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Chronic toxic impacts of fenthion on the profiles of enzymes in the fresh water fish *Cyprinus carpio* (linn.)

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ABSTRACT
The effect of chronic exposure to a sublethal concentration of the fenthion pesticide, on enzyme activities in liver, brain, and muscles of the freshwater teleost fish, *Cyprinus carpio*, was studied after period of two months. The comparative study between control and the exposed fish revealed that there was a slight difference in enzyme activity at the lowest concentration while severe inhibition of all enzymes activity were observed at the concentrations, 0.38 and 0.193 mg/1. Among the three concentrations selected, the highest concentration seems to be the most highly potent and cause significant change in enzyme activity. The inhibitory effect on LDH and SDH activity of *C. carpio* is dependent on the concentration of insecticide during 60 days exposure test period The activity of GOT and GPT decreased significantly in both, the muscle and liver of Fenthion exposed fish *C. carpio.* There was a decrease in acid and alkaline phosphatase activity in muscle and liver of Fenthion treated fish *C. carpio* Adenosine triphosphatase activity was reduced in both liver and muscle tissues of exposed fish *C. carpio.* The inhibition of AChE was remarkable in all the experimentally exposed fishes depending upon the concentration of Fenthion and exposure period. The results suggest that anaerobic metabolism was favoured and aerobic oxidation of pyruvate was impaired in fish exposed to fenthion.

Keywords: Fenthion, LDH, SDH, GOT, GPT, ATP, AChE.

1. Introduction
Enzymes are an invention of the nature designed to accelerate and control numerous chemical reactions in a specific manner that determine the metabolism and vital activities of a cell and thus of an organism. However, the orderly balance in the physiological process is constantly under attack by the environmental adversities. The study on disturbance in enzyme activities with respect to a change in environment makes the attractive index of stress, hence, could be due to this reason enzyme analysis are becoming increasingly important for the determination of toxic effects of chemical pollutants in the field of environmental toxicology. Isreal [15] studied biochemical changes in certain tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cyprniformes) exposed to fenthion. Baby Joseph [3] studied the impact of pesticide toxicity on selected biomarkers in fishes. Mastan [18] studied sub-lethal effect of pesticides on the distribution of glutaminases in the brain of *Labeo rohita* (Ham.). Abilash [1] studied toxic, physico-morphological and behavioural responses of *Oreochromis mossambicus* exposed to commercial grade endosulfan and reported alterations in the cellular morphology of pesticide treated fish. Satyanarayan [28] studied impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto.* Dhasharath [10] studied about the effect of endosulfan and butachlor on the digestive enzyme and proximate composition of the fish, *cyprinus carpio.* Bhatnagar [4] worked on rogor induced changes in alkaline phosphatase activity in few tissues of *Mystus vittatus.* Rao [24] studied the effect of methyl parathion on esterases of fresh water teleost *T. mossambica* and reported that acetyl cholinesterase activity decreased in muscle, gill, liver and brain tissues. Sastry and Malik [31] reported acute and chronic effects of Diazinon on activities of dehydrogenase of fresh water teleost *Channa punctatus*. Verma [39] studied changes in alkaline phosphatase activity in fish *Channa gachua* exposed to Thiodan and Rogor. Rao [23] reported impact of Methyl parathion on lactate dehydrogenase iso-enzymes of a teleost *Tilapia mossambica*.

