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Toxic effect of carbohydrate metabolism changes on carp (*Cirrhinus mrigala*) exposed to deltamethrin (synthetic pyrethroid)

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Abstract
The present work was designed to determine toxicity on carp *Cirrhinus mrigala* exposed to Deltamethrin and the effect of carbohydrate metabolism changes observed during both lethal and sub-lethal concentration. Acute toxicity LC_{50} of Deltamethrin detected 8 µl/l and $1/10^{th}$, i.e. 0.8 µl/l of the acute toxicity selected as sub-lethal for sub-acute test concentration. The current experiment to study the impact of Deltamethrin on certain aspects of carbohydrate metabolism were studied and estimated the levels of activities of glycogen phosphorylase, glucose-6-phosphatase, LDH and SDH in the fish organs like gills, liver and kidney of the *Cirrhinus mrigala*. Exposures of the lethal and sub-lethal concentrations of Deltamethrin taken at different exposure periods were considered. The main effects were studied during experimental tenures and the results were obtained in the present study which indicates clearly that the carbohydrate metabolism is disturbed when the fish, *Cirrhinus mrigala* is exposed to Deltamethrin. The results were seen to be valuable tool that should be incorporated to a battery of biomarkers.

Keywords: *Cirrhinus mrigala*, deltamethrin, glycogen phosphorylase, glucose-6-phosphatase, LDH, SDH and biomarker

Introduction
In this present scenario the major function of carbohydrates in the metabolism is as fuel to be oxidized to provide energy for other metabolic processes. In this role carbohydrate is utilized by cell mainly in the form of glucose (Harper *et al.*, 1979) [16]. Carbohydrates of all the energy reserves are more rapidly utilized and undergo speedy exhaustion. Carbohydrates play not only a structural role in the cell but may serve as a reservoir of chemical energy to be increased and decreased according to organisms needs. The energy derived from the oxidation of carbohydrates is of prime importance for the survival of the animal. The energy resource available at the disposal of animals, carbohydrates form the chief constituents to be readily utilized. The carbohydrate metabolism is broadly divided into two segments (a) anaerobic segment or glucose through Embden-Meyerhof pathway and (b) aerobic segment which consists of oxidation pyruvate to acetyl CoA to be utilized through citric acid cycle. This is coupled with utilization and mobilization of reduced coenzymes (NADH or NADPH) for synthesis of ATP molecules through electron transport system coupled by oxidative phosphorylation (Lehninger, 1990) [27].
Energy a vital force for the biological systems, occupies a key position in the metabolic machinery. The breakdown of organic constituents, mainly carbohydrates, releases the required energy to the cell (Prosser and Brown, 1973) [40], thus the carbohydrate metabolism gained importance in the physiology of an animal where in it releases the electromagnetic energy locked up in the monosaccharide, by breaking down to simple carbon dioxide and water. The free energy liberated by the breakdown of food stuffs, as a rule is transformed into the phosphate bond energy (ATP) before its utilization (Kreb, 1954) [24]. The ATP synthesis in biological systems mainly depend on the phosphorylation of ADP associated with glycolysis and biological oxidation involving the citric acid cycle and electron transport system (Lehninger, 1990) [27]. Any perturbations in these cycles lead to alterations in the energy budget of the cell (Prosser and Brown, 1973) [40]. Pollutants affect not only the rate of carbon flow in a given metabolic pathway, but also the contribution of different metabolic pathways

to the total metabolism of an animal. Impairment of carbohydrate metabolism has been observed in a variety of physiological disorders and pathological conditions (Harper *et al.*, 1979) [16]. This may prove to be of negative survival value for the affected organisms. Investigations were conducted earlier on carbohydrate metabolism during pathological conditions in different animals following exposure to various pesticides (Love, 1980) [28], (Al-Kahem, 1993) [2].

