Sub-lethal Effect of Pesticides on the Distribution of Glutaminases in the Brain of Labeo rohita (Ham.)

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Citation

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Abstract

In the present study, attempts have been made to investigate the sub-lethal effect of Organophosphates on the various enzymes such as phosphate activated glutaminase and L-Keto acid activated glutaminase in the different regions of brain of Labeo rohita. A total of 360 specimens of Labeo rohita (weight 90±5 gms and 18-20 cm in length were used in the present study. They were divided into 6 groups and each group has 30 fishes. They were then exposed to sub-lethal concentrations of dichlorvas, monocrotophos and phosphamidon for acute and chronic studies. In exposed fishes, phosphate glutaminase and X-ketoacid glutaminase registered significant changes in different brain regions under both acute and chronic studies

INTRODUCTION

A sizeable amount of organophosphates have been in use to boost agricultural yield. The run-off's from treated lands are known to interfere with nutrio-economically important animal growth in water bodies by altering and disrupting the different physiological processes. Even though there exists a mechanism in the animal body to counteract the toxic impact of organophosphates by catecholamine formation, but, this is insufficient to totally nullify the effect (Shaffi, 1980; 1982; Shaffi & Dubey, 1989, Shaffi, 1999 & Shaffi et al. 2000). Brain plays the most vital role in the metabolism of an animal so in the present investigation brain has been used as the key organ to understand the toxic impact of organophosphates on the animal body. Biochemical heterogeneity of brain regions is well established in different fish species, but the investigation on the sub-lethal effect of toxicants on the regional neuro-chemistry is imperative to identify the real site of action of the organophosphates. This may further help to find out remedies to protect the economically and nutritionally important organisms, on the other and to provide a congenial atmosphere to achieve maximum growth that will help boost the national economy (Shaffi & Habibulla, 1977; Shaffi et al 1977; Shaffi et al 1977; Shaffi, 1978; 1979; 1980; 1981 and 1982).

Brain is the centre of reasoning, regulation, translation and coordination and is aided by glutanegic and cholinergic nerve. The organophosphate (Monocrotophos, dichlorvas and phosphamidon) effect has been investigated on all aspects of fish metabolism expect biochemical regionalization of an organ (Shaffi & Jeelani, 1985; 1986; Jeelani & shaffi, 1988; Shaffi & Dubey, 1989, Shaffi, 1999). Thus in the present study the authors have made an attempt to investigate the sub-lethal effect of organophosphates on the various enzymes (phosphate activated glutaminase and L-Keto acid activated glutaminase) in the different regions of brain in Labeo rohita under acute and chronic exposures.

MATERIALS AND METHODS

Disease free, healthy and alive Labeo rohita (Ham) (18-20 cm standard length, 90±5 gms. Standard weight) were procured from some selected local ponds (to avoid ecological variations) and acclimatized to the laboratory conditions for a week and fed on ab-libitum, commercial pellet food. The tap water with alkalinity 100 mg/l, carbondioxide 1.98 mg/l, dissolved oxygen 8.0 to 8.6 mg/l, hardness of water 11 mg/l and temperature 23±20C was used.

SOURCES OF ORGANOPHOSPHATE AND LC 50 VALUE

Analytical grade organophospahtes were procured from M/s Cibageigy Ltd., Bombay, India. Labeo rohita were exposed to seven serial concentration were repeated seven times with relevant controls. The observed mortality data was subjected to probit analysis in order to determine LC 50 value (Finney, 1971).

