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METHYL PARATHION 50% EC INDUCED CHANGES IN ENZYMATIC ACTIVITIES OF THE FISH *Channa punctatus* (BLOCH)

KEYWORDS: Methyl parathion, Acute toxicity tests, enzymatic changes, and *Channa punctatus*

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ABSTRACT *The acute toxicity tests for 24, 48, 72 and 96 h to the fish Channa punctatus were conducted with an organophosphate pesticide methyl parathion 50% EC in both static and continuous flow-through methods. The LC_{50} values obtained were 0.6661, 0.6473, 0.6200 and 0.5949 ppm respectively for static, and 0.5736, 0.5592, 0.521 and 0.5364 ppm respectively for continuous flow-through method. The 1/10th of static 96 h LC50 value was taken as sub-lethal concentration and the fish were exposed to both lethal and sub-lethal concentrations for 8 days. The vital tissue of the fish viz; gill, liver, brain, muscle and kidney were isolated and analyzed for study of changes in enzymatic activity of amino-transferases, Aspartate Amino Transferase (AAT) and Alanine Amino Transferase (ALAT); Lactate dehydrogenase (LDH) and acetyl cholinesterase enzymes after 24, 96 h and 8 days of exposures. All the enzymatic activities were elevated under both lethal and sub-lethal concentrations, whereas the activity level of ACh. E activity was decreased. The changes in activity levels are more in lethal concentration compared to sub-lethal exposure. The changes in enzymatic activity are directly proportional to the increase in exposure period. These changes were in agreement with the earlier reports. The results obtained in all were discussed with the available literature.*

Introduction:

The organophosphorus (OP) insecticides were developed before and during World War II. The history of their development has been reviewed (Koelle, 1981and Holmstedt, 1963). Organophosphorus (OP) compounds represent the most widely used group of pesticides for the control of agricultural pests and disease causing vectors (Maitra and Mitra, 2008). Increasing demand for organic pesticides in public health and agriculture (Long, 1971) resulted in the production of numerous formulations (Macdonald and Deichmann, 1970), which causing imbalances in the ecosystems (Mark, 1969). Methyl parathion ($C_8H_{10}NO_5PS$) is an OP insecticide with a broad range of activity which inhibits acetyl cholinesterase activity (WHO, 1996). Due to the wide availability of OP compounds, poisoning is common (Garcia *et al*., 2003). Biological monitoring is one of the ways developed to effectively maintain the quality of the environment at socially and biologically desirable level. The idea of using aquatic biota for toxicity monitoring is not a recent phenomenon. Henderson and Pickering (1963), Jackson and Brungs (1966) have used fish for their monitoring studies. The usual measure of the environmental effect of any pollutant on an animal is mortality; however, other effects which are more delicate and indicate of physiological changes may ultimately detrimental to a population survival. The impairment of behavioral, physiological functioning could result in a gradual reduction in the adaptive capacity of species, leading to a decrease in their survival ability and population level.

Although the effects of pesticides on fish are extensively studied and has also been reviewed (Holden, 1973; McKin *et al*., 1976; Brungs *et al*., 1977; Edwards, 1983 and Gupta, 1986), but yet there is a need for information from the physiological angle. Incidentally, a great deal of attention has been paid to evaluate the hazardous effects of various pesticides on physiology of many non-target organisms by Tejendra *et al*., (1990), Sprague (1971), Sastry and Siddiqui (1984), Sreenivasa Moorthy *et al*., (1985).

Aminotransferases mobilize the aminoacids into carbohydrate and lipid metabolism. There exists a rapid turnover of free aminoacids from cell to cell, tissue to tissue through the circulating fluid and utilize for various purposes through inter-conversions. Transaminases form an important group of enzymes mediating carbohydrates, protein and lipid metabolism. Transamination represents the mechanism causing eventual deposition of nitrogenous waste products like ammonia and urea resulting in the production of carbon compounds, which contribute towards gluconeogenesis and fatty acid formation. AAT and ALAT are two important enzymes mainly involved in the interconversion of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and aminoacids thus bringing the protein and carbohydrate metabolism on one hand and alanine, aspartic acid and glutamic acid on the other (Moore, 1964; Knox and Greengard, 1965). Aminotransferases also act as precursors of gluconeogenesis and probably during the period of stress, they meet the energy demands by channeling aminoacids into carbohydrate metabolism (Watts and Watts, 1974; Martin *et al*., 1983). The aspartate aminotransferase catalyses the interconversions of aspartic acid, and α-ketoglutaric acid to oxaloacetic acid and glutamic acid, while alanine, amino transferase catalyses the interconversion of alanine and α-ketoglutaric acid to pyruvate and glutamic acid.