Glucose is the principal sugar in blood of fishes, serving the tissues as a major metabolic fuel. Besides yielding energy through glycolysis and TCA cycle, pentose sugars are also formed in the hexose monophosphate shunt from glucose, which are important constituents of nucleotides, nucleic acids and many coenzymes. In general glucose level in the blood of circulating fluid is maintained in an animal through active absorption of glucose from the digested food stuffs, and also is formed from glycogen, amino acids and glycerol through glycogenolysis and gluconeogenesis under certain stress conditions. In several fishes, blood glucose level has been correlated to their level of activity and hence to their level of metabolism. There are evidences that in fish blood glucose level shows most striking alterations in response to the change in environmental factors (Umminger, 1975) [50], (Hattinght, 1977) [17]. The levels of it may even be affected under toxic stress, which reflects the variations in the entire carbohydrate metabolism (Tewari *et al.*, 1987) [49]. Glycogen, commonly called as animal starch, is the main storage polysaccharide and a great source for blood glucose. Maintenance of glycogen reserves is one of the important features of the normal metabolism (Mong and Polland, 1981) [34]. Alterations in liver and Kidney glycogen under situations of stress have been reported, and a significant depletion in tissue glycogen is said to reflect the state of strenuous activity on the part of the fish (Tewari et al., 1987) [49].

Glycogen phosphorylase is an enzyme that exists in two forms, one activated with adenosine monophosphate (AMP) and the other activated without it, which are inter-convertable by phosphorylation and dephosphorylation mechanism (Cori and Cori, 1945) [8], (Cori et al., 1955) [9], (Majewski and Giles, 1981) [31]. This, being an enzyme concerned with the metabolic breakdown of glycogen, assumes considerable importance in studies involving liver and kidney glycogen levels. In addition to phosphorylase activity in the liver, glucose-6-phosphatase acts as a catalyst in the conversion of glucosc-6-phosphatc to glucose. Hydrolysis of glucosc-6-phosphate is a key step in the process of gluconeogenesis. During stress conditions the alterations in blood glucose and glycogen levels in liver and kidney in fishes affect the rate of activity of these two enzymes. The studies of (Macleod, 1960) [30] brought to light some fundamental but significant facts regarding glycolysis and glycolytic enzymes in fishes. Those studies not only establish the presence of lactate dehydrogenase in a number of fish tissues such as heart kidney, skeletal kidney, liver and kidney but also indicated that the activity of glycolytic enzymes, except the hexokinase and phosphofructokinase, which in the skeletal kidney is 10-100 times higher than that in the other tissues. The studies of (Martin and Tarr, 1960) [33] on fish tissues indicated that the entire machinery for the rapid breakdown of glycogen to lactate is present in the skeletal Kidney as well as in the other organs of fish. Succinate dehydrogenase is one of the important key enzymes, and exhibits an important function in energetics. Succinate dehydrogenase (SDH), a flavin-linked enzyme of Kreb's cycle catalyses the reversible oxidation of succinate to fumarate and serves as a link between electron transport system and oxidative phosphorylation. This enzyme is localized at the inner surface of the mitochondrial membrane, and contains FAD as the prosthetic group (Singer, *et al.*, 1973) [45]. Hence; the activity of it can be taken to reflect the rate of operation of TCA cycle in different organs of the fish under situations of stress.

