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Full Length Research Paper

Impact of sublethal concentration of triazophos on regulation of protein metabolism in the fish *Channa punctatus* (Bloch)

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The fresh water fish is an important human food source in India and the fish is constantly exposed to pesticides which are used extensively to control agricultural pests. Insecticide triazophos organophosphates (OP) altered protein metabolism in the liver, brain and kidney tissues of the fish *Channa punctatus* (Bloch). The total protein when exposed to different time periods decreased and free amino acids increased significantly. The enzymes which are involved in protein metabolism were altered significantly. The increase in activity of deaminases reveals the breakdown of nucleo-tides to yield excess energy to overcome the toxic pressure. Exposure to sublethal doses of triazophos extract caused significant ($p < 0.05$) time and dose dependent reduction in the levels of total protein, acetylcholinesterase (AchE) and significant enhancement in the levels of total free amino acids, glutamine, adenosine monophosphate (AMP) deaminases, adenosine deaminases, glutamate dehydrogenase (GDH), aspartate aminotransferase (AAT), alanine aminotransferase (ALAT) and alkaline acid phosphatases, and the activity of enzyme protease in both liver, brain and kidney tissues of fresh water fish *C. punctatus*.

Key words: Triazophos (OP), Lc_{50}, protein metabolism, enzyme activity, *Channa punctatus*.

INTRODUCTION

Indiscriminate and extensive use of insecticides to protect crops posess a serious threat to humans and the surrounding environment. Almost all pesticides are volatile in nature when applied to crops. These pesticides can be circulated into different ecosystems by different agents (Weber, 1977) after entering into the environment like air, water, different food chains, soil and other agents (Farmer et. al., 1972). The pesticides which are liberated into the aquatic environment have a deterious effect on fish and subsequently to man (Metelev et al., 1983). A review of the toxicological literature reveals that exposure of chemical can produce unexpected effects (Alession 1996; Feron et.al., 1995). Many researchers were baffled by these unexpected effects following exposure of individuals to low levels of pesticides and other chemical toxins (Lee et al., 1997; Chun-Yun et.al., 2003). According to Waliszewski et al. (2003), Aronson et al. (2000) and Abdul (2003) most pesticides may enter into the food channels and cause physiological damage. Among all pesticides, the organophosphates (OP) are widely used to control pests because of their rapid effectiveness and easy biodegradation (Mahboob and Siddiqui, 2002). A number of recent clinical studies revealed that most of the OP and other toxic chemicals could alter the immune system (Barcarolli and Martinez, 2004; Thangavel et al., 2004; Chen et al., 2004). Triazophos is an organophosphate

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Abbreviations: OP, Organophosphates; **AchE,** acetylcholinesterase; AMP, adenosine monophosphate; **GDH,** glutamate dehydrogenase; **AAT,** aspartate aminotransferase; **ALAT,** alanine aminotransferase.

compound being widely used in India to control agricultural pests. The sublethal exposure of OP compounds has produced several changes in energy metabolism of fish (Rani et al., 1989). The present study is undertaken to evaluate the toxic effects of triazophos pesticide during the exposure time periods on the protein metabolism in liver, brain and kidney tissues of *Channa punctatus* (Bloch).

MATERIALS AND METHODS

Healthy fresh water fish *C. punctatus*, an edible fish with average weight 80-120 gr and 15 - 25 cm length, were collected from a local market. These fish were kept in cement tanks (6 x 3 x 3 feet) at least 3 weeks for acclimatization under continuous water flow. The average temperature of water was 22 ± 1.0°C. They were fed *ad libitum* with groundnut cake along with commercial feed pellets (1 - 1.5% body weight). Prior to the experiment, the fish were starved for one day (Butterworth, 1972). Technical grade (90% Purity) sample triazophos (O,O-diethyl, 0,1 phenyl, 1H, 1,2,4, Triazol 3yl-phosphorothio) was used in sublethal concentration. Triazophos was introduced into the water tubs by dissolving in acetone (0.5% w/v). The Lc_{50} (0.019ppm) for 48 h was determined by the method of Bayne et.al. (1977). Batches of six fish were exposed to sub acute to acute periods in sublethal concentration 0.006 ppm of triazophos in tap water for 24, 48, 72 and 96 h. After removing the fish at the stipulated time interval, liver, brain and kidney tissues were quickly isolated and kept in ice-Jacketed petri-dishes for biochemical estimations. The physico-chemical parameters of tap water in which fish acclimatized are as follows: Temperature 30 - 35°C; pH 7.2; electrical conductivity 0.052 milliohms; calcium 5 mg/l; sodium 2.1 mg/l; biocarbonates 142 mg/l; total alkalinity 69 mg/l; sulphates 7.1 mg/l; biological oxygen demand (BOD) 1.6 mg/l; chemical oxygen demand (COD) 0.008 mg/l; fluoride 0.03 ppm.