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

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of a cell and thus of an organism. However, the orderly balance in the physiological process is constantly under attack by the environmental adversities (Leena muralidharan, 2014).

Methyl parathion is an organophosphorus insecticide that was first synthesized in the 1940s. It is relatively insoluble in water, poorly soluble in petroleum ether and mineral oils, and readily soluble in most organic solvents (Laville, 2006). It is used increasingly in agriculture and public health management, as an effective replacement of its ethyl analogue, parathion (o,o-diethyl o-p-nitrophenyl phosphorothioate), which has been banned in many countries because of its higher mammalian toxicity. Methyl parathion was originally developed by the German pesticide company Bayer. It is a non-systemic pesticide that kills pests by acting as a stomach poison.

In the present study an attempt has been made to evaluate the effect of methyl parathion on the enzymatic activities of the fresh water fish *Channa punctatus*

Materials and Methods:
The capture freshwater snake headed fish *Channa puntatus* with a size range of 8-10 cm, irrespective of their sex, have been chosen as the test organism in the present study. The fish were obtained from the fish market, Guntur, Andhra Pradesh, India which is 14 km away from the University. The fish were acclimatized to the laboratory conditions in large plastic tanks with un-chlorinated ground water for two weeks at a room temperature of 28±2°C. During the period of acclimatization fish were fed daily with fish meal on an average of 3% of their body weight. Feeding was stopped one day prior to the experimentation. All the precautions laid down by APHA (1998) were followed.

The test toxicant methyl parathion 50% EC Emulsifiable Concentrate (EC) stock solution was made with acetone as solvent. In the present study 1/10th of 96 h LC_{50} value was taken as sub-lethal concentration to study the effect on enzyme: aminotransferases (AAT and ALAT), Lactic dehydrogenase (LDH), acetylcholinesterase (AChE). The activity of AAT and ALAT were determined by the method of Reitman and Frankel (1957). The Lactate Dehydrogenase activity (LDH) was estimated by the method of Srikanthan and Krishna Murthy (1955). AChE enzyme assays were performed spectrophotometrically by the method of Ellman *et al*., (1961).

Results and Discussions:
The studies of toxicity clearly indicate that methyl parathion was highly toxic to the test fish C.punctatus. The LC50 values obtained were 0.6661, 0.6473, 0.6200 and 0.5949 ppm respectively for 24, 48, 72 and 96 h for static, 0.5736, 0.5592, 0.521 and 0.5364 ppm respectively for continuous flow-through method. When a comparison is made between the static and continuous flow-through systems, LC50 values are low when compared to the static values, this may be due to the constant maintenance of concentration of toxicant in flow-through system and higher values in static system are due to bioaccumulation by fish, pesticide absorption to toxicant chamber and degradation of the compound. The 1/10th of static 96 h LC50 value was taken as sub-lethal concentration and the fish were exposed to both lethal and sub-lethal concentrations for 8 days and the vital tissue of the fish viz; gill, liver, brain, muscle and kidney were isolated and analyzed for study of changes in enzymatic activity of amino-transferases, Lactate dehydrogenase (LDH), and acetyl cholinesterase enzymes after 24, 96 h and 8 days of exposures.

Amino transferases (AAT and ALAT):
The calculated values of amino-transferases per cent change over control were graphically represented in Fig. I, Fig. II and Fig. III of 1and 2. The changes in the levels of AAT and ALAT were observed in different tissue of brain, liver, muscle, gill and kidney in the test fish *Channa punctaus* under sub-lethal and lethal concentrations of test toxicant after 24, 96 h and 8days of exposure. The values were expressed mg of formazon formed /mg protein /h.

Under exposure to sub-lethal and lethal concentrations of methyl parathion 50% EC for 24 h,96h and 8days highest per cent change in the of activity AAT was observed in muscle and

Fig.1.I. Change in the specific activity levels of Aspartate amino transferase (AAT) (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentration of Methyl parathion 50% EC for 24 h.

