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Fish Cholinesterases as Biomarkers of Sublethal Effects of Organophosphorus and Carbamates in Tissues of *Labeo rohita*

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Abstract.- The present study was undertaken to assess the sub lethal toxic effects of carbosulfan and parathion on acetylcholinesterases (AChE) and butyrylcholinesterases (BChE) in various tissues of *Labeo rohita* and was compared with control. The fingerlings of *Labeo rohita* (L= 90 ±6 mm, W= 30.00 ± 2.00 g) were exposed to three sublethal concentrations of parathion (0.038, 0.019 and 0.012 mg/L) and carbosulfan (0.198, 0.099 and 0.066 mg/L) in a three replicates for the total period of 60 days. The minimum AChE activity was recorded as 1.06 ± 0.03, 0.7 ± 0.04,1.91 ± 0.06,1.42 ± 0.30, 4.41 ± 0.11, and 1.71 ± 0.02 µmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with an exposure of concentration of 0.06 mg / L of parathion. The minimum BChE activity was observed as 8.22 ± 0.09, 2.60 ± 0.37,1.96 ± 0.09, 4.53 ± 0.09,17.28 ± 0.28 and 7.90 ± 0.11 µmol/min/g protein in the brain, gills, flesh, kidney, liver and blood, respectively with an exposure of 0.06 mg / L of parathion. The minimum AChE activity was recorded as 4.91±0.10, 4.77±0.31, 4.66±0.19, 3.80±0.18, 20.41±0.21 and 5.44±0.08 µmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with exposure concentration of carbosulfan (0.28 mg/L), whereas, the minimum BChE activity was 14.90 ± 0.60, 3.10 ± 0.20, 4.48 ± 0.18, 20.41±0.17,6.91 ± 0.17 and 6.78±0.02 µmol/min/g protein in brain, gills, flesh, kidney, liver and blood, respectively with the exposure of 0.28 mg/L carbosulfan. It has been concluded that parathion was found to be the potent inhibitor of cholinesterases as compared to cabofuran.

Key words: Parathion, carbosulfan, acetylcholinesterase, butyrylcholinesterase, cholinesterase inhibition.

INTRODUCTION

Due to reasons that last for decades, environmental monitoring of pesticides is an urgent need. Contamination by pesticides is an important public health problem, mainly in developing countries. It is estimated that only 0.1% of the applied pesticides in fact reach the target pests, while the rest spreads throughout the environment (Hart and Pimentel, 2002). In addition, among the 500,000 deaths a year related to pesticides in the developing world, approximately 200,000 occur due to the use of organophosphorus (OP) and carbamates (CB) pesticides (Eddleston *et al.*, 2008). These are among the most important classes of insecticides/acaricides in usage and billing (Nauen and Bretschneider, 2002). The increasing pollution of water resources in Pakistan and the consequential effects on human health as well as the environment is a matter of grave concern. The drinking and surface water in densely populated areas is polluted due to various anthropogenic activities (Ahmed, 2006). Owing to be an agricultural country, pesticide usage and of course import of pesticides has increased to a great extent in Pakistan, from 225,176 tons to 305,938 tons in the last two decades (Anonymous, 2011). The intensity and amount of pesticide import as well as its expanded use for pest control and improvement of crops in intensive agriculture are posing a serious hazard to terrestrial as well as fragile aquatic ecosystems and biota including fish (Mahboob *et al.*, 2011; Yaqin and Hensen, 2010; Mora *et al.*, 1999).

Presently there is an increasing concern over the accumulation and persistence of pesticides in the aquatic ecosystem posing a serious threat to biological life including human beings (Faruk, 2008). Chemical pesticides with persistent molecules (long half-life periods) comprises a threat to fish and also to the human population consuming the affected fish. Being strictly aquatic, fish are directly exposed to these pesticides by absorption through skin, breathing and oral intake of pesticide-