EXPERIMENTAL DESIGN

L. rohita were kept in six separate aquaria (4x1.5x2 feet, 40 ltr, water capacity) with 30, L. rohita in each. Aquaria 1to3 contained dechlorinated water and were treated under as control group. To aquaria 4,5 and 6 were added sub-lethal concentrations of moncrotophos, dichlorvas and phosphamidon respectively, for a period of 48 hrs under acute studies. Similar number of Labeo rohita were kept in six different aquaria as control (1-3) and treated (4-6) groups, respectively, for a period of 45 days under chronic studies. Seven Labeo rohita from each aquarium control (Aquaria 1-3) and treated (Aquaria 4-6) were removed and dissected separately after 12, 24 and 36 hours in case of acute studies and 15, 30 and 45 days in case of chronic studies. The fishes were fed on ab-libitum, a commercial pellet food during the experimental period. The aquaria were examined after every four hours during the acute and chronic studies to remove the faeces, uneaten food and dead fish. On every occasion food was put in each aquarium till L. rohita was stopped feeding. L. rohita was starved during the first 24 hrs and the last 24 hrs of the exposure to check metabolic differences, if any, due to differential feeding. The water of treated and untreated aquaria was changed daily after feeding the exposure to check metabolic differences, if any, due to differential feeding. The water of treated and untreated aquaria were changed daily after feeding.

BRAIN COMPARTMENTATION

The Labeo rohita from control (1-3) and treated (4-6) aquaria were removed after 12, 24 and 36 hrs (acute studies) and 15, 30 and 45 days (chronic studies) exposure and the brain was exposed and separated into cerebrum, diencephalan, cerebellum and medulla oblongata (Shaffi 1978, 1993, 1995, 1999, Shaffi et al 1999).

ENZYME AND PROTEIN ASSAYS

The activity of phosphate activated glutaminase and L-keto acid activated glutaminase was determined by experiment the ammonia formed by the diffusion technique of Conway and Byrne (1993). The reaction mixture for phosphate activated glutaminase consisted of 0.06m tris-maleate buffer (pH 8.5) and 0.004M L-glutaminase and tissue homogenate in the final volume of 3 ml. The reactants were incubated at 37C for 15 minutes prior to the addition of substrate and the incubation was terminated after appropriate time (15 minutes).

The reaction mixture for L-keto acid activated glutaminase consisted of 0.06M tris -buffer (pH 7.4) 0.004M L-glutamine tissue and 0.03M z-oxoglutarate in a volume of 3 ml. The enzyme was incubated for 5 days at 37C prior to the addition of L-glutamine. The reaction was terminated after 60 minutes of incubation. The protein amount in the enzyme was estimated by Lowry et al (1951).

STATISTICAL ANALYSIS

The experiment was repeated with seven separate Labeo rohita samples for acute and chronic studies and the data was subjected to test of ANOVA.

RESULTS

Phosphate glutaminase and X-ketoacid glutaminase registered significant changes in different brain regions (cerebrum, diencephalon, cerebellum and medulla oblongata) exposed to the sub-lethal concentrations of dichlorvas, monocrotophos and phophamidon under both acute and chronic studies in Labeo rohita (Table 1-4).

ACUTE STUDIES

Under the acute studies the highest fall in phosphate glutaminase was noticed with dichlorvas followed by monocrotophos and then phophamidon among brain regions. The highest fall was in cerebrum (12 hrs) followed by medulla oblongata (24 hrs), diencephalon (36 hrs) and cerebellum (36 hrs) in decreasing order (Table 1).

Under acute studies the highest fall in X-ketoacid glutaminase was noticed with dichlorovas followed by monocrotophos and then phosphamidon. Among brain regions the highest fall was in cerebrum (24 hrs) followed by medulla oblongata (24 hrs) diencephalon (36 hrs) and cerebellum (36 hrs) in decreasing order (Table 2).

