SUBLETHAL HAEMATOLOGICAL EFFECTS OF FENTHION ON THE FRESH WATER FISH *Cyprinus carpio.*

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The present work was conducted to evaluate the haemato-biochemical alterations such as plasma levels of glucose, lactic acid, protein, haemoglobin, along with clotting time in *Cyprinus carpio* exposed to the three different sub- lethal doses (0.38 mg/l, 0.193 mg/l & 0.096 mg/l) of fenthion for 60 days.

Blood serum of exposed and unexposed *C. carpio* was analysed to determine glucose (Folin- Wu method), lactic acid (Berker & Summerson method), protein (Lowry method) and haemoglobin contents (Acid haematin method) as well as coagulation time (Lee & White method) after 60 days test period

Sublethal exposure(0.38mg/l) of fenthion showed increase of 57% blood glucose and 34% lactic acid levels and reduced the protein and haemoglobin content to 28%. The clotting time was also reduced to 32% in the exposed fish.

In the present investigation, it was observed that stressed *C. carpio* exhibited gill damage caused by tissue hypoxia at 0.38 mg/ml. It is likely that hypoxia stimulated the increase in blood glucose and lactic acid levels for providing anaerobic energy source during fenthion stress. Decrease in protein level may be due to its degradation and its utilization for gluconeogenesis to cope with the fenthion- related high energy demand. Decrease in haemoglobin content and clotting time may be due to histo- pathological damages observed in the liver. Reduction in protein, haemoglobin content and clotting time with elevation of blood glucose and lactic acid levels in fenthion- induced fish indicates shift in metabolism to compensate the toxicity stress.

Discharge of toxic elements from industrial process, mining and agriculture developments may have detrimental effects on aquatic animals (Beijer and Jornelo 1979; Hamilton and Mehrle 1986). Pesticides cause great harm to aquatic animals including fishes. Fenthion O,- O–dimethyl–04 methyl thio.m–toyl phosphorothionate, an organo phosphate insecticide, is a contact and stomach insecticide with a useful penetration and persistent action. It is registered in 37 countries. This pesticide is used to control variety of household mosquitoes. Primarily in house hold use and agricultural crops (Cox 2000; Cong. *et .al* 2009). Similarly fenthion is toxic because it inhibit the activity of enzymes. (Fulton and Key 2001; Oruce and Usta 2007, Verma and Gupta 1976). Very few studies have demonstrated the affect of fenthion on fish (Thomson & Murthy 1976). Kitmura *et al* 2000, 2003, Leena. Gopi (1998) have performed an extensive work on effect of fenthion on the liver function of *Cyprinus carpio.* Fish form an important part of human food (Oruce and Usta, 2007). It appear to possess the same biochemical pathway to due with the toxic effects of endogenous and exogenous agent as do mammalian species.

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(Cachner 1998). Gills are the first organ to get affected by pollutants (Gallagher and Digiulio, 1992). Kidney is a major target of toxic chemically and cause permanent or temporary derangement of homeostatsis (Miller *et. al*. 2002).

Blood is a unique mirror in which are the initial processes taking place in an organism are reflected. The observation on different haematological parameters provide a good deal in diagnosing the effect of environmental stress on an animal and in fact would give an insight into changes induced in the circulating fluid. Study of haematological parameters can indicate physiological response to a contaminated environment. (Dethloft. *et. al*., 2001). Biochemical parameters can show the impact of water pollution on fish (Bucher and Hofer, 1990). *C.carpio* is an economically important fresh water fish and commonly cultured in many parts of the world. In the study, an attempt was made to investigate the toxicity on glucose, lactic acid, protein, haemoglobin and clotting time in the blood plasma of *C.carpio* exposed to different sublethal concentrations of fenthion for 60days exposure.