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contaminated water or pesticide contaminated prey (Mathur and Singh, 2006). Among the various biomarkers of pesticide exposure, the family of cholinesterase *viz.*, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has widely been used as biomarkers to evaluate the noxious effects of pesticides *i.e.* CB and OP. The primary and most known target for the action of OP and CB compounds is a family of enzymes (Cholinesterases; ChEs) formed by acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). The first is synthesized in hematopoiesis, occurs in the brain, endplate of skeletal muscle, erythrocyte membrane, and its main function is to regulate neuronal communication by hydrolyzing the ubiquitous neurotransmitter acetylcholine (ACh) in the synaptic cleft (Quinn, 1987; Silman and Sussman, 2005). The second is synthesized in the liver and is present in plasma, smooth muscle, pancreas, adipocytes, skin, brain and heart (Çokugras, 2003). Aquatic vertebrates (terrestrial, aquatic) and invertebrates give the nippy signal of pesticide intoxication long before their death so are taken as model organisms (Archana *et al.*, 2011; Adedejio, 2011; Ferreira *et al*., 2010; Torre *et al*., 2002; Rodriguez-Fuentes and Gold-Bouchot, 2000). AChE plays an important role in neurotransmission at cholinergic synapses and neuromuscular junction by rapid hydrolysis of the neurotransmitter ACh to choline and acetate. Other ChE including BChE are crucial for different parts of the immune system. Though its physiological functions are not well defined, BChE is considered as one of the core detoxifying enzymes (Assis *et al*., 2010; Shea and Berry, 1984; Soreq and Zakut, 1993; Behra *et al.*, 2002). Some of the investigations hypothesize that BChE protect AChE against xenobiotics like pesticides (Whitaker, 1986). Cholinergic biomarkers are generally divided into two main classes: AChE and BChE in most of the organisms extensively used as a diagnostic tool for ecotoxicological assessment of pesticides and other xenobiotics in aquatic environments (Torre *et al*., 2002). Pesticides are generally applied in crop plants for the mitigation of pests, but only 0.1% of the applied doses reach the target pests. The remaining one sustains or spreads throughout the environment (Hart and Pimentel, 2002).

The exposure concentrations of pesticides that are not lethal to fish, may affect their physiology and behavior, ultimately demolishing survival, reproduction, metabolic disturbances and growth (Kegley *et al*., 1999; Murty, 1986). The prevalence of most of the CBs (carbofuran, carbosulfan, carbaryl) and OPs (triazophos, chlorpyrifos, profenofos, endosulfan, methamidophos, diazinon, parathion methyl and malathion) has been studied in aquatic biota, water and sediments in Punjab, Pakistan (Mahboob *et al.*, 2009, 2011). The OPs and CBs had been found to pose deleterious effects on biota including fish, determined in term of various biomarkers, growth and general health. The present study was hypothesized that OPs and CBs are major agrochemicals, sternly affecting different neuro enzymes and ultimately the growth of various indigenous fish species of Pakistan, commonly cultured and consumed. Therefore, the primary objective of the study was to assess the effect of sublethal concentration of arathion and carbosulfan on the activity of AChE and BChe and associated risk in *Labeo rohita*.

MATERIALS AND METHODS

Live specimens of fingerlings of *Labeo rohita* (L= 90 ± 6 mm, W= 30.00 ± 2.00 g) were transported from the Fish Seed Hatchery, Faisalabad and were acclimatized in glass aquaria (70 liters). Fish were fed with commercial feed @ 3% body weight during acclimatization period (15 days). Water parameters were analyzed and maintained on a daily basis. Pesticide of technical grades of parathion 98% and carbosulfan 90% was obtained from Ali Akbar Enterprises, Lahore, Pakistan.

Pesticides exposure tests

Sublethal toxicity tests were performed after determination of acute toxicity. Three sub lethal concentrations of all pesticides were prepared in a suitable solvent *i.e.*, parathion in acetone (Merck, Germany), carbosulfan in ethanol (Merck, Germany) as $1/5^{th}$, $1/10^{th}$ and $1/15^{th}$ part of LC_{50} (predetermined). Fish were exposed to these lower

concentrations of pesticides in triplicates with 20 fish in each, for a period of 60 days. The fish were fed daily with commercial diet at the rate of 3% of their body weight in two fractions with the time interval of eight hours. The aqueous solution was renewed after every four days to maintain a continuous supply of pesticides to fish. The fish were taken out from each aquarium at the end of the experiment and anesthetized with MS-222 (Finquel®). They were dissected to get brain, gills, liver, kidney and meat samples which were quickly removed, frozen in liquid nitrogen and were stored at -20°C. AChE and BChE activity was determined following the procedure described by Ellman *et al.* (1961) and Kuster (2005) with certain modifications. Total soluble proteins were determined by Bradford (1976) standard method in order to assess activity of enzymes/g of protein. Differences among treatments were tested using ANOVA followed by the Turkey HSD test.