Lactic dehydrogenase or LDH, Succinic dehydrogenase or SDH enzymes are associated with carbohydrate metabolism. LDH represents Embdten-Meyerhof Parnas Pathway. Under anerobic condition this enzyme converts pyruvic acid to lactic acid. SDH are one of the important enzymes involved in Kreb's cycle. The oxidation of succinic acid to fumaric acid are regulated by this enzyme. Aspartate aminotransferases (AAT) or Glutamate oxaloacetic transaminase (GOT).Alanine amino transferase (ALAT) or Glutamic pyruvic trans- aminase (GPT) are enzymes involved in protein metabolism. GOT are the most active and widely distributed transaminase. GPT are active and more abundant in liver compared to other tissue Acid phosphatase (ACP), Alkaline phosphatase (AIP), Adenosine triphosphatase (ATP) enzymes are associated with certain reactions involved in energy production, and are the most active phosphatase, with optimum pH of 9.0 .ACP has an' optimum pH of 5 and has limited activity but has a function similar to that of AIP. ATPase catalyses the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and phosphoric acid bringing about the release of enormous energy. It is also involved in osmoregulation. In some cases ATPase activity is activated by Mg^{+2} and in other by $Ca^{+}2$ and Mg^{+2}. Another form of ATPase is stimulated by Na^{+} and K^{+}. The enzyme acetylcholinesterase (AChE) is highly specific and rapidly hydrolyses acetylcholine that is formed during the passage of nerve impulse. AChE is often called true cholinesterase. Since Scanty information is available on enzymatic alteration in connection with pesticides toxicity in *C. carpio* the present investigation has been undertaken to study the effect of sub1ethal concentrations of Fenthion on enzyme: dehydrogenases, aminotransferases, acetylcholinesterases and phosphatases.

2. Materials and Methods:

Acute toxicity study revealed that toxicity of Fenthion does not increase with time. Therefore toxicant concentrations selected were 3/4, 1/2, 1/4, 1/8 an 1/16 of 96 hr LC_{50} i.e. 1.162, 0.775, 0.387, 0.193 and 0.096 mg/l. All the various concentrations were prepared on same day. Parallel acetone groups (controls) were maintained in similar way. The test fish were exposed to different test concentrations selected for a period of two months. The fish *Cyprinus carpio* (Linn.) all measuring about 13-14cms and 15-20gms weight were procured from Arey pond, Mumbai. They were free of pollutants and were acclimatized to laboratory conditions for three weeks. This method helped the investigator to select the, healthy looking fish. The fish were selected for the test as mentioned in APHA [2] and exposed to five different sublethal concentrations of Fenthion for a period of two months. Besides, control groups were also simultaneously maintained for confirming the results. In order to maintain the concentration of toxicants throughout the period of experiment and to avoid the accumulation of metabolic wastes, entire water was replaced by fresh aged tap water every alternate day. The water analysis for determining pH, acidity, alkalinity, hardness, dissolved oxygen was carried out regularly twice a week following the standard methods described in APHA [2]. The average values of all these parameters are presented in table.

At the end of experimental period, four surviving fish from each group were sacrificed to obtain brain, liver and muscle tissue. The homogenates were prepared with distilled water and centrifuged at 10,000 rpm. for 10 minutes. The clear supernatant was used as an enzyme source after proper dilution. Lactic dehydrogenase (LDH) activity was analysed by following the method given by Bergmeyer [5]. Optical density of the mixture was determined on spectronic 20 at 340 mp (Model Baush & Lomb, Model No. Cat. no. 33-31-72). Succinic dehydrogenase (SDH) activity was estimated by the spectrophotometric method described by Slatter and Bonner [34]. Optical density was taken at 340 mp on spectronic 20.Aspartate amino transferase (GOT or AAT) activity in the homogenate was assayed according to the method described by Bergmeyer and Bernt [6]. Optical density was read at 546 mμ on spectronic 20. Alanine amino transferase (AlAT or GPT) the method described by Bergmeyer and Bernt [7] was followed for analysing the AlAT activity in the tissue homogenate of test fish. Optical density of the solution was read at 546 mp on Spectronic 20. Acid phosphatase (ACP) the method of Shinowara [33] was employed for the assay of, the enzyme ACP. Optical density of the samples were read at 660 mp on Spectronic 20. Alkaline phosphatase (AlP) the method described by Bodansky [8] was employed for the assay of this enzyme. Optical density was read at 660 mp on Spectronic 20.Adenosine triphosphate (ATP) activity in tissue homogenate was determined by Dubois [12] method. In this reaction ATP was hydrolysed to ADP and the inorganic phosphate thus liberated was measured by the method of Fiske and Subbarao [13]. Acetyl cholinesterase (AChE) the activity of AChE in the brain homogenate was determined according to the method of W. Plitz [42]. Optical density was measured at 490 mμ on Spectronic 20.

