JOURNAL OF AGRICULTURE & SOCIAL SCIENCES
1813–2235/2007/03–2–52–54
http://www.fspublishers.org

The Effect of Diazinon on Glutathine and Acetylecholinesterase in Tilapia (*Oreochromis niloticus*)

NAGWA HASSAN ELNWISHY[1], MOHAMMED TAWFIC AHMED, MOHAMED SAAD EL-SHERIF AND MOHAMMED ABD EL-HAMEED
Biotechnology Research Center, Suez Canal University, (New University), Ismailia, Egypt
[1]Corresponding author's e-mail: nwishy@yahoo.com

ABSTRACT

The influence of organophosphorus (OP) Diazinon on gluatathione (GSH) content, acetylecholinesterase (AChE) in Tilapia (*Orechromis niloticus*) was studied. Uniform (40 g) male tilapia were divided in 3 replicates for two concentrations ($LC_{33.5}$ & LC_{10}) for 30 days and compared with control (un-treated) fish. Samples of muscles and liver were randomly taken before fish was transferred to untreated aquaria for 7 days of recovery then similar samples were taken. Results revealed that the *in vivo* chronic exposure of Tilapia to sub-lethal concentrations of Diazinon for 30 days caused a reduction in total GSH and AChE activity. Both total GSH and AChE activity were increased at the end of the recovery intervals. It was concluded that GSH could be a valuable biomarker to mirror pollutant status in the aquatic systems.

Key Words: Diazinon; *Oreochromis niloticus;* Gluatathione; Acetylecholinesterase

INTRODUCTION

Lakes, rivers and sees became illegally the end point of the discharge of pollutants. Unlike heavy metals, concentrations of organophosphate (OP) pesticides potentially correlate with the length and weight of fish (Zehra *et al.*, 2003). Thus, by continues accumulation various environmental problems like reduced reproduction, hematological changes (Köprücü *et al.*, 2006) and nervous system damage (Hamm *et al.*, 1998) in fishes have occurred. Thus, a double action of increasing people awareness about diseases caused by environmental pollution (Hussain *et al.*, 2003) and producing environment friendly chemicals (Yousaf *et al.*, 2004) is a must. In Egypt, some reports documented the influence of such heavy reliance on pesticides on environmental and human welfare (Tawfic & Ismail, 1991; Tawfic *et al.*, 2002). Meanwhile, Diazinon, after azinophosmethyl and parathion (Alabaster, 1969) is the most toxic OP pesticide used in Egypt. In this study, the biochemical and physiological function in fish after a chronic exposure to Diazinon was investigated to come up with a biomarker to mirror pollution status in the aquatic systems; and to test whether recovering can detoxify fish to normal status regardless of toxicants concentration in water.

MATERIALS AND METHODS

In this study 150 uniform (40 g) male tilapia fishes were stocked at 2 fish m^{-3} in glass aquaria, provided with air supply and dechlorinated water. Fish was fed commercial pellets 40% protein twice/day and sustained in natural photoperiod conditions. Water was adjusted at 25 ± 1C° and pH 7.7 ± 0.5.

Toxicity studies. Fish was divided in aquaria and exposed to series of concentrations of Diazinon (100, 50, 10 & 5 mg L^{-1}) for 96 h to determine its lethal concentration (LC_{50}). Control fish (un-treated) was maintained under similar conditions. Then LC_{10} (treatment 1) and $LC_{33.5}$ (treatment 2) were calculated and used for 30 days chronic exposure on fish. Samples of muscles and liver were randomly taken from the fish in the aquaria, then fish was removed to un-treated aquaria for 7 days recovery, which ended with collecting the similar samples.

Biochemical determinations. Determination of Diazinon was made on (1) Total GSH content in Liver (Beutler *et al.*, 1963), and (2) acetylecholinesterase (AChE) Activity (Den *et al.*, 1983). Then results were tested by multivariate ANOVA analysis ($p < 0.05$) using SPSS 13.0, 2004.