RESULTS AND DISCUSSION

Characteristics of water

The quality of water was kept constant. Aquaria were continuously aerated, so that the dissolved oxygen level was maintained at 5.00-5.50 mg/L. The electric conductivity, pH and water temperature were 2.27–2.51 mS, 7.6–8.7 and 25°C-27°C, respectively.

Median lethal concentration (LC_{50}) of parathion and carbosulfan

A dose dependent increase and time dependent decrease was recorded in the mortality rate. As the exposure time increased from 24 to 96 h, the LC_{50} was reduced. At 96h LC_{50} for parathion was 0.33 mg/L (0.25- 0.39) and for carbosulfan 1.41 mg/L (0.97-2.03) (Fig.1).

ChE activity

The effects of parathion and carbosulfan have been evaluated on ChE activity in various organs of *Labeo rohita* (Table I).

ChEs are the biomarkers that have widely been used in various organisms. Inhibition of ChE has been studied in several systems and organs with a focus on brain tissues (Pena-Llopis *et al.*, 2003; Fernandez-Vega *et al.*, 2002). In the present investigation along with the other organs, ChE activities were observed in the brain under exposure of both pesticides, brain AChE decreased to 1.06–14.95 µmol/min/g protein with different concentrations of parathion compared to control group. BChE activity was also reduced against various concentrations of parathion. In *Labeo rohita*, the BChE decreased to 8.22±0.09–29.91±0.09, after exposure to different concentration of parathion (Table I). The sensitivity of fish to pesticide exposure, especially OP is dependent on the level of brain AChE activity. Murphy *et al.* (1968) is of the view that the fish

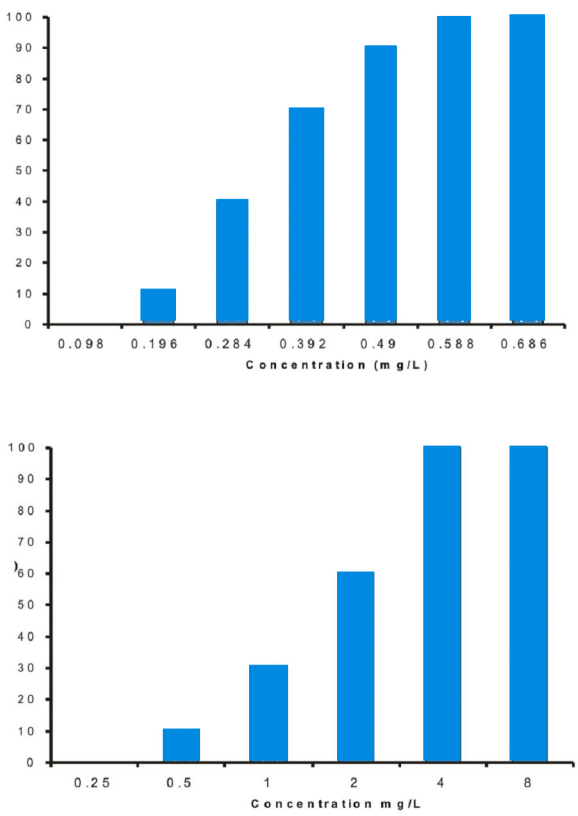

Fig. 1. Effect of different concentrations of (mg/L) of parathion and carbosulfan on the mortality (% age) of *Labeo rohita* 96 hours after exposure.