3. Results and Discussion:

The results of selected enzymes in muscle and liver tissues of exposed & control fish C. carpio are presented in table. 1&.2. The comparative study between control and the exposed fish revealed that a slight difference in enzyme activity at the lowest concentration while severe inhibition of all enzymes activity can be observed at the concentrations, 0.38 and 0.193 mg/1. The changes in LDH and SDH in muscle and liver tissues of *C. carpio* due to chronic exposure of fish to Fenthion are presented in table no. 1&2. The inhibitory effect on LDH and SDH activity of *C. carpio* was dependent on the concentration of insecticide during 60 days exposure test period. Among the three concentrations selected, the highest concentration seems to be the most highly potent and cause significant change in enzyme activity.

Joshi [18] and Gaikwad [14] reported similar inhibition of LDH activity in *G. affinis and T. mossambica* exposed to Thiodan and reported that it may be due to anaerobic condition. Verma [38] observed similar reduction in LDH and SDH activities in chronically treated fish *N. notopterus* to various insecticides. Moffett and Yarborough [20] reported that DDT, Toxaphene and Dieldrin inhibited SDH activity in mitochondria with disrupted membrane in both resistant and susceptible *G. affinis*. According to Sastry and Malik [31] in *C. Punctatus*, lactate dehydrogenase (LDH) activity was inhibited in liver, kidney, intestine, gills and muscle but in brain, the activity was elevated when expse to diazinon. Ranganatha Koundiya and Ramamurthi [26] reported that inhibition of SDH activity could be due to depression of cellular oxidation. In the present study, inhibition in LDH and SDH activities indicates the blockage of anerobic and aerobic metabolism to meet the energy demand due to toxicity stress.

Transformation represents one of the principle metabolic pathways for the synthesis and deamination of amino acids. It allows an interplay between carbohydrates, fat and protein metabolism, the activity which can serve the changing demand of an organism. The activity of GOT and GPT decreased significantly in both, the muscle and liver of Fenthion exposed fish *C. carpio* (Table1, 2). Similar inhibition was observed by. Verma [37, 38], Gaikwad [15] and Joshi [18] was due to cellular disturbance and damage to these organs causing leakage of, these enzymes from the tissue into circulatory system. Jee [17] found an increase in levels of serum glutamic oxaloacetic acid transaminase, glutamicpyruvic acid transaminase, glucose and alkaline phosphatase and a decrease in the concentration of plasma total protein, albumin, cholesterol and lysozyme in Korean rock fish *Sebastes schlegeli* Hilgendorf exposed to cypermethrin. Soman [30] also reported similar reduction in GOT and GPT activity in Lebaycid-1000 exposed fish, *Colisa fasciata* and suggested that it could be due to damage caused to tissue leading to the leakage of enzymes. The present report on *C. carpio* supports the view of above workers and it may be suggested that decrease in enzyme activity of transaminase might be due to leakage of these enzymes from the damaged tissues into the serum under Fenthion stress.

Alkaline phosphatase is the brush border enzyme associated with maintenance of orthophosphate pool, the transfer of phosphyl group, the hydrolysis and stratification of metabolities moving across the membrane within the cell and between the extra cellular spaces Seev [33]. Gaikwad [14] reported reduction in alkaline phosphatase activity in *T. mossambica* exposed to Thiodan and suggested that it could be due to leakage of enzyme from damaged hepatocytes into the circulatory fluid. Verma [39] reported fall in alkaline phosphate activity in *Channa gachua* exposed to sublethal concentrations of Thiodan and Rogor. Bhatnagar [4] reported that inhibition in alkaline phosphatase activity in *Mystus vittatus* exposed to Rogor could be due to uncoupling of phosphorylation. In the present study also reduction in alkaline phosphatase activity has been observed (Table no. 1,2). This could be from leakage caused due to considerable damage to tissues and or it can be suggested that it may be due to the disruption in the process of oxidative phosphorylation during the formation of energy rich compounds.