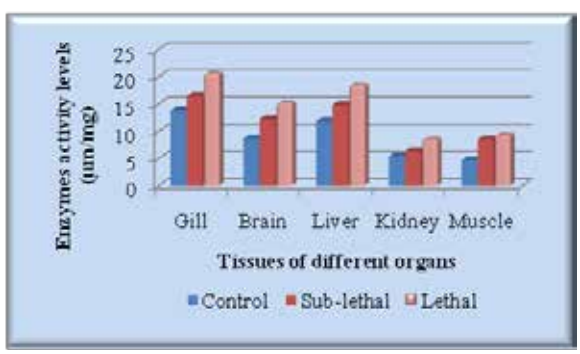

Fig.1.II. Change in the specific activity levels of Aspartate amino transferase (AAT) (µ Moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentration of Methyl parathion 50% EC for 96 h.

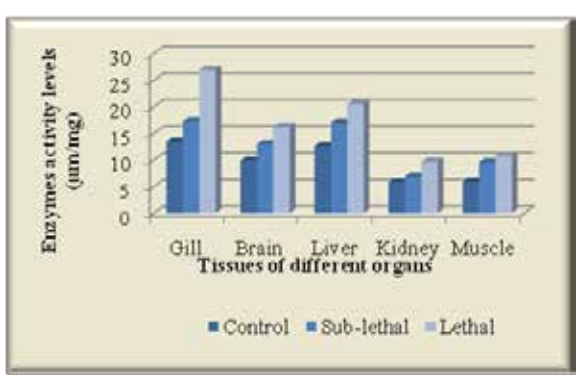

Fig.1.III. Changes in the specific activity levels of Aspartate amino transferase (AAT) (µ moles of pyruvate formed/ mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentration of Methyl parathion 50% EC for 8 Days.

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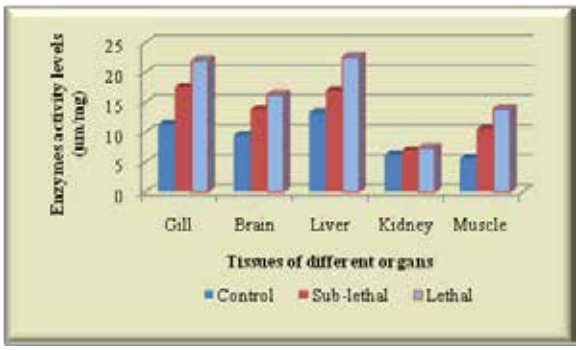


Under exposure to sub-lethal and lethal concentrations of methyl parathion 50% EC for 24 h,96h and 8days highest per cent change in the of activity AAT was observed in muscle and brain; muscle and gill and kidney in *Channa punctatus*. The activity levels of AAT increased significantly during 8 days of exposure. The highest per cent change of ALAT was observed in kidney for 24h, 96h and 8days. The changes of AAT were graphically represented in fig.1.I, fig.1.II and fig.1.III, for ALAT in fig.2.I, fig. 2.II and fig.2.III. Since the pesticide stress was known to induce significant change in protein metabolism, it is likely that the aminotransferase were also considerably affected. The highest per cent change in the activities of AAT and ALAT in different tissues of fish suggest either increased operation of transamination or increased

synthesis of amino acids from other sources like glucose or fatty acids during methyl parathion intoxication. The increase in activities of aminotransferases as observed in the present study were in agreement with earlier reports, demonstrating a consistent increase in the activities of these enzymes under conditions of enhanced gluconeogenesis. AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell. A close relation appears to exist between the mitochondrial integrity and transaminase levels and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. The alteration in the activities of AAT and ALAT as observed in the present study may also be due to the mitrocondrial distruption and damage as a result of methyl parathion induced stress. The elevation of AAT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT and ALAT in different tissues of brain, liver, muscle gill and kidney of the fish *Channa punctatus* can be considered as a response to the stress induced by methyl parathion to generate ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the excess energy demand under the toxic manifestations.

Fig.2.I. Change in the specific activity levels of Alanine amino transferase (ALAT) (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to Methyl parathion 50% EC for 24 h.

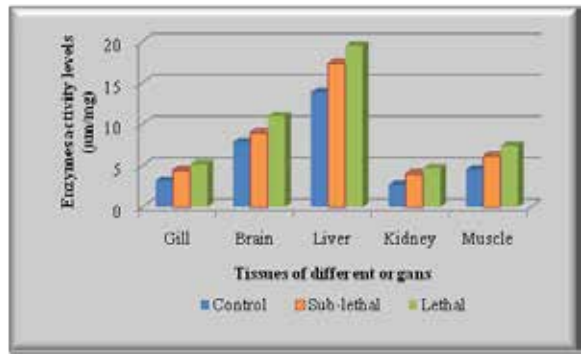


Fig.2.II. Change in the specific activity levels of Alanine amino transferase (ALAT) (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to Methyl parathion 50% EC for 96 h.