Reports are available on the effects of heavy metals on the activities of glycogen phosphorylase, glucose-6-phosphatase, LDH and SDH in freshwater fishes. (Hinton *et al.*, 1973) [19] Observed a decrease in SDH activity in liver and kidney of the fish *Ictalurus punctatus* after exposure to acute doses of methyl mercuric chloride. (Norbonne *et al.*, 1975) [38] Reported decrease in liver glycogen and an increase in blood glucose levels in *Cyprinus carpio* on exposure to lead nitrate. In rainbow trout *Salmo gairdneri*, dorsal white muscle glycogen level decrease on exposure to the lethal concentration of zinc (Hodson, 1976) [20]. (Larsson *et al.*, 1976) [25], (Larsson, 1977) [26] while working on the effects of cadmium on some hematological and biochemical aspects of freshwater fishes reported that cadmium caused anemia and phyperglycermia in those teleosts. A decrease in glycogen level of liver and muscle was reported in *Heteropneustes fossilis* subjected to mercury intoxication (Quayyum and Shaffi, 1977) [41]. (Christensen *et al.*, 1977) [7] Observed an increase in LDH activity and a decrease in plasma glucose in the brook trout *Salvelinus fontinalis* exposed to 0.06 to 6.35 µg/l of cadmium chloride. (Benerjee *et al.*, 1978) [5] Have shown impairment of the carbohydrate metabolism in *Clarias batrachus* and *Tilapia mossambica* exposed to cadmium. (Dokholyan and Akhmedova, 1978) [11] Have reported a decrease in blood glucose level in the Caspian roach *Rutilus rutilus* treated with 5 and 10 µg/l of mercuric chloride. Decrease in glucose-6-phosphatase activity was also reported in *Clarias batrachus* exposed to cadmium (Benerjee *et al.*, 1978) [5] and in *Channa punctatus* exposed to mercury (Sastry and Gupta, 1979) [44]. (Helmy *et al.*, 1979) [18] Observed hypoglycemia in fish Liza macrolepis exposed to lead nitratrate and also the Cadmium chloride exposed shows toxic effect on fish by (Shivaraj *et al.*, 2018) [54].

The above cited literature clearly reflects the impact of heavy metal on some aspects of carbohydrate metabolism in different species of fishes. Reports on the effect of Deltamethrin (Synthetic Pyrethroid) on carbohydrate metabolism of a freshwater fish taking the size, concentration of the Deltamethrin and period of exposure put together as one aspect, in order to understand the possible shifts in the energetics of the fish are not available. Hence, an attempt is made in the present investigation to study the influence of Deltamethrin on certain aspects of carbohydrate metabolism by estimating the activities of glycogen phosphorylase, glucose-6-phosphatase, LDH and SDH in the organs of the freshwater fish, *Cirrhinus mrigala* following exposure to the lethal and sub-lethal concentrations of Deltamethrin taking different exposure periods into consideration.

Materials and methods

Biological Species collection, maintenance and acute toxicity test

The biological test system *Cirrhinus mrigala* weighing about 5 ± 2 g and measuring an average length of 4-5 cm were collected from the local fish farm Dharwad, Karnataka India and these fishes were kept in large aquarium. The experimental fishes were acclimatized to laboratory

conditions for 15 days in tap water whose physico-chemical characters were examined by following the international guideline method, American Public Health Association (APHA, 2005) [3]. Water media was renewed every day and maintained a 12-12 h photoperiod during acclimatization and test periods. The fishes were fed regularly with commercially available fish food pellets during acclimatization and test tenures but feeding was stopped two days prior to treatment to test medium for acute toxicity test. The commercial grade deltamethrin (Decis, 30%EC) was obtained from Bayer Crop Science, India Ltd., Gujarat, India. The Quantity of deltamethrin at a concentration of (3 µl/l to 9 µl/l) was prepared and exposed to ten fish per concentration along with 20 L of water for each concentration with control replicates and (LC_{50}) of deltamethrin was found to be 8 µl/l. The LC_{50} value at (24h, 48h, 72h & 96 hours) was determined by following the method of (Finney, 1971) [13]. Based on the results of LC_{50}, the fishes were exposed sub-lethal concentrations for (1, 5, 10 and 15 days) One tenth ($1/10^{th}$, i.e. 0.8 µl/l) of the acute toxicity value was selected as the sub-lethal concentration for sub-acute test. The concentrations of the test compound used in short term definitive tests were between the highest concentration at which there was 0% mortality and the lowest concentration at which there was 100% mortality.