MATERIALS AND METHODS

Specimens of *C.carpio* were procured from a fish farm located at Arey, Mumbai, India. The length and weight ranged from 13 to 14cm and 15-20gm respectively. They were kept in aquaria for two weeks to get acclimatized to laboratory conditions. During the period of acclimations all fishes were fed with commercial fish food twice daily. The water condition like temperature, pH, dissolved oxygen and hardness analyzed weekly were 29 ± 1^0C, 7.8 ± 0.5, 6.8 ± 0.3, 232 ± 4.2 mg/l as calcium carbonate respectively.

After two weeks of acclimation ,30 aquaria each containing 30L water were stocked with ten fish per aquarium. Different concentration of fenthion (0.01, 0.1, 0.2, 0.40, 0.6, 0.8, 1.0 mg/l), purchased from Bayer India Ltd, were prepared by adding the required volume of acetone. A control set was run with the same number of fish and the same volume of acetone without adding fenthion. The experiments were then run in triplicate. The water in the aquaria was altered daily. The lethal concentration Lc50 for 24, 48, 72 and 96hr was computed by the probit method.

Acute toxicity study revealed that toxicity of fenthion does not increase with time. Therefore toxicant concentration selected were, 3/4, 1/2, 1/4, 1/8 & 1/16 of 96 hr Lc50 (0.162, 0.775, 0.387, 0.193 and 0.096mg/l). All the concentrations were prepared on the same day and parallel control group were maintained in similar way.

Fishes from the aquarium were removed after 60days of exposure during the experimental period. Blood samples were collected in heparinized vials by cutting the caudal peduncle .Collected fresh sample of blood was used for analysis .Sample of clotted blood were discarded. Haemoglobin content in the blood was estimated by acid haematin method (Hawk *et.al*.1965) coagulation time was determined by Lee White method (Dacie and Lewis 1979).

The remaining blood was centrifuged at 6000rpm for 10min at 4^oC and the collected plasma was stored at -20^oC till analyzed. The glucose, proteins and lactic acid content was assayed by Folin Wu method (Hawk *et.al.* 1965); Lowry *et.al.*(1951) and Berker and Summerson method (1941) mentioned by Hawk *et.al.* (1965).

For statistical analysis the one way analysis of variance (ANOVA) was applied to test the significance of difference among the different values. Pvalues less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Lc50 value were calculated from the mortality rate recorded for 24, 48, 72 and 96 hrs by straight line graphical interpolation method. The 95% confidence limit of Lc50 value and slope function of the line have been calculated according to the method suggested by Litchfield and Wilcoxon (1949) (Fig.1). The magnitude of toxic effects of pesticides also depend on length and weight, corporal surface/bodyweight ratio and breathing rate (Singh

