.061 ± 0.0001) ,while the activity increased in phenol exposed fish (0.72 ± 0.01 and 0.076 ± 0.001 U mol / min mg protein.). However, the activity of glutathione –S transferase in liver was increased significantly in malathion, lannate and phenol exposed fish after 3 weeks of exposure with induction of 13.0 % , 51.5 % and 102 % and specific activities of 1.12 ± 0.08 , 1.5 ± 0.23 and 2.0 ± 0.35 U mol / min / mg protein respectively .

DISCUSSION

The pharmacological effect of organophosphorus and carbamate insecticides is one of the main causes of disruption of nerve impulse transmission in the central and peripheral nervous system by inhibition of acetylcholinesterase (Daabees *et al.,* 1992).

Inhibition of this enzyme lead to building up of acetylcholine at sites of cholinergic synapses and hence to permanent nerve stimulation with extreme lethal results .

Results of the present study showed that inhibition of AChE activities in brain, serum and liver significantly increased in malathion poisoned fish (75 , 39.8 and 55.9 %) and least in lannate and phenol (62.5 ,40.5 , 30.2 % and 25 , 24.5, 23.3 %) after1, 2 and 3 weeks of exposure respectively .

The significant inhibition of acetylcholinesterase by malathion (organophosphorus) and lannate (carbamate) may be due to phosphorelation or carbamilation of esterase site, serine hydroxyl group of the enzyme .Thus, the anticholinesterase potency depends largely on the phosphorylating or carbamylating ability of either organophosphate or carbamate ester respectively .

The inhibition of AChE activities in brain, serum and liver of *O. niloticus* in the present investigation is in accordance with that recorded in the brain of Zebrafish exposed to 0.5 -1.1 ppm malathion for 7 days (Ansari and Kumar, 1984), in brain of *Cyprinus carpio* exposed to dimethoate (Satyadevan *et al.*, 1993) , in brain of *O .niloticus* exposed to malathion and bayluscide for 30 days (Danasoury *et al*., 1997) and in brain and muscle of Chinook salamon exposed to chloropyrifos for 96 hr. (Wheelock *et al* .,2005)).

Since organophosphorus and carbamates have a relatively short half life, the assessment of cholinesterase inhibition is a useful tool to evaluate their environmental impact on aquatic biota, even when they are no longer detectable in the environment (Valbonesi *et al*., 2003). The inhibition of neural acetylcholinesterase by organophosphorus and carbamate pesticides is the primary toxic mechanisms of these compounds .

In Mammals , glutathione – S – transferase are thought to play a physiological role in the inhibiting the detoxification of potential alkylating agents including drugs, pesticides and other non polar environmental agents. These enzymes catalyze the reactions of such compounds with the SH group of glutathione (GSH), there by neutralyasing their electrophilic sites and rendering the products to more water soluble .Glutathione conjugates are through to be metabolized further by cleavage of the glutathione and glycine residues (Daabees *et al* .,1992).

The present results revealed inhibition of brain and serum glutathione -S transferase activities of *O .niloticus* by malathion and lannate compared with the control group after 3 weeks of exposure, while induction of glutathione- S transferase in brain, serum and liver were recorded in phenol treated fish after three weeks of exposure . The induction of glutathione S- transferase in liver , brain and serum of *O . niloticus* exposed to phenol to protect biological macro molecule from potential toxicity of phenol and its metabolites .

The increased glutathione -S transferase activities indicate that phenolic compound oxidized macromolecule and activates antioxidant defense system, which may be useful as general biomarkers of phenolic compounds exposure .

Induction of glutathione –S transferase activities in liver of *O. niloticus* exposed to phenol is greater in brain and serum . The inhibition of glutathione-S transeferase activities in brain and serum of

O. niloticus exposed to malathion and lannate may be due to reduction of enzymes ability to protect the fish from certain electrophilic toxins which are metabolized by the transferase .

Induction of glutathione –S transferase activities in brain serum and liver of *O. niloticus* exposed to phenol in the present investigation is in accordance with that recorded in brain , serum and liver of *Clarias lazera* exposed to synthetic pesticides (Daabees *et al*., 1992) ,in liver and kidney of *O. niloticus* captured from sewage polluted sites (Hamed *et al* ., 2003) , in liver of Rainbow trout exposed to cadmium (Ait –aissa *et al*., 2003), in liver of *Cyprinus carpio* exposed to polychlorinated biphenyles (Schmidt *et al.*, 2004) and in liver of rainbow trout (*Oncorhynchus mykiss*) injected by 100,200 and 400 mg/kg body weight trinitrotoluene for 72 hr (Ek *et al.*, 2005).