Proteins levels were estimated by the method of Lowry et.al. (1951) using bovine serum albumine as standard. Homogenates 2 ml (w/v) cold distilled water was prepared in 30% TCA; values are expressed as mg/100 mg wet wt of tissue.

Free amino acids (FAA) were estimated using the ninhydrin method (Moore and Stein, 1954). Homogentes (5% w/v) were prepared in 10% (w/v) TCA, centrifuged at 300 rpm and supernatent was used for amino acid estimation. FAA is expressed as mg/100 mg wet wt of the tissue.

Glutamine was estimated using the method of acid hydrolysis described by Colowick and Kaplan (1967). Homogenates ((10%, w/v) cold distilled water were) was prepared in 10% H_2SO_4. Values are expressed as moles of glutamine/g wet wt of the tissue.

Aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) were estimated using the method of Reitman and Frankel (1957). Tissue homogenates (10% w/v) were prepared in cold 0.25 M sucrose solution and centrifuged at 3000 rpm for 15 min to obtain a clear supernatent which was used as enzyme source. Values are expressed in micro moles of pyruvate formed/mg protein/h.

Glutamate dehydrogenase (GDH) activity levels were estimated following the method of Lee and Lardy (1965). Homogenates (10% w/v) were prepared using 0.25 M sucrose solution, centrifuged at 3000 rpm and supernatent was used for the enzyme source. Values are expressed as micro moles of formazan formed/mg protein/hr of tissue.

Adenosine monophosphate (AMP) deaminase was estimated by using the method Weil-Malherbe and Green (1955) and modified by Wagelin et.al. (1978). Homogenates (10%, w/v) were prepared in cold distilled water and centrifuged at 3000 rpm and supernatent was used for enzyme source. AMP deaminase has been expressed as micro moles of ammonia formed/mg protein/h.

For assaying adenosine deaminase activity by the method of Agarwal and Parks (1978), tissue homogenates (10% w/v) were prepared in ice cold distilled water and centrifuged at 3000 rpm for 15 min to obtain a clear supernatent which was used as enzyme source.

Acetylcholinesterase (AChE) was estimated using the method of Ellman et.al. (1961). Homogenates (2% w/v) tissue were prepared in 0.25 M sucrose solution. The enzyme activity was expressed as micro moles AChE hydrolyzed/mg protein/hr of tissue. Alkaline and acid phosphatases were estimated by the method of Kind and King (1954). Calculated activity from standard graph values are expressed in micro moles of iP formed/ml/h. The enzyme assays were made after preliminary standardization regarding linearity with respect to time of incubation of enzyme concentration.

RESULTS AND DISCUSSION

The protein content was decreased in liver, brain and kidney tissues during triazophos treatment (Tables 1, 2 and 3). According to Nelson and Cox (2005), Harper (2005) and Sathyanarayana (2005), the physiological activity of animal was indicated by the metabolic status of proteins. Jrueger et al. (1968) reported that the fish can get its energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and pos-sible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis. The toxicants may affect the hormonal balance which could directly or indirectly affect the tissue protein levels (Singh et.al., 1996; Morthy and Priyamvada, 1982; Khilare and Wagh, 1988).

FAA pool was increased in the tissues of fish during exposure to triazophos. The enhanced FAA levels may be channelled for energy synthesis and other metabolic reactions (Kovacs and Seglen, 1981). The elevated FAA levels are utilized for energy production by feeding them as keto acids into the TCA cycle through amino-transferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh et.al., 1996). It is also attributed to lesser use of amino acids (Seshagiri et.al., 1987) and their involvement in the maintenance of an acid-base balance (Moorthy et.al., 1984). Natarajan (1985) suggested that stress conditions induce elevation in the transamination pathway. The increase in FAA levels of tissues indicates stepped up proteases activities and fixation of ammonia into keto acids (Srinivasa murthy et.al., 1986; Ali, 2003). Tripathi and Singh (2003) reported that the enhanced FAA may be due to depletion of reserved glycogen so that the fish can try to yeild metabolic energy by gluconeogenesis process. Similar findings were observed by Carlson (2003), Vijuen and Steyn (2003), and Anupama (1989) in various animals during different toxic conditions.