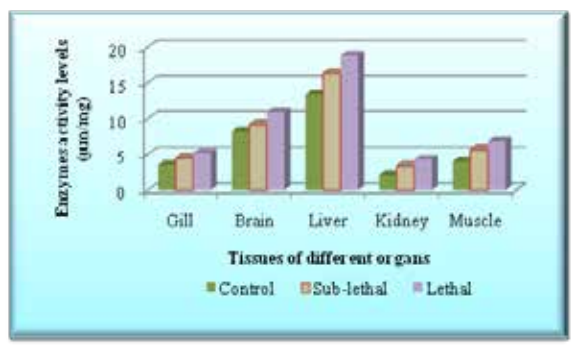
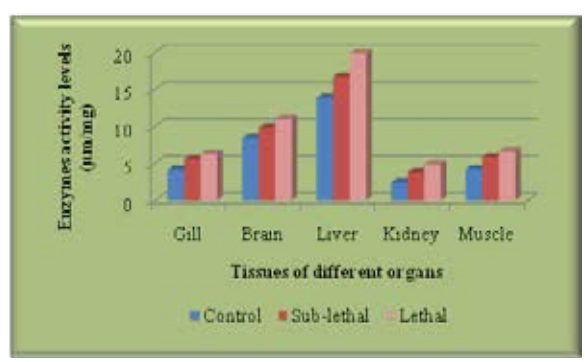


Fig.2.III. Change in the specific activity levels of Alanine amino transferase ALAT (µ moles of pyruvate formed/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to Methyl parathion 50% EC for 8 days.



The depletion of proteins under the stress of methyl parathion toxicity observed in different tissues of C.punctatus indicates the proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. The alterations in the levels of activity of aminotransferases induced by the pesticide methyl parathion clearly indicate that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems.

Both AAT and ALAT levels increased in tissues of fish suggesting the conversion of aminoacids released by the proteolysis into keto acids for energy production. The increase in the activities of AAT and ALAT as observed in the present study may also be due to the mitochondrial disruption and tissue damage, as a result of methyl parathion

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induced stress. Under the altered protein metabolism, the transfer of amino group is very essential for the reutilization of amino acids and to counter-check the excess proteolysis under pesticide stress. The present observed elevation of AAT and ALAT activity was supported by the earlier workers. Increase in aminotransferases activity was reported in Tilapia mossambica, under different pesticides exposure (Girija, 1987 and Radhaiah 1988). Increased levels of GOT and GPT in liver, muscle and blood under cadmium toxicity in *Channa punctatus* was observed by Sastry and Shukhla (1990). Kaviraj and Das (1994) reported an increased activity of aminotransferases due to lindane toxicity on Anabas testudineus during pesticide stress.

Lactate Dehydrogenase activity (LDH):
In the present study, it was observed that the activity of LDH was highly elevated following methyl parathion 50% EC exposure indicating increased anaerobic respiration to meet the energy demands where, aerobic oxidation is lowered. Lactate dehydrogenase (LDH) converts

the lactate to pyruvate and has very important role in carbohydrate metabolism. The LDH activity depends on its five isoenzymes and the activity changes under pathological conditions (Martin *et al*., 1983). The elevation in enzyme activity of *Channa punctatus* exposed to sub-lethal and lethal concentrations of methyl parathion (50% EC) was highly significant ($p<0.05$).

Fig.3.I. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentration of Methyl parathion 50% EC for 24 h.

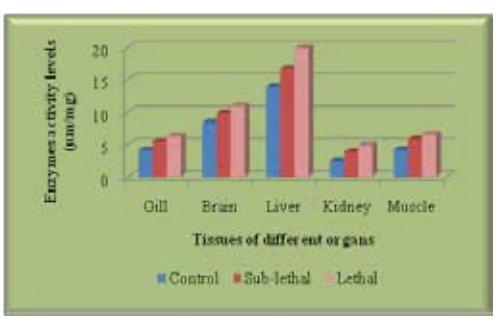


Fig.3.II. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentrations of Methyl parathion 50% EC for 96 h.