Figure 1

Table – 1 Effect of sub-lethal dose of pesticides on brain - phosphate glutaminase in (Ham.) - Acute studies

Regions of the brain	Period of Exposure											
	Dichlorvas				Monocrotophos				Phosphamidon			
	Cont.	12hrs	24hrs	36hrs	Cont.	12hrs	24hrs	36hrs	Cont.	12hrs	24hrs	36hrs
Cerebrum	45.8±0.2	33.2±5.4	31.1±4.9	23.6±3.9 (51.92)	46.2±7.5	37.6±6.2	28.2±4.1	22.4±4.6 (48.48)	44.6±0.2	31.8±6.4	25.2±2.8	18.2±3.2 (40.53)
Diencephalon	37.6±6.6	29.9±7.2	24.9±3.8	15.4±2.6 (40.96)	37.2±4.8	30.2±5.4	24.6±8.2	13.4±3.9 (36.50)	36.8±4.3	32.6±3.6	23.9±1.9	11.2±2.2 (30.43)
Cerebellum	24.6±8.1	20.8±8.7	14.8±4.8	7.8±1.2 (31.70)	22.6±3.6	17.8±3.9	12.8±2.4	6.6±1.8 (29.20)	25.8±3.4	20.6±4.8	14.20±2.4	6.9±1.1 (26.74)
Medulla oblongata	30.4±5.9	24.6±4.5	15.8±2.9	13.3±1.8 (43.75)	32.6±4.4	21.7±2.5	14.2±3.4	13.8±2.2 (42.33)	30.6±4.6	18.7±3.2	12.6±1.7	10.2±1.9 (35.33)

Values (µ mole ammonia formed/60 min/mg protein) are ± SEM mean of seven replicates Super Scripts a-c indicate that P>0.002, P<0.10 & P<0.001, respectively. Figures in

parenthesis indicate the percentage of fall.

Figure 2

Table – 2 Effect of sub-lethal dose of pesticides on brain - ketoacid glutaminase in (Ham.) - Acute studies

Regions of the brain	Period of Exposure											
	Dichlorvas				Monocrotophos				Phosphamidon			
	Cont.	12hrs	24hrs	36hrs	Cont.	12hrs	24hrs	36hrs	Cont.	12hrs	24hrs	36hrs
Cerebrum	89.3±11.4	59.6±7.9	44.2±6.8	39.8±4.8 (57.14)	68.8±10.6	60.9±8.6	46.0±6.9	37.1±3.9 (53.92)	89.4±9.6	58.6±8.8	42.0±6.2	34.3±3.9 (949.42)
Diencephalon	52.6±10.3	46.2±8.6	39.4±6.9	24.6±3.8 (46.78)	52.9±9.8	45.2±7.4	36.1±6.8	22.2±4.2 (41.96)	53.1±8.2	46.1±5.9	38.6±4.9	20.1±2.6 (37.83)
Cerebellum	35.2±9.6	29.3±4.8	22.8±4.4	13.8±2.6 (39.20)	35.6±6.8	28.6±5.9	24.1±6.8	12.1±2.8 (33.98)	34.2±7.4	31.3±4.8	24.9±3.2	10.9±1.9 (31.87)
Medulla oblongata	42.3±8.8	38.2±5.6	26.8±5.2	20.8±1.9 (49.40)	41.8±4.9	35.3±9.8	22.8±6.2	19.2±1.8 (45.85)	45.2±6.8	38.1±8.2	21.9±4.2	17.2±2.4 (39.81)

Values (µ mole ammonia formed/60 min/mg protein) are ± SEM mean of seven replicates Super Scripts a-c indicate that P>0.002, P<0.10 & P <0.001, respectively. Figures in parenthesis indicate the percentage of fall.

CHRONIC STUDIES

Under chronic studies, the highest fall in phosphate glutaminase has noticed with dichlorvas followed by monocrotophos and then phosphamidon. Among brain regions the highest fall was in cerebrum (15 days) followed by medulla oblongata (15 day) ,diencephalon (30 days) and cerebellum (45 days) in decreasing order (Table 3), with dichlorvas in L. rohita then with monocrotophos and phosphamidon (Table 3). The fall in L-ketoacid glutaminase was highest in cerebrum (20 days), diencephalon (30 days) and cerebellum (45 days). (Table 4).