The elevated levels of glutamine in liver, brain and kidney tissues reveal an enhancement of the biosynthesis of

Table 1. Effect of Lihocin on the protein metabolism in the liver tissue of *C. punctatus* (Bloch).

Parameters	Control	Triazophos			
		24 h	48 h	72 h	96 h
Total proteins	163.6 ± 2.11	153 ± 1.29* PC = -6.22	143.33 ± 1.69* PC = -12.5	137.24 ± 5.21 PC = -16.11	121.80 ± 2.71 PC = -25.55
Free amino acids	719 ± 8.96	813 ± 6.80 PC = 13.07	973 ± 20.41 PC = 35.32	1145 ± 80.11 PC = 59.24	1452 ± 18.43 PC = 101.94
Glutamine	84.60 ± 6.42	89.15 ± 4.32* PC = 5.91	106.20 ± 12.40 PC = 25.56	117.50 ± 8.50 PC = 38.93	129.80 ± 6.88 PC = 53.60
Alkaline phosphatases	9.33 ± 0.99	12.38 ± 0.80 PC = 32.69	15.77 ± 1.44 PC = 69.02	19.46 ± 1.08 PC = 108.57	21.85 ± 3.66 PC = 134.19
Acid phosphatases	8.36 ± 0.36	9.24 ± 0.11* PC = 10.52	13.08 ± 0.85 PC = 56.45	15.08 ± 0.28 PC = 80.38	17.75 ± 0.85 PC = 112.32
AlAT	23.5 ± 1.60	28.43 ± 1.62 PC = 20.97	32.33 ± 2.13 PC = 37.57	33.20 ± 1.85 PC = 41.27	40.16 ± 1.46 PC = 170.89
AAT	19.75 ± 0.75	22.78 ± 1.18 PC = 15.34	28.36 ± 1.36 PC = 43.59	31.46 ± 4.65 PC = 59.29	33.84 ± 1.71 PC = 71.34
GDH	0.71 ± 0.02	0.83 ± 0.01 PC = 16.90	1.06 ± 0.02 PC = 49.29	1.12 ± 0.12 PC = 57.74	1.28 ± 0.23 PC = 80.28
AMP deaminase	0.39 ± 0.02	0.42 ± 0.01* PC = 7.69	0.62 ± 0.01 PC = 58.97	0.68 ± 0.03 PC = 74.35	0.74 ± 0.042 PC = 89.74
AChE	6.34 ± 0.30	5.20 ± 0.29 PC = -17.98	4.71 ± 0.17 PC = -25.70	4.15 ± 0.19 PC = -34.54	3.76 ± 0.16 PC = -40.69
Adenosine deaminase	0.132 ± 0.04	0.145 ± 0.016* PC = 9.84	0.196 ± 0.023 PC = 48.48	0.203 ± 0.001 PC = 53.78	0.283 ± 0.002 PC = 114.39

Each value is mean SD of 6 (Six) observations. All values are statistically significant from control at 5% level ($p < 0.05$). PC denotes percent change over control. *Not significant; Units as per Table 3

glutamine. The elevated glutamine may be utilized in the formation of amino acids in protein synthesis. According to Narsaimha and Ramana (1985), the organochlorine pesticides may initiate the synthesis of glutamine during toxic conditions. Present finding suggests that the fish has inherent tissue specific resistance potentiality to withstand ambient pesticide toxicity by suitably modulating its metabolic profiles.

The increase in AMP deaminsae and adenosine deaminase activities could contribute ammonia to the tissue through the purine nucleotide metabolism. According to Nelson and Cox (2005), these deaminases contribute little amount of nucleotides in the different tissues through purine nucleotide metabolism. Enhanced activity of deaminases may be due to tissue damage under xenobiotic action.