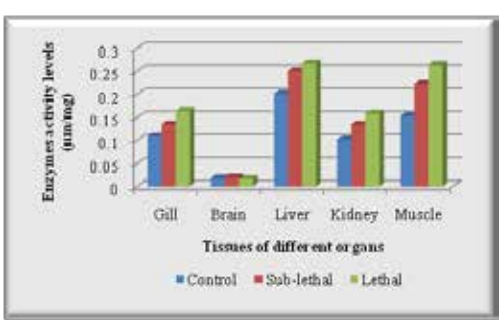
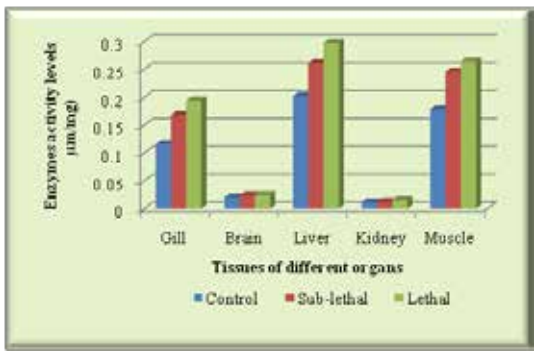


Fig.3.III. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µmoles of formazan/mg protein/hr) and % change over the control in different tissues of *Channa punctatus* on exposure to sub-lethal and lethal concentration of Methyl parathion 50% EC for 8 days.



The increase of LDH activity during conditions favoring anaerobic respiration to meet the energy demands lowers the aerobic respiration (Martin *et al*., 1983). The earlier reports on Tilapia mossambica (Anastasi and Bamistor (1980); Radhaiah 1988) support the present study.

Natarajan, (1984), reported increased LDH activity in gill, brain, muscle and liver tissues of Labeo rohita exposed to sub-lethal concentrations of metasystox.The earlier reports on Tilapia mossambica (Anastasi and Bamistor (1980); Radhaiah 1988) support the present study. Natarajan, (1984), reported increased LDH activity in gill, brain, muscle and liver tissues of Labeo rohita exposed to sub-lethal concentrations of metasystox. Ghosh, (1987) reported that sub-lethal toxicity of organophosphate (phosphamidon) on Clarios batractus showed an elevated LDH activity in gill, brain, liver and muscle tissues. Fluke (1972), explained that the raise of LDH activity increases the permeability of cells as well as necrosis. Sub-lethal copper toxicity on Labeo rohita, elevated the LDH activity levels (Venkataramana *et al*., 1990). Similar observations on LDH activity were made under pesticides stress by Veeraiah (2001), Tathaji (2007); Japamalai (2012); and Prasada Rao (2012).

Changes in Acetylcholinesterase Activity (ACh. E):
The ACh. E activity was estimated in different tissues like muscle, brain, liver, gill and kidney of the test fish *Channa punctatus* exposed to sub-lethal and lethal concentrations of methyl parathion for 24h, 96h and 8 days and the per cent change results were graphically represented through Fig.4.I, Fig.4.II and Fig.4.III. In the present study, the ACh. E activity was

Fig.4.I. Change in the specific activity levels of Acetyl Cholinesterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/gr tissue/min) and % change over the control in different tissues of *Channa punctata*, on exposure to sub-lethal and lethal concentration to Methyl parathion 20% EC for 24 h.

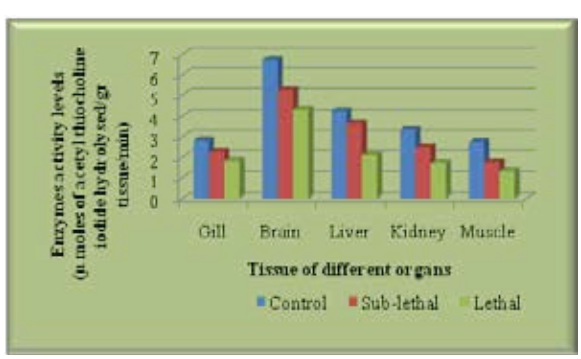


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Fig.IV.28. Change in the specific activity levels of Acetyl Cholinesterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/gr tissue/min) and % change over the control in different tissues of *Channa punctatus*, on exposure to sub-lethal and lethal concentration to Methyl parathion 50% EC 96 h.