Table I.- Comparison of means (±S.E.) for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity (µmol/min/g protein), of control group and three exposure concentrations (mg/L=ppm) of parathion and carbosulfan in different organs of *Labeo rohita.*

InsecticideS	Enzymes	Treatments (mg/L)	Brain	Gills	Flesh	Kidney	Liver	Blood
Parathion	AChE	Control	41.47 ±0.03 A	20.41±0.05A	18.21±0.07A	24.44±0.07A	97.23±0.55A	27.71±0.09A
		0.06	1.06±0.03D	0.7±0.04D	1.91±0.06D	1.42±0.30D	4.41±0.11D	1.71±0.02D
		0.03	7.95±0.07C	3.27±0.16C	2.69±0.04C	6.92±0.07C	24.29±0.60C	11.60±0.28C
		0.02	14.95±0.06B	6.07±0.15B	7.41±0.28B	8.42±0.28B	43.61±0.09B	14.84±0.42B
	BChE	Control	42.51±0.22A	7.44±0.22A	19.81±0.37A	42.31±0.70A	170.11±0.38A	48.41±0.37A
		0.06	8.22±0.09D	2.60±0.37C	1.96±0.09D	4.53±0.09D	17.28±0.28D	7.90±0.11D
		0.03	27.11±0.33C	4.47±0.11B	4.87±0.11C	11.22±0.08C	33.71±0.07C	15.66±0.29C
		0.02	29.91±0.09B	4.91±0.22B	12.61±0.51B	14.55±0.33B	96.42±0.11B	24.44±0.08B
Carbosulfan	AChE	Control	41.51±0.09A	21.91±0.33A	19.22±0.26A	25.53±0.22A	99.61±0.23A	27.77±0.28A
		0.28	4.91±0.10D	4.77±0.31D	4.66±0.19D	3.80±0.18D	20.41±0.21D	5.44±0.08D
		0.14	10.22±0.08C	6.31±0.45C	6.99±0.42C	8.91±0.23C	34.04±0.35C	13.71±0.17C
		0.09	18.42±0.23B	11.55±0.33B	12.70±0.16B	14.22±0.11B	45.64±0.24B	19.92±0.412B
	BChE	Control	41.61±0.44A	7.78±0.26A	20.61±0.18A	41.33±0.21A	169.00±0.77A	50.22±0.41A
		0.28	14.90±0.60C	3.10±0.20D	4.48±0.18D	7.81±0.19D	20.41±0.17D	6.91±0.17D
		0.14	27.45±0.11B	4.12±0.17C	14.71±0.27C	17.22±0.18C	37.56±0.45C	19.71±0.22C
		0.09	26.68±0.17B	6.68±0.46B	15.31±0.34B	20.41±0.31B	98.66±0.26B	27.67±0.44B

Means with different letters for each fish in a column are highly significantly different (P<0.01). S.E. =standard error

possessing greater activity of enzymes, showed an increased inhibition of the enzyme, when exposed to pesticides. Our results are in agreement with the findings of above mentioned workers. Carbosulfan has also caused severe inhibition of ChEs (AChE and BChE) in brain of fish up to 69% to 874% compared to control group under various exposure concentrations of carbosulfan with a highly significant difference (P<0.01) (Tables I, II). Biomarkers are useful indicators of pollution because of their high sensitivity and presenting the first detectable signs of sublethal stress response in organisms (Stegeman *et al.*, 1992; Chamber and Boone, 2002). Inhibition of ChE in either nervous system or flesh has been acknowledged as the adverse effect on the organisms because the enzyme activity of the target tissues is known to contribute in the neurotransmission (Padilla, 1995). Most of the observations of AChE inhibition in fish have been emphasized in the brain only as the intoxication of the brain contributes a lot towards behavioral changes (Jaffery and Keizer, 1995). Although catfish brain seemed to be the most sensitive to the exposure of aldicarb, the fish with 90% inhibition of AChE was alive with moderate

Table II.- Analysis of variance (ANOVA) for esterase activities (µmol/min/g of protein), of control group and three exposure concentrations (mg/L=ppm) of parathion and carbosulfan in different organs of *Labeo rohita.*

Organs	Mean of squares	
	Parathion	Carbosulfan
Acetylcholinesterase		
Dedgree of freedom	3	3
Brain	921.44**	777.221**
Gills	260.22**	161.44**
Flesh	170.81**	118.91**
Kidney	297.67**	251.21**
Liver	4722.61**	3531.47**
Blood	343.94**	288.22**
Butyrylcholinesterase		
Dedgree of freedom	3	3
Brain	537.6^{NS}	370.22**
Gills	16.33^{Ns}	15.37**
Flesh	187.66**	129.22**
Kidney	771.80**	560.63**
Liver	14722.6**	13767.44**
Blood	967.11**	988.69**