Carbohydrate metabolism estimations

Estimation of glycogen phosphorylase (l-4-glucanorthophosphate glucosyl transferase, e.c. 2.4.1.1) activity in the fish

Phosphorylase activity in the tissues of the fish was estimated using; the method described by (Sutherland, 1955) [48]. Homogenates (5%) was prepared in 0.1 M sodium fluoride solution (pH 6.5). The homogenate was centrifuged at 1500 rpm for 15 min, thus extracting the enzyme into the supernatant. The supernatant was diluted four times with the cold sodium fluoride solution. The incubation mixture consisted of 0.2 ml of 2% glycogen and 4 ml of diluted enzyme. This mixture was incubated at 37 °C and the reaction was started by adding 0.2 ml of 0.016 M glucose-6-phosphates and 0.004 M adenosine-5-monophosphate (1:1 ratio). The reaction was arrested after 15 min by adding 5 ml of 10% trichloroacetic acid. A blank was also run similarly. Finally, the inorganic phosphates liberated were estimated by the method of (Fiske and Subba Rao, 1925) [14]. For this, to an aliquot of the above reaction mixture 4.5 ml of 0.44% ammonium molybdate and 0.2 ml of I-amino-2-naphthol-4-sulphonic acid (ANSA) were added. The contents were mixed well and heated in boiling water for 10 min. After cooling the volume was made to 10 ml with distilled water and the colour developed was measured in a spectrophotometer at a wavelength of 660 nm and the activity expressed as µM Pi liberated/mg protein/h, using phosphate standards.

Estimation of glucose-6-phosphatase (e.c. 3.1.3.9) activity

Glucose-6-phosphatase activity in the tissues of fish was estimated using the method of (Young, 1970) [53]. A 5% homogenate (w/v) was prepared in 0.25 M ice-cold sucrose solution. 0.5 ml of 0.2 M tris-malate buffer, 0.2 ml of 0.05 M glucose-6-phosphate and 0.2 ml of distilled water were incubated at 37 °C for 5 min and to this 0.1 ml of homogenate was added and incubated exactly for 10 min. The reaction was stopped by adding 1.0 ml of 10% trichloroacetic acid. A blank was also run similarly. Finally the inorganic phosphates liberated were estimated by the method of (Fiske and Subba Rao, 1925) [14] at a wavelength of 660 nm and the activity is expressed as µM Pi liberated/mg protein/h.

Estimation of succinate dehydrogenase (succinate acceptor oxido reductase, e.c. 1.3.99.1) activity (SDH)

Succinate dehydrogenase activity in the organs was estimated using the colorimetric method of (Nacholas *et al.*, 1960) [36]. A 5% homogenate (w/v) was prepared in 0.25 M ice cold sucrose solution, centrifuged at 3000 rpm for 10 min and the supernatant was taken as the source of enzyme. The incubation mixture consisted of 0.2 ml of 0.4 M phosphate buffer (pH, 7.7), 0.2 ml of 0.2 M sodium succinate, 0.1 ml of 0.004 M 2-(p-indophenol)-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT), 0.1 ml of 0.005 M phenazine methosulphate and 0.5 ml of 5% enzyme preparation. The mixture was incubated at 37 °C for 30 min and the reaction was stopped by adding 6.0 ml of glacial acetic acid. The formazon formed was extracted into 6.0 ml of toluene overnight at 50 °C and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 495 nm. A blank taking 0.5 ml of distilled water and control taking 0.5 ml of boiled enzyme were also run similarly. INT standards were prepared alongside for comparison. The enzyme activity is expressed as µM formazon/mg protein/h.