Acid phosphatase is a lysosomal enzyme associated with growth differentiation and the lysis of cells .There was a decrease in acid phosphatase activity in muscle and liver of Fenthion treated fish *C. carpio* (Table no. 1, 2). According to Gaikwad [14] reduction in acid phosphatase activity could be due to unbalanced catabolism of enzyme protein. Verma [39] reported that acid and alkaline phosphatase activity increased in lethal and decreased in sublethal concentrations of Thiodan and Rogor when *Channa gachua* was exposed to them. Thus, in the present investigation fall in acid phosphatase activity could be due to uncoupling of oxidative phosphorylation and cellular damage, as viewed by other workers.

Adenosine triphosphatase activity was reduced in both liver and muscle tissues of exposed fish *C. carpio.* (Table no.1, 2). ATP is an enzyme mediating membrane transport. ATPase is located in cell membrane and has been implicated in active transport of Na^+ and K^+ across the cell membrane Skov [31]. Active transport systems serve to concentrate nutrients within the cell to maintain the proper level of inorganic electrolytes to correct osmotic pressure and volume of intracellular fluid. The cell activities 'require the passage of solutes against concentration gradient and are primarily dependent on ATP for energy. When ATP is hydrolysed by action of ATPase, ADP and inorganic phosphatase are produced and energy from high energy phosphate bond is released. This energy is utilised to drive the active transport system. Dalela [12] who studied the effects of ATPase in *Channa gachua* pointed out that endosulfan interferes with various energy recurring processes in the fish. Gaikwad [15] and Joshi [16] reported similar inhibition of ATPase activity in *T. mossambica* and *Gambusia affinis* exposed to Thiodan. Muralidharan [21] reported on ionic imbalance caused by the effect of pesticide on *Cyprinus carpio.* According to Rao [25] fall in ATPase activity was due to profound effect on oxidative metabolism by the fish caused due to stress of pesticide. In the present study, the reduction observed in ATPase activity could be due to its extensive use in energy demanding process and or in oxidative metabolism or may also be due to ionic imbalance as reported by Muralidharan [21] caused by the effect of pesticide. Thus, it may be suggested that the observed inhibition of acid and alkaline phosphatase and ATPase in liver and muscles of *C. carpio* may be due to depression of cellular oxidation of the uncoupling of oxidative phosphorylation.

Acetylcholinesterase is an enzyme that modulate the amount of neurotransmitter acetylcholine as suggested by O'Biren [22]. Measurement of the activity of this enzyme in aquatic animals not only offers a means of detecting serious pollution by AChE agents but it has the potential for indicating extent of poisoning the animal in actual environment Coopage [9] .Variations in AChE activity in the brain tissue of exposed fish are presented in table.3. The inhibition of AChE is remarkable in all the experimentally exposed fishes depending upon the concentration of Fenthion and exposure period. According to Weiss [40, 41] organophosphorous pesticide affected fish dies when AChE activity ranges between 5.4% and 92% of normal activity. Joshi [18] and Soman [30] reported similar inhibitory effects in different species of fishes exposed to various organophosphorous insecticides. According to Rath and Mishra [27] declining trend of brain and liver AChE activity in *Tilapia mossambica* exposed to Dichlorovos increased with increase in exposure and inhibition was due to its interference with AChE. Ujjal Banerjee [36] reported that change in concentrations of sodium and potassium in the CNS due to toxic stress impairs the ionic balance in nerve membrane thus, ultimately blocking the nerve conduction due to non-release of acetylcholine and inhibition of acetylcholinesterase activity finally causing paralysis in the insect body .According to Sheela Susan jacob [29] high toxicity of Fenthion is because of its interference with normal mechanism of nerve impulse and enzyme cholinesterase. The ability of Fenthion to inhibit AChE activity as observed in the present study may be due to high accumulation of Fenthion in brain tissue and could also be due to intake of Fenthion with ChE group. This may lead to impairment of the normal functioning of nerve impulse causing physiological and behavioural modifications that can lead to reduction in survival ability.