The triazophos pesticide cause increased in GDH activity in the tissues during initial periods of exposure. The important function of GDH is that the amino group of most amino acids is transferred to α-ketoglutarate to produce glutamate. The increased GDH activity may indicate increased rapid utilization of amino acids (Nelson and Cox, 2005; Sathyanarayana, 2005). The oxidation of glutamate in the Kreb's cycle leads to increased energy though small (Narasimha and Ramana, 1985).

There were enhanced activity of AAT and AlAT during the toxic exposure of triazophos pesticides. The elevated activities of AAT and AlAT were observed by Bakthavasthalam and Srinivasa (1984) in *Anabas testudineus* during the treatment of OP pesticides, Narasimha et.al. (1986) in *Tilapia mossambica* under lindane toxicity and by Sajal et al. (1988) in gastropoda *Thiara lineata* during methyl parathion toxicity.

In the present study, the AChE activity was observed during the toxicity of triazophos in liver, brain and kidney tissues of *C. punctatus*. The AChE was decreased in liver, brain and kidney tissue during triazophos treatment. According to Cavanagh (1973) OP compound has the ability to induce delayed toxic neuropathy in animal. Holmested (1959) concluded that the most of organophosphate compounds which act as acute cholinergic poisons inhibit AChE in the central and peripheral nervous system after metabolism. The decreased activity of AChE is because of the phosphorylation of the OP compounds. Toxicity of another Carbamate pesticide dieldrin caused higher residues in liver than tissues of *Calrias batrachus* (Lamai, 2003).

The alkaline and acid phosphatases were enhanced

Table 2. Effect of lihocin on the protein metabolism in the brain tissue of *C. punctatus* (Bloch).

Parameters	Control	Triazophos			
		24 h	48 h	72 h	96 h
Total Proteins	103.83±2.26	94.16 ± 1.06* PC = -9.13	88.83 ± 1.57* PC = -14.44	72.36 ± 4.54 PC = -30.30	63.24 ± 3.52 PC = -39.09
Free amino acids	562±20.46	588 ± 6.41 PC = 4.62	678 ± 17.08 PC = 20.64	736 ± 16.84 PC = 30.96	798 ± 20.00 PC = 41.99
Glutamine	58.34±5.99	64.83 ± 7.85* PC = 11.12	74.62 ± 6.32 PC = 27.90	89.13 ± 8.49 PC = 52.77	98.46 ± 7.93 PC = 68.76
Alkaline Phosphatases	7.03±0.93	8.66 ± 0.43 PC = 23.18	11.19 ± 1.63 PC = 59.17	13.94 ± 0.17 PC = 98.29	16.40 ± 1.38 PC = 119.06
Acid Phosphatases	6.25±0.73	7.24 ± 0.07 PC = 15.84	9.40 ± 1.20 PC = 50.40	12.80 ± 0.15 PC = 104.8	14.66 ± 1.11 PC = 134.56
AlAT	12.16±1.21	14.35 ± 0.73 PC = 18.00	19.16 ± 1.34 PC = 57.56	22.80 ± 1.10 PC = 87.50	27.00 ± 1.64 PC = 122.03
AAT	8.48±0.95	9.48 ± 0.99 PC = 11.79	12.55 ± 1.35 PC = 47.99	16.45 ± 1.89 PC = 93.98	19.53 ± 1.24 PC = 130.30
GDH	0.43±0.02	0.62 ± 0.01 PC = 44.18	0.81 ± 0.01 PC = 88.37	0.921 ± 0.01 PC = 114.18	1.03 ± 0.02 PC = 139.53
AMP deaminase	0.29±0.01	0.36 ± 0.02 PC = 24.14	0.37 ± 0.021 PC = 27.58	0.44 ± 0.03 PC = 51.72	0.64 ± 0.001 PC = 120.68
AChE	10.45 ± 0.45	9.21 ± 0.36* PC = -11.86	8.64 ± 0.26 PC = -17.32	8.01 ± 0.16 PC = -23.34	7.32 ± 0.21 PC = -29.95
Adenosine deaminase	0.098±0.001	0.108 ± 0.011* PC = 10.20	0.123 ± 0.026 PC = 25.51	0.143 ± 0.012 PC = 45.91	0.193 ± 0.001 PC = 96.93

Each value is mean SD of 6(Six) observations. All values are statistically significant from control at 5% level ($p < 0.05$). PC denotes percent change over control. *Not significant; units as per Table 3.