Estimation of lactate dehydrogenase (1 - lactate nad oxide reductase e.c. 1.1.27) activity (LDH)

Lactate dehydrogenase activity in the organs was estimated using the method of (Srikanthan and Krishnamoorthi, 1955) [46] as modified by (Govindappa and Swami, 1965) [15]. A 5% homogenate (w/v) was prepared in 0.25 M ice cold sucrose solution, centrifuged at 2500 rpm for 15 min and the supernatant was taken as the source of enzyme. The incubation mixture consisted of 1.0 ml of 0.4 M phosphate buffer (pH 7.4), 0.5 ml of M lithium lactate, 0.1 ml of 0.0001 M. nicotinamide adenine dinucleotide (NAD), and 0.1 ml of 0.004 M 2-(p-indophenol)-3-p-nilrophenyl-5-phenyltetrazotium chloride (INT) and 0.5 ml of 5% enzymes preparation. The mixture was incubated at 37 °C for 30 min and then the reaction was stopped by adding 6.0 ml of glacial acetic acid. The formazon formed was extracted into 6 ml of toluene overnight at 50 °C. The optical density colour developed was measured in a. spectrophotometer at a wavelength of 495 nm, A blank taking 0.5 ml of distilled water and control by taking 0.5 ml of boiled enzyme were also run similarly. INT standards were prepared alongside for comparison. The enzyme activity is expressed as µM formazon/mg protein/h.

Statistical Data Analysis

Each experiment was repeated six times and the mean value was calculated. The data obtained are analysed statistically by following Duncan's multiple range test (Duncan, 1955) [12].

Results

Glycogen phosphorylase and glucose-6-phosphatase

Glycogen phosphorylase and Glucosc-6-phosphatase (liver only) activity in the fish, *Cirrhinus mrigala* under median lethal concentration of deltamethrin are presented in Figure 1 and Figure 2. Among the exposure periods increase in the activities of glycogen phosphorylase and glucose-6-phosphatase of fish was observed. Glycogen phosphorylase

activity was less at day 1 (gill -1.101, kidney 6.591 and liver 20.137), more at 4th day (gill 5.234, kidney 21.451 and liver 49.215). Under sub-lethal concentration increasing trend in the activity of both glycogen phosphorylase and glucose-6-phosphatase.was observed up to day 15. Maximum activity of glycogen phosphorylase was noticed in kidney on day 15 (13.125) and minimum (-0.231) activity was observed in liver on day 1. Glucose-6-Phosphatase activity in liver depicted maximum increase of 40.934% noticed on day 10 and minimum increase (2.397%) was observed on day 1.

Succinate dehydrogenase (SDH) activity

From the data presented in Figure 3 it is seen that, SDH activity decreased significantly in the gill, kidney and liver of the fish, at all the exposure levels studied in the median lethal concentration of deltamethrin. In sub-lethal concentration also a significant decrease was observed in the SDH activity (Figure 3). Among the four exposure periods of the median lethal concentration, the decrease in SDH activity in the organs of the fish was less at day 1 (gill-27.246%, kidney -16.229% more at 4th day (gill -83.623%, kidney -38.196% and liver -35.258%) with significant difference in between, in the order 1 < 2 < 3 < 4 days. In sub-lethal concentration the decrease was less at day 1 (gill -10.000%, kidney -9.672% and liver -13.373%) and more at 10th day (gill -22.898%, kidney -16.229% and liver -22.998%) in the organs of fish in the order 1 < 5 < 10 > 15, with significant difference (P > 0.05) difference between 1 to 15 days. The difference among the organs was in the order; gill > kidney > liver.

Lactate dehydrogenase (LDH) activity

From the data presented in the Figure 4, it is seen that LDH activity significantly increased in gill, kidney and liver of the fish at 1, 2, 3 and 4 days of exposure to the median lethal concentration of deltamethrin. In sub-lethal concentration also LDH activity increased in the organs of the fish in all the exposure periods (Figure 4). However, this increase was relatively less compared to the increase in the median lethal concentration. Among the exposure periods in the organs of the fish exposed to the median lethal concentration was in the order 1 < 2 < 3 < 4 days. In the sub-lethal concentration the increased LDH activity at 1st day progressed further at 15 days. Thus the increase was in the order 1 > 5 > 10 > 15 days. Based on the percent values it is observed that the decrease in the LDH activity differed in degree in the organs studied. However, these differences were not much and also not consistent among the exposure periods.