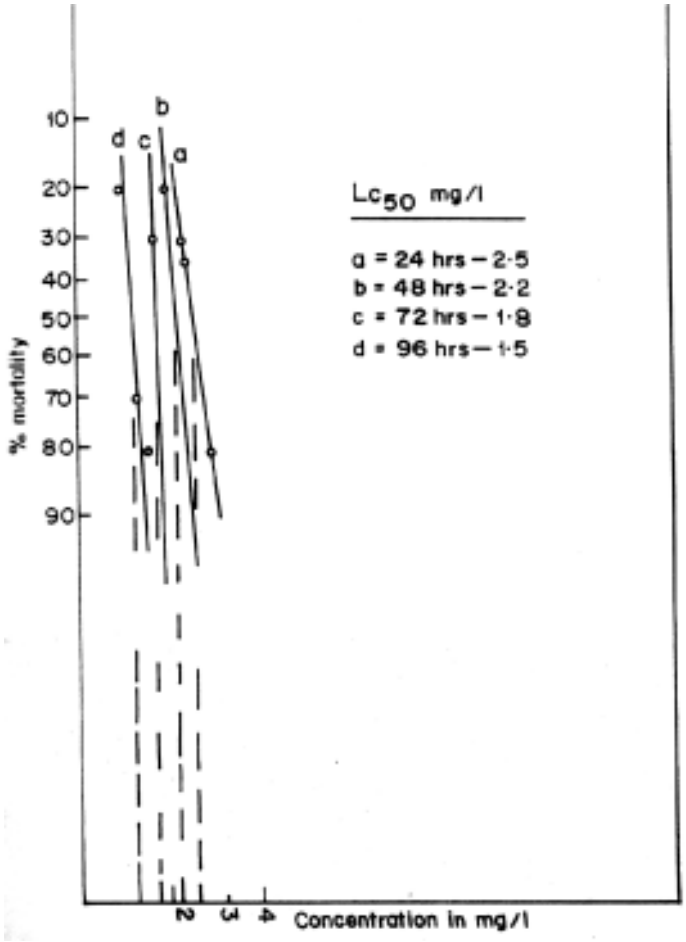

Fig 1: Graphical presentation of Lc50 values of fenthion

and Narain 1982, Murthy 1986). According to the report given by Sheela Susan Jacob *et. al* (1982) fenthion is a neurotoxic agent. Manoharran and Subbaiah 1982; Oh *et. al* 1991, Murthy (1986) reported that toxicity is due to detoxification and absorption. Toxicity varies with respect to species, size of fish and duration exposure. (Dutta *et. al.* 1995).

The blood is a unique mirror in which all the internal process taking place in an organism are reflected. The observation on different haematological parameters provide a good deal in diagnosing the effects of environmental stress on an animal and infact would give an insight in the changes induced in the circulating fluid. Some investigators have also identified changes in several haematological parameters as indicators of metal exposure. (Cyriac *et. al.* 1989). However present findings indicate that in *Cyprinus carpio* sublethal chronic exposure to fenthion altered the blood parameters.

The fish exposed to different concentration of fenthion showed increase in glucose and lactic acid and decrease in protein, haemoglobin and clotting time. Values compared to the control fish (Fig. 2). It is documented that under stress condition, fish become hyperactive perhaps to get out of the stressful medium and would require an increased amount of