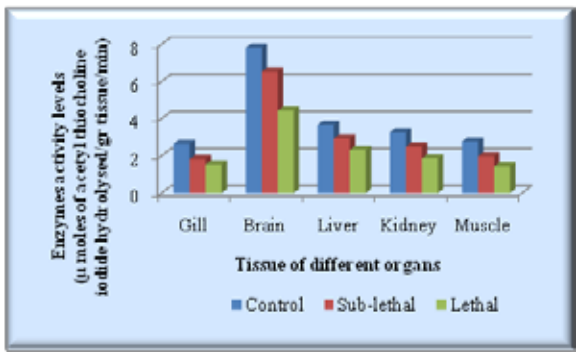
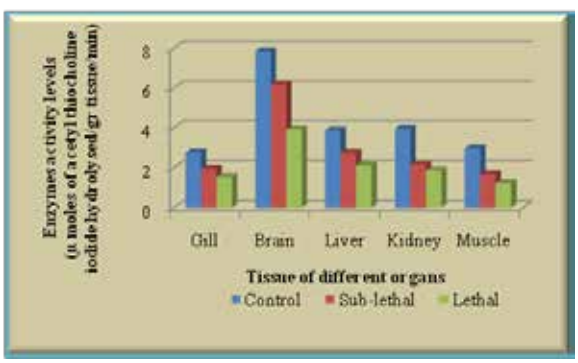


Fig.IV.29. Change in the specific activity levels of Acetyl Cholinesterase (ACh.E) (µ moles of acetyl thiocholine iodide hydrolysed/gr tissue/min) and % change over the control in different tissues of *Channa punctatus*, on exposure to sub-lethal and lethal concentration to Methyl parathion 50% EC for 8 days.



inhibited more in lethal concentration than in sub-lethal concentration of methyl parathion 50% EC, it may be due to the occupation of the OP compound with the active site of ACh. E, followed by phosphorylation of the phosphorus of the OP to the oxygen of the hydroxyl group of serine at the active site (Michel & Krop (1951). The greater inhibition of ACh.E in the fish was in brain. This may be due to the organophosphate compounds activity on neural activity. Since these OPs are neuro-toxic compounds the activity levels of ACh. E was inhibited in brain.

Acetyl cholinesterase enzyme involves in the maintenance of the structural and functional integrity of cellular membranes. The action of this enzyme and metabolism of individual groups of compounds were reviewed by O'Brien (1960). The extent of brain ACh.E reduction was proportional to the concentration of the substance (Weiss, 1958). Ach.E Enzyme inhibition is higher in lethal exposure than in sub-lethal exposure (Coppage s., 1975). Pinfish exposed to Malathion 58 µg/l 40 to 60% of the fish died and the extent of inhibition of ACh.E was in the range of 72 to 79% (Coppage *et al.*, 1975). Methyl parathion in shiners caused 58.6 to 83.7% inhibition of ACh.E within 10 hours after exposure (Gibson and Ludke, 1973). The maximum level of inhibition of ACh.E was reached within 2 to 4 hours with methyl parathion and after 20 h with methyl parathion (Benke *et al.*, 1974).

The cholinesterase activity in the erythrocytes, gill, heart and serum of rainbow trout was reduced within 3 hrs of exposure to acephate and 1 hr after exposure to fenitrothion (Duangsawasdi and Klaverkamp, 1979). In Tilapia, a highest level of ACh.E inhibition was noticed in brain followed by muscle, gill and liver (Kabeer *et al.*, (1979). Species related differences in the sensitivity of brain ACh.E were noticed by Yamin *et al.*, (1994). Monocrotophos caused a significant inhibition of the brain acetyl-cholinesterase activity (Siddiqui *et al.*, 1991). The different degrees of acetylcholinesterase inhibition were always accompanied by marked diminution of rat brain nor-epinephrine level by organophosphorus insecticides (Brezezinski *et al.*, 1980).

Brain ACh.E activity was significantly inhibited when fathead minnow Pimephales promelas exposed to chlorpyrifos (Olson and Christensen, 1980). The ACh.E activity inhibited when Cyprinus carpio exposed to dimethoate (Manju Tembhre and Santhosh Kumar, 1994) in various fish with different pesticides (Bhaktavatsalam and Sreenivasa Reddy, 1982) in Oreochromis exposed to phosalone (Devaraj *et al.*, 1991) also support the present work.

Conclusion:

In the present study, it was proved that the organophosphate methyl parathion 50% EC in sub-lethal and lethal concentrations had induced changes in the activity levels of AAT, ALAT, LDH and ACh.E enzymes of the freshwater snake headed fish *Channa punctatus*.

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