RESULTS AND DISCUSSION

Toxicity results showed that LC_{50} of Diazinon for 96 h was 2.8 mg L^{-1}, LC_{10} and $LC_{33.5}$ were calculated and equaled 0.28 mg L^{-1} and 1.89 mg L^{-1}. Total GSH in livers was significantly reduced to 52.63% and 40.98% of its normal level in treatments 1 and 2, which then recovered to 68.06% and 75.49.6%, respectively (Fig. 1 & Table I). The difference between the reduction levels in both treatments was significant, which was related to toxicity. The reduction was probably due to liver's attempts to detoxify the body from the toxicant; as GSH can offer protection against OP insecticides by active subsequent conjugation with GSH (Kostaropoulos *et al*., 2001).

AChE activity was significantly inhibited to 70.15 and 64.01% of its normal level in fish exposed to treatments 1 and 2, respectively. AChE activity increased in recovered fish of both treatments to 78.24% and 78.81%, respectively (Fig. 2 & Table I). The difference of inhibition level between treatments was significant, indicating that the inhibition in AChE was correlated with the concentration of

Table I. LSD multiple comparisons

	Dependent Variable	Parameter	Mean	Std. Error	Sig. [1]	sig.[2]	95% Confidence Interval	
							Lower Bound	Upper Bound
Treatment 1	GSH	Treated fish	1720.33	±46.83	0.001	0.001	1619.89	1820.77
		Recovered fish	2224.67	±46.83	0.001		2124.23	2325.11
		Untreated fish	3268.60	±51.30			3158.57	3378.63
	ACHE	Treated fish	809.67	±24.95	0.001	0.001	756.16	863.17
		Recovered fish	903.00	±24.95	0.001		849.49	956.51
		Untreated fish	1154.20	±27.33			1095.58	1212.82
Treatment 2	GSH	Treated fish	1393.50	±128.30	0.001	0.001	1118.33	1668.67
		Recovered fish	2567.33	±128.30	0.001		2292.17	2842.50
		Untreated fish	3400.80	±140.54			3099.37	3702.23
	ACHE	Treated fish	781.00	±44.97	0.001	0.001	684.55	877.45
		Recovered fish	961.67	±44.97	0.001		865.22	1058.12
		Untreated fish	1220.20	±49.26			1114.55	1325.85

The mean difference is significant at the .05 level.
1 significance within treatment; compared to control ,
2 significant between treatments (Wilks' Lambda)

Fig. 1. Total GSH Content in Liver

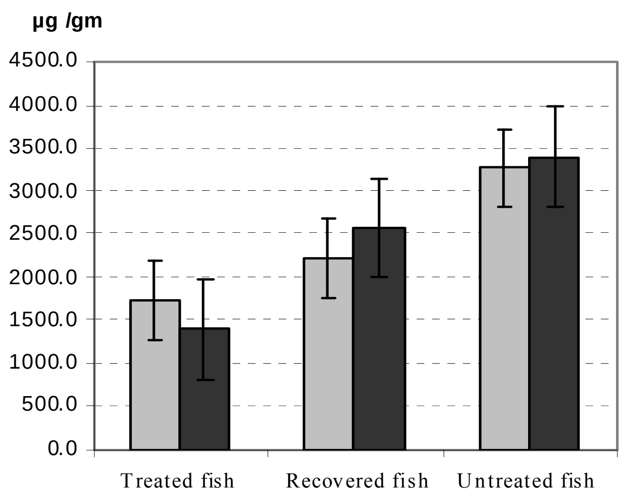
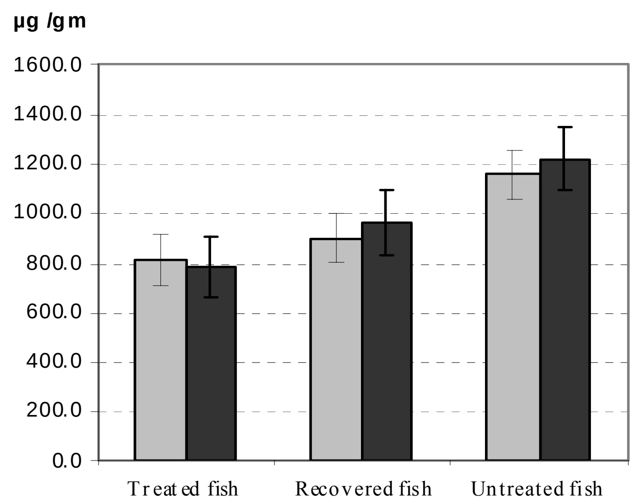


Fig. 2. AChE Activity



Treatment 1 Treatment 2

the toxicants. Reduced AChE activity was mostly caused by Diazinon as neurotoxins.