In conclusion , the results revealed that inhibition of acetylcholinesterase activities in brain serum and liver of fish used as useful indicator of aquatic pollution by organophosphorus and carbamate insecticides (biomarker of effect), Moreover induction of glutathione – S – transferase enzyme in fish used as an indicator of aquatic pollution by phenolic compounds (biomarker of defense) .

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Table (1): Physical and chemical characteristics of the used dechlorinated tap water

Characteristic	Values
Temperature (C)	24.0 ± 2.00
pH	7.8 ± 0.03
Dissolved oxygen	7.1± 0.50
Total alkalinity	170.000 ± 21.000
No2	0.12 ± 0.004
No3	0.00 ± 0.001
NH3	0.8709 ± 0.03
Conductivity (U mhos/Cm)	370.00 ± 10.00

Values except pH, temperature and conductivity, are expressed in mg/L
Values are mean ± standard deviation

Table (2) : Brain , serum and liver acetylcholinesterase activities of Oreochrmis niloticus exposed to malathion (1.75 mg/L) ,Lannate (0.65 mg/L) or phenol (9 mg/L) for three weeks .

Pollutants		**Exposure time**		
		1` week	**2 weeks**	**3 weeks**
		S.A I %	**S.A I %**	**S.A I %**
Brain	Control	8.2 ±0.3	7.9±0.25	8.0±0.30
	Malathion	3.0±0.4* 63.4%	2.3±0.13* 70.8%	2.0±0.55* 75%
	Lannate	5.2±0.2 * 36.5%	4.5±0.26* 43.0%	3.5±0.31 * 62%
	Phenol	7.0±0.3 12.1%	6.7±0.11 15.1%	6.5±0.20 * 25 %
Serum	Control	2.7 ±0.01	2.91±0.01	2.65±0.02
	Malathion	1.9 ±0.16 * 31.1%	1.80±0.09 32.0%	1.75±0.1* 39.85%
	Lannate	2.0 ±0.03 * 2.3%	1.70±0.02 24.5%	1.73±0.03* 40.5%
	phenol	2.3±0.01 17.5%	2.1±0.04 27.8%	2.0±0.06 24.5%
Liver	Control	2.5+0.12	2.80 +0.32	2.6+ 0.16
	Malathion	1.5±0.34 * 37.6%	1.3±0.13* 52.5%	1.1 ±0.2 * 55.9%
	Lannate	1.9±0.13 * 25.4%	2.0±0.23* 28.5%	1.8 ±0.33* 30.2%
	Phenol	2.0±0.45 21.5%	2.1±0.36 25.0%	2.0±0.14 23.3%

S. A : Specific activity expressed as U mol/min /mg protein .
I % :Percent of inhibition.
Results are expressed as mean ± standard error (S.E) of 6 fishes
* Significant at $p < 0.05$.

Table (3) : Brain , serum and liver glutathione S – transferase activities of *O. niloticus* exposed to malathion (1.75 mg/L) ,lannate (0.65 mg/L) and phenol (9 mg/L) for three weeks .

Pollutants		Exposure time		
		1 week	2 weeks	3 weeks
		S.A I %	S.A I %	S.A I %
Brain	Control	0.63±0.22	0.65±0.12	0.60±0.13
	Malathion	0.58±0.01 7.9%	0.50±0.03 23.0%	0.44±0.12 20.6%
	Lannate	0.59±0.02 6.3%	0.55±0.01 15.3%	0.49±0.01 18.3%
	Phenol	0.73±0.42 * 15.8%	0.68 ±0.34 4.61%	0.72±0.01* 20.0%
Serum	Control	0.062±0.001	0.059±0.01	0.061±0.001
	Malathion	0.053±0.003 14.5%	0.061±0.01 3.3%	0.047±0.002 22.9%
	Lannate	0.059±0.002 4.3%	0.056± 0.01 5.0%	0.050±0.002 18.0 %
	phenol	0.071±0.01 * 14.5%	0.063±0.02* 6.77%	0.076±0.001* 24.5%
Liver	Control	0.9±0.01	0.9±0.01	0.99±0.01
	Malathion	1.1±0.8 0 * 15%	1.1±0.09 20.4%	1.12±0.08 * 13%
	Lannate	`1.0±0.12 * 8.%	1.4±0.13 * 42.8%	1.5±0.23 * 51%
	Phenol	1.6±0.23* 73.%	1.8±0.13* 83.6%	2.0±0.35 * 100 %

S.A : Specific activity expressed as U mol/min/mg protein.
I% :Percent of inhibition or induction.
Results are expressed as mean ± standard error (S.E) of 6 fishes.
* Significant at P <0.05