Under chronic studies the highest fall in L-keto acid glutaminase was noticed with dichlorvas followed by monocrotophos and then phophamidon. Among brain regions highest fall was in cerebrum (15 days) followed by medulla oblongata (15 days), diencephalon (30 days) and cerebellum (45 days) in decreasing order (Table 4). Among the enzymes, L-keto acid glutaminase registered more fall than phosphate glutaminase in different brain regions in Labeo rohita in both acute and chronic studies.

Figure 3

Table – 3 Effect of sub-lethal dose of pesticides on brain-phosphate glutaminase in (Ham.) - Chronic Studies

Regions of the brain	Period of Exposure											
	Dichlorvas				Monocrotophos				Phosphamidon			
	Cont.	15days	30days	45days	Cont.	15days	30days	45days	Cont.	15days	30days	45days
Cerebrum	46.2±7.8	32.8±6.9	26.5±8.4	18.4±2.9 (39.82)	46.8±9.2	34.9±5.4	28.1±4.4	16.2±3.2 (34.60)	45.9±3.9	30.7±6.9	25.1±3.4	12.2±2.4 (26.57)
Diencephalon	37.8±8.6	30.6±4.8	19.4±3.9	11.2±1.8 (29.62)	39.6±6.9	32.5±3.8	17.4±3.4	10.6±1.9 (26.78)	36.4±4.2	30.4±5.8	16.3±1.6	8.2±1.9 (22.52)
Cerebellum	25.2±5.8	21.6±3.9	18.1±2.8	6.4±1.1 (25.39)	25.8±5.6	23.4±4.9	18.2±2.1	5.4±0.9 (20.93)	24.8±3.8	23.0±5.2	17.4±1.2	5.8±0.8 (35.52)
Medulla oblongata	31.2±4.9	20.2±7.2	14.2±3.3	10.8±2.1 (33.97)	31.9±4.4	20.9±3.8	12.6±10.9	8.4±1.5 (29.46)	30.6±2.7	16.7±3.6	10.8±2.9	7.6±1.1 (24.83)

Values (µ mole ammonia formed/60 min/mg protein) are ± SEM mean of seven replicates, Super Scripts a-c indicate that P>0.002, P>0.10 & P >0.001 respectively. Figures in parenthesis indicate the percentage of fall.

Figure 4

Table – 4 Effect of sub-lethal dose of pesticides on brain - ketoacid glutaminase in (Ham.) - Chronic Studies

Regions of the brain	Period of Exposure											
	Dichlorvas				Monocrotophos				Phosphamidon			
	Cont.	15days	30days	45days	Cont.	15days	30days	45days	Cont.	15days	30days	45days
Cerebrum	70.1±12.2	66.2±8.2	48.0±6.4	34.8±3.6 (49.64)	71.1±13.1	61.1±5.9	43.0±7.2	32.2±4.6 (45.28)	69.8±10.4	61.3±8.2	39.0±4.6	29.2±3.1 (41.83)
Diencephalon	55.1±9.8	46.2±6.4	33.2±3.9	21.2±4.2 (39.92)	53.7±7.2	45.3±4.3	29.1±3.8	19.2±2.8 (35.75)	52.6±6.9	46.4±7.6	28.5±6.2	17.2±4.2 (32.69)
Cerebellum	34.8±7.4	28.6±3.3	20.8±6.1	10.8±2.9 (31.03)	35.2±3.9	29.8±3.1	20.2±2.8	10.1±1.2 (28.69)	34.1±3.6	29.2±6.0	19.2±4.1	8.4±3.0.9 (24.63)
Medulla oblongata	42.9±6.2	36.5±5.4	24.1±4.4	18.8±3.1 (43.82)	43.1±6.2	36.4±5.8	20.7±4.6	17.8±2.2 (41.29)	41.8±4.9	35.0±3.2	18.5±5.2	15.1±2.8 (36.12)

Values (µ mole ammonia formed/60 min/mg protein) are ± SEM mean of seven replicates Super Scripts a-c indicate that P>0.002, P>0.10 & P >0.001, respectively. Figures in parenthesis indicate the percentage of fall.