In animal bodies, Diazinon is converted to diazoxon, which is the strong inhibitor of AChE (Eisler, 1986) and approximately 20 times more toxic than Diazinon (Tsuda *et al.,* 1997). However, insignificant difference of AChE activity was found between the recovered fish of both treatments, which indicated the difficulty and/or slowness of recovering the damaged neurotransmission in fish.

CONCLUSION

Even though aquatic organisms can survive in small levels of pollutants, their biochemical functions would be damaged regardless of how small these concentrations are. Damage as neurotransmission (AChE activity) might be difficult to recover. Total GSH can be an effective biomarker to identify aquatic pollution level with Diazinon.

REFERENCES

Alabaster, J.S., 1969. Survival of fish in 164 herbicides, insecticides, fungicides wetting agents and miscellaneous substances. *Int. Pest Control,* 11: 25–35

Beutler, E., O. Duron and B.K. Kelly, 1963. Improved method for the determination of bloodglutathione. *J. Lab. Clin. Med.,* 61: 882–8

Den, B.D., W.A. Poppe and W. Trischler, 1983. Manual of AChE determination Kit. *J. Clin. Chem. Biochem.*, 21: 381–6

Eisler, R., 1986. *Diazinon Hazards to Fish, Wildlife and Invertebrates: A Synoptic Review,* Report No. 85, pp: 1-9. U.S. Fish Wild-life Serve Biology Reproduction

Hamm, J.T., B.W. Wilson and D.E. Hinton, 1998. Organophosphate induced acetylecholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (*Oryzias latipes). Neurotoxicology,* 19: 853–70

Hussain, S., M.Z. Hassan, Y. Mukhtar and B.N. Saddiqui, 2003. Impact of Environmental pollution on human behavior and up-lift of awareness level through mass media among the people of faisalabad city. *Int. J. Agric. Biol.,* 5: 660–1

Kostaropoulos, I., A.I. Papadopoulos, A. Metaxakis, E. Boukouvala and E. Papadopoulou-Mourkidou, 2001. Glutathione S-transferase in the defense against pyrethroids in insects. *Insect. Biochem. Molec. Biol.,* 31: 313–9

Köprücü, S., K. Köprücü, M.Ş. Ural, Ü. İspir and M. Pala, 2006. Acute toxicity of OP pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochem. Physiol.,* 86: 99–105

Tawfic, M. and S. Ismail, 1991. Residues of some organochlorine compounds in fish, crab and sediment from El Temsah lake, Suez Canal and their effect on the metochondrial ATPase of the new zealand white rabbit. *J. Egyptian Public Health Association,* 1: 557–75

Tawfic, M., M. Loutfy and E. El Shiekh, 2002. Residue levels of DDE and PCBs in the blood serum of women in the Port Said region of Egypt. *J. Hazard Materials,* 89: 41–8

Tsuda, T., T. Inoue, M. Kojima and S. Aoki, 1997. Pesticides in water and fish from rivers flowing into lake Biwa. *Bull. Environ. Contam. Toxicol.,* 57: 442–9

Yousaf, R., M. Cheema and S. Anwars, 2004. Short Communication: Effects of pesticide application on the health of rural women involved in cotton picking. *Int. J. Agric. Biol.,* 6: 220–1

Zehra, I., T. Kauser, E. Zahir and N.I. Imam, 2003. Determination of Cu, Cd, Pb and Zn concentration in edible marine fish *Acanthopagurus berda* (DANDYA) along Baluchistan coast–Pakistan. *Int. J. Agric. Biol.,* 5: 80–2

(Received 13 December 2006; Accepted 23 February 2007)