Table 3. Effect of lihocin on the protein metabolism in the kidney tissue of *Channa punctatus* (Bloch).

Parameters	Control	Triazophos			
		24 h	48 h	72 h	96 h
Total proteins micro grams/100 mg wet. wt	213.83±2.60	197.5 ± 2.21* PC = -7.63	186.83 ± 6.64* PC = -12.62	179.24 ± 1.80 PC = -16.17	168.30 ± 0.68 PC = -21.29
Free amino acids micro grams/100mg wet. wt	342±12.59	375 ± 2.48 PC = 9.64	418 ± 16.34 PC = 22.22	469 ± 14.20 PC = 37.13	498 ± 10.36 PC = 45.61
Glutamine micro grams/100mg wet.wt	36.85±6.21	43.63 ± 3.89 PC = 18.39	49.25 ± 7.29 PC = 33.64	52.80 ± 6.89 PC = 43.28	59.39 ± 6.84 PC = 61.16
Alkaline Phosphatases ip formed/mg protein/hr	9.64±1.08	10.03 ± 0.26* PC = 4.04	11.51 ± 0.48 PC = 19.39	13.08 ± 0.28 PC = 35.68	15.76 ± 1.47 PC = 63.48
Acid Phosphatases ip formed/mg protein/hr	5.50±0.53	6.62 ± 0.12 PC = 20.36	8.07 ± 0.15 PC = 46.72	9.42 ± 0.28 PC = 71.27	10.24 ± 0.87 PC = 86.18
AlAT micro grams/100mg wet.wt	17.33±1.37	18.29 ± 0.24* PC = 5.53	20.83 ± 1.34 PC = 20.19	24.22 ± 0.18 PC = 39.75	26.5 ± 2.14 PC = 52.91
AAT micromoles of pyruvate formed/mg protein/hr	14.29±1.99	15.42 ± 0.86 PC = 7.90	18.07 ± 0.53 PC = 26.45	19.34 ± 0.46 PC = 35.33	21.91 ± 1.53 PC = 53.32
GDH micromoles of formazon formed/mg protein/hr	0.09±0.01	0.13 ± 0.01 PC = 44.44	0.22 ± 0.01 PC = 144.44	0.263 ± 0.02 PC = 192.22	0.312 ± 0.03 PC = 246.66
AMP deaminase micromoles of ammonia formed/mg protein/hr	0.24±0.001	0.29 ± 0.03 PC = 20.83	0.35 ± 0.017 PC = 45.83	0.38 ± 0.06 PC = 58.33	0.41 ± 0.025 PC = 70.83
AChE	2.01 ± 0.41	1.79 ± 0.10* PC = -10.94	1.65 ± 0.12 PC = -17.91	1.31 ± 0.17 PC = -34.82	1.29 ± 0.11 PC = -35.82
Adenosine deaminase micromoles of ammonia formed/mg protein/hr	0.045±0.002	0.072 ± 0.012 PC = 60.00	0.096 ± 0.002 PC = 113.33	0.120 ± 0.01 PC = 166.66	0.136 ± 0.001 PC = 202.22

Each value is mean SD of 6 (Six) observations. All values are statistically significant from control at 5% level ($p < 0.05$). PC denotes percent change over control. *Not significant.

during toxic exposure period and under stress condition. The elevated levels of phosphatases may indicate the increase in the rate of phosphorylation and transport of molecules across the cell membrane. The enhanced phosphatases activity reveals increase transportation of metabolites through cellular membrane. Abdul et.al. (2004) and Venkateshwarlu et al. (1990) also reported that the pesticides cause significant increase in cellular damage which cause enhanced activity of phosphatases activity. Carla et al. (2005) reported altered phosphatases activity in Kupffer-melanomacro phagic cells of *Rana esculenta* during environmental pollution. The fish can utilize stored proteins to overcome the toxic stress. On to toxic stress, the levels of key enzymes involved in proteins metabolism changed. The present study concludes that triazophos in sublethal concentration alters tissue protein metabolism in *C. panctatus* as a consequence of triazophos toxicity, the fish shifts to alternate methods of metabolism to overcome the toxic stress and maintain its survival in the polluted environment.

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