**, highly significant (P<0.01)

symptoms of intoxication (Everett *et al.*, 2000). Our findings have shown less than 90% inhibition in brain under any of the exposed concentrations of both pesticides, whereas the maximum inhibition was observed in flesh when fish was exposed to the maximum concentration (0.06 mg/l) of parathion. The activity of BChE in gills was reduced to 2.60±0.37 µmol/min/g protein compared to 7.44±0.22 µmol/min/g protein of control group when exposed to 0.06 mg/l of parathion. Carbosulfan inhibitory effect on flesh AChE ranged from 39% to 80% under different concentrations compared to the control group. The BChE activity of flesh was recorded as 15.31 ± 0.34 µmol/min/g protein when fish were exposed to 0.09 mg/l carbosulfan (Table II). Toni *et al*. (2010) also reported a decrease in flesh ChE activities in fish. The flesh ChEs represent the largest pool of ChE in the body. It is also important to control the muscular function since the loss of muscular control can have many problems for fish such as loss of swimming control and blockage of opercular movement. This may result in reduced oxygenation of the blood and consequently lead to hypoxia induced death (Zinkl *et al.,* 1987). This may also attribute towards the changes in behavior of fish when exposed to pesticides. The AChE activities were also found to be reduced in gills (98.12% and 77.54%, respectively) after the exposure of parathion (0.06mg/l) and carbosulfan (0.28mg/l). The BChE activity was reduced to 2.60±0.37 µmol/min/g protein after the exposure of parathion (0.06 mg/l), which is significantly less than the control group activity (7.44±0.22 µmol/min/g protein). This inhibition was 66.88% in gills with 0.28 mg/l of carbosulfan (Table II). In sublethal toxicity, the AChE and BChE activities in blood were significantly reduced after two months of exposure to parathion and carbosulfan (Tables I, II). Hence, the reduction in the activity of ChE in gills may be attributed to reduced respiratory activity as demonstrated by the Chamber and Carr (1995), who reported that the primary cause of AChE inhibition-induced death in mammals is generally related to respiratory failure (Chamber and Carr, 1995).

In addition to the brain, gills, flesh and blood, AChE activity were also observed to be reduced in liver and kidney. The maximum inhibition was observed in kidneys (95.2%) and in liver (96.9%) when exposed to maximum concentration of parathion (Table I). Less inhibition in both the organs was observed, when fish were exposed to various concentrations of carbosulfan as mentioned in Table I. BChE activity also followed the similar trend with a maximum inhibition up to 88.7% in liver caused by exposure of parathion (0.06 mg/l) followed by carbosulfan exposure (0.0.28 mg/l) as 89.6%. A significant difference between maximum inhibition caused by parathion and carbosulfan, was observed in the kidney (Table I). As far as the effects of both of the pesticide are concerned, we found that the highest concentrations of parathion and carbosulfan had affected the cholinesterase level maximum in all the tissues. Heath *et al.* (1993) studied the effect of carbosulfan on newly hatched striped bass larvae and decrease in ChE activity was observed at high concentration of carbosulfan. Our findings are in line with the results of various (Heath *et al.,* 1993: Pena-Llopis and Ferrando, 2003; Fernandez-Vega *et al.,* 2002). More than 50% inhibition of ChE (AChE and BChE) activities were observed in all the organs of *Labeo rohita* under the highest exposure concentration (0.06 mg/l), medium exposure concentration (0.03 mg/l) of parathion and the highest exposure concentration of carbosulfan (0.298 mg/l), The variation in inhibition was observed under other concentrations of both the pesticides. ChEs has been considered as a good indicator of intoxication (Coppage and Mathews, 1974; Westlake *et al.,* 1981).The current findings are in line with the findings of Dembele *et al.* (2000), who reported the variable inhibition of AChE by chlorpyrifos, chlorfevinophos, diazinon (OPs) and carbosulfan (CB) in common carp.

CONCLUSIONS

It is concluded that parathion was more toxic compared to carbosulfan in the context of inhibition of AChE and BChE in brain, blood, gills, flesh, kidneys and liver of *Labeo rohita*. This enzyme inhibition is likely to affect the functions including metabolism and neurotransmission etc. of all these organs

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