DISCUSSION

The bio-chemical organization reflects the physiological status of an organ and chemical regionalization indicates its role in a specified area of metabolism. Conversion of one type of substrate into another would indicate the ability and adaptation of that region of the organ under changed life style (Shaffi, 1993, 1995, 1999, Shaffi et al 1999, Shaffi et al 2000).

The variations in the phosphate and L-ketoacidglutaminase in cerebrum, diencephalon, cerebellum and medulla oblongata in Labeo rohita exposed to sub-lethal concentrations of monocrotophos, dichlorvas and phosphamidon may be attributed to the differential precipitation of pesticides into various brain regions and subsequent interference with different chemical components and in the present investigation such a process could have taken place and consequently pesticides registered inhibition of L-ketoacidglutarate glutaminase and phosphate activated glutaminase (Berl et al , 1975, Balazas & Cremer 1973, Berl & Clarke 1975, Fonnum, 1975, Lajtha 1970, Goodman & Gilman 1980).

The fall in L-ketoacidglutarate glutaminase and phosphate activated glutaminase may also be ascribed to the hydrophobic nature of pesticides (Nag 1992, Roberts 1960, Shaffi & Habibulla 1977a, b, Shaffi et al 1977). Among the three pesticides monocrotophos is less polar and much more penetrable in comparison with dichlorvas and phosphamidon in L. rohita. Hence, in the present study the fall in L-ketoacidglutarate activated glutaminase and phosphate activated glutaminase is much higher with monocrotophos in

comparison with dichlorvas and phosphamidon and the variations in glutaminases level can be ascribed to the above approach.

Severe memory impairment, concentration deficiency, lower osmotic pressure can be an indication of suppressive nature of the pesticides and such a mechanism could have been happened in the present investigation and the maximum enzyme fall recorded in the cerebrum of Labeo rohita in comparison to other brain regions (like diencephalon, medulla oblongata and cerebrum) confirm the interference and inhibitory nature of the pesticides as cerebrum is concerned with behaviour, thought, learning, memory, coordination and voluntary activities.

Organophosphate pesticides can meddle with the metabolic cycle of glutamic and glutamine and perhaps it may disturb the homeostasis equilibrium of the neurotransmission and related substances and further influence the permeability of cell membrane which in turn helps to enhance free calcium, causes breakdown of cytoskeletal elements and degeneration of myelin sheath. A shift in the homeostasis is concerned with the activity of both glutaminases. Similar sequence of changes could have taken place in the present investigation and the fall in L-ketoglutarate glutaminase and phosphate activated glutaminase in cerebrum, diencephalon, medulla oblongata and cerebellum may be disturbance in the biochemical and physiological equilibrium. The fall of the enzymes in different brain regions in L. rohita exposed to acute and chronic duration may be due to alterations in the changed atmosphere (Balazas & Cremer 1973, Berl et al 1975, Berl & Clarke 1975, Goodman & Gilman 1980, Melnikov 1971, Shaffi et al 2000).

Amounts of glutamate, catecholamine, acetylcholine and 5-hydroxy tryptamine and glutaminases in different brain regions indicate the physiological status and a change in the amount in the above parameters due to organophosphate exposure (monocrotophos, dichlorvas and phosphamidon) can be taken as the index of nervous activity in the present study. The fall of the above said in different brain regions in L . rohita can be an indicator of biochemical alterations and subsequent fall in the neural activity in L. rohita (Goodman & Gilman, 1980, Shaffi, 1993, 1995, Shaffi et al 1999, 2000).

Variations in acetylcholine, catecholamines and derivatives of amino acids was noticed in rat brain treated with monocrotophos by Shaffi (1980) and it may be treated as a corollay to our finding in brain regions of L. rohita.

Differential response of glutaminases to monocrotophos, dichlorvas and phosphamidon in different brain regions in L. rohita might be concerned with their involvement in metabolism and degree of response to the above pesticides and vice-versa. Similar course may be the evidence for the present changes.

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