Table 1: Changes in enzyme activity in the muscle tissue after 60 days exposure to fenthion

Determination	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
LDH µmole/hr/mgprotein	0.023 ± 0.004	0.02 ± 0.0023 - 13.04%	0.0098 ± 0.005 - 57.3%	0.0076 ± 0.002 - 66.9%
SDH µmole/hr/mgprotein	0.077 ± 0.002	0.058 ± 0.003 -24.6%	0.041 ± 0.004 -46.7%	0.022 ± 0.01 -71.4%
GOT unit/hr/mgprotein	79.34 ± 0.47	60.0 ± 0.32 - 24.3%	50.3 ±0.42 - 36.6**%**	34.4 ± 0.57 - 56.6%
GPTunit/hr/mgprotein	48.63 ± 0.72	40.88 ± 0.77 -16.10%	30.25 ± 0.68 -37.7%	19.58 ±0.43 -59.73%
AcP µg p/hr/mgprotein	0.667 ± 0.01	0.460 ± 0.013 -31.0%	0.379 ±0.01 -44.2%	0.372 ±0.012 -43.6%
AIP µg p/hr/mgprotein	0.661 ± 0.01	0.521 ± 0.13 -21.1%	0.501 ± 0.2 - 24.2.0%	0.497 ± 0.013 - 24.8%
ATP µg p/hr/mgprotein	14.58 ± 1.4	14.42 ±0.9 -1.09%	13.86 ± 1.0 -4.93%	10.50 ± 1.3 -27.9%

Table 2: Changes in enzyme activity in the liver tissue after 60 days exposure to fenthion

Determination	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
LDH µmole/hr/mgprotein	0.094 ± 0.013	0.078 ± 0.004 - 17.02%	0.054 ± 0.006 - 42.5%	0.0198 ± 0.008 - 78.9%
SDH µmole/hr/mgprotein	0.188 ± 0.012	0.089 ± 0.007 -52.6%	0.068 ± 0.005 -63.8%	0.047 ± 0.004 -75.0%
GOT unit/hr/mgprotein	155 ± 5.47	151.3 ± 3.32 - 2.5%	140 ±4.42 -9.6**%**	131.0 ± 2.57 - 15.4%
GPTunit/hr/mgprotein	235 ± 10.72	230.3 ± 3.77 -2.12%	225 ± 4.8 -4.25%	194.58 ±3.43 -17.4%
AcP µg p/hr/mgprotein	0.401 ± 0.11	0.39 ± 0.13 -2.74%	0.363 ±0.01 -9.72%	0.341 ±0.04 -14.9%
AIP µg p/hr/mgprotein	0.52 ± 0.11	0.519 ± 0.03 -0.192%	0.419 ± 0.08 - 19.4%	0.410 ± 0.06 - 21.1%
ATP µg p/hr/mgprotein	23.01 ± 1.4	20.71 ±1.9 -9.9%	19.28 ± 1.4 -16.2%	18.09 ± 1.3 -21.3%

Table 3: Changes in acetyl cholinesterase activity in the brain of Cyprinus carpio exposed to fenthion for 60 days

Enzyme	Control	0.096mg/l	**0.193mg/l**	0.38mg/l
Acetyl cholinesterase µ eq of enzyme hydrolysed/mg protein	78.66±6.36	68.76±5.3 -12.5%	50.9±4.6 -35.2%	28.7±2.8 -63.5%

±Standard deviation for 5 determinations in %
(%) = percentage change from Control.

4. Conclusion:
Inhibition of LDH, SDH, GOT GPT, AcP, AlP, ATP. and AChE lndicates toxicity stress of Fenthion.

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