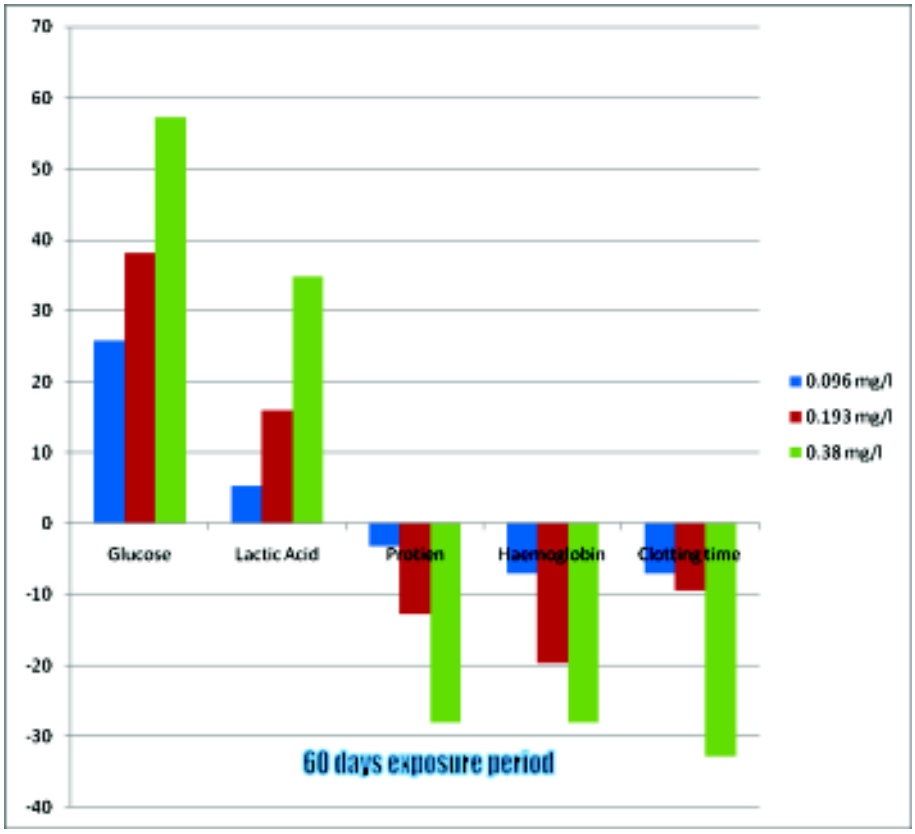

Fig 2 : Change in protein content of blood plasma of *Cyprinus carpio*

oxygen to meet their energy demand, secondly fish secrete increased amount of mucus to coat the body especially gills to get relief from the irritating pollutants. This in turn reduces the gaseous exchange through the gill. Thus an increased utilization of oxygen and reduced supply of it may cause a hypoxic condition in fish (Pandey *et. al.* 1979 and Alkahem et. al 1998). Decrease in hemoglobin values of diazinon exposed fish was reported by Bananee *et. al* (2008, 2011) and related it to the destruction of cells and / or decrease in size of cells due to the adverse effects of pesticide. Decrease in haemoglobin was due to disruption of iron synthesis machinery caused by inhibition of aerobic glycolysis in tilapia exposed to fentirothion. Signficant elevation of glucose level may be due to the breakdown of glycogen into glucose. It is a fact that stress stimuli repeat rapid secretion of glucocorticoids and catecholamines from adrenal tissues of the fish. Both hormones are known to produce hyperglycemia in fish. This condition causes glycolysis in the liver and muscles of daizinon exposed fish.

The change in the protein content in the blood plasma of *C.carpio* due to chronic exposure of fish to fenthion are presented in Fig.2. The reduction in protein content or as dependent on the concentration of fenthion. Among the three concentrations selected the highest concentration (0.38mg/l) seem to be the most highly potent and cause significant change (-28.10%) in protein level. The chary suggests the impairment of protein synthesis. Verma *et.al* 1979, Goel and Garg, 1980, Singh *et.al*.1982, Dutta *et.al*. 2003, Bucher *et.al.* 1990, reported similar decrease in protein content in blood of different species of fish when treated with different pollutants. Similar response was noted in other fresh water fish exposed to acute toxic level of pesticides (Svoboda *et. al*. 2001; Rao 2010). The pesticide may change the function of vital organs like liver and kidney disrupting the homeostatic condition of the body. Decrease in protein content may be due to their degradation and the product which may be fed to TCA cycle through amino transferese system to cope up with the fenthion stress. Secondly reduction in protein content level could also be attributed to histopathological damage to kidney leading to its impaired functioning.

Elevation in lactic acid level was due to anaerobic pathway lead by the fish to meet the increased demand for energy under pollution stress. Similar increase in lactic acid content in Heteropneustes fossils was reported by Sastry and Subhadra (1982). It is noted that fish was hyperactive and this has attributed to impaired gill function.

Disturbance in coagulative mechanism whether acquired or inherited may not only from alteration of coagulation but also from abnormal fibrinolytic activity of the plasma. Depression in coagulation time between 125 and 85 sec in saccobranchus fossils and between 140 and 95sec in *Labeo rohita* was observed after exposure to chloradane by Verma *et. al.* 1979 and Bansal. *et. al* 1979. The decrease in clotting time can be attributed to deletion in protein content and also due to histepathological damage caused to liver.

CONCLUSION

From the 96hr Lc50 recorded it can be concluded that fenthion is moderately toxic to *C.carpio*. Exposure to chronic sublethal concentration of fenthion resulted in significant haemato biochemical alterations. These changes suggest that the treated fishes are faced with severe metabolic stress. The result clearly indicate that usage of fenthion in the field

may be a threat to fishes as well as human. The fact is that fenthion is metabolized to toxic form in fish presumably has environmental and health implication for its use as pesticide.

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