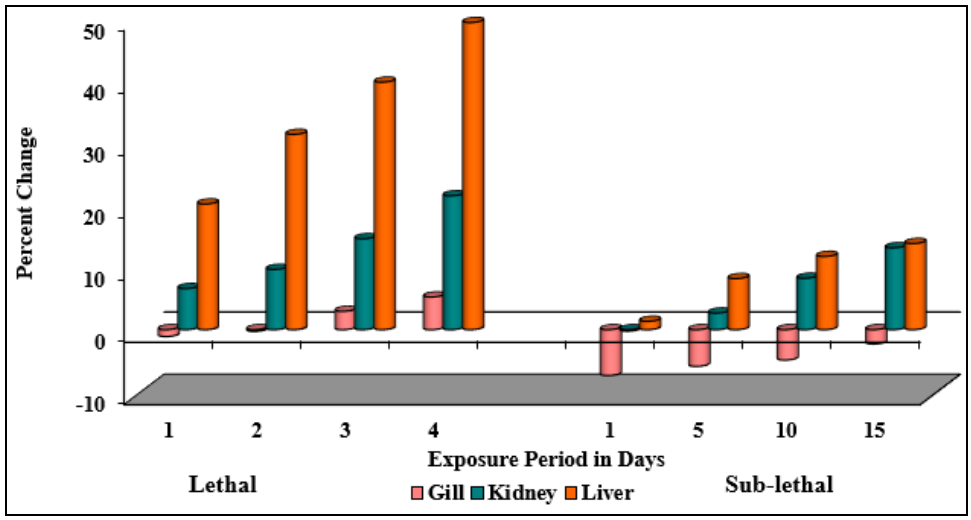

Fig 1: Per cent change over control in glycogen phosphorylase activity (µM of Pi formed/mg protein/h) in the tissues of fish, *Cirrhinus mrigala* on exposure to the lethal and sub-lethal concentrations of deltamethrin.

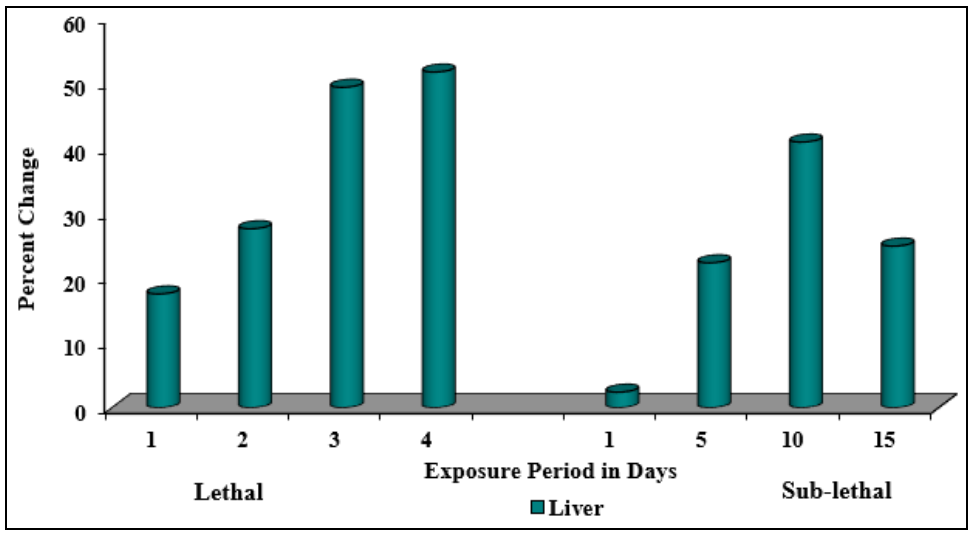

Fig 2: Per cent change over control in glucose-6-Phosphatase activity (mM of Pi formed/mg protein/h) in the fish, *Cirrhinus mrigala* on exposure to the lethal and sub-lethal concentrations of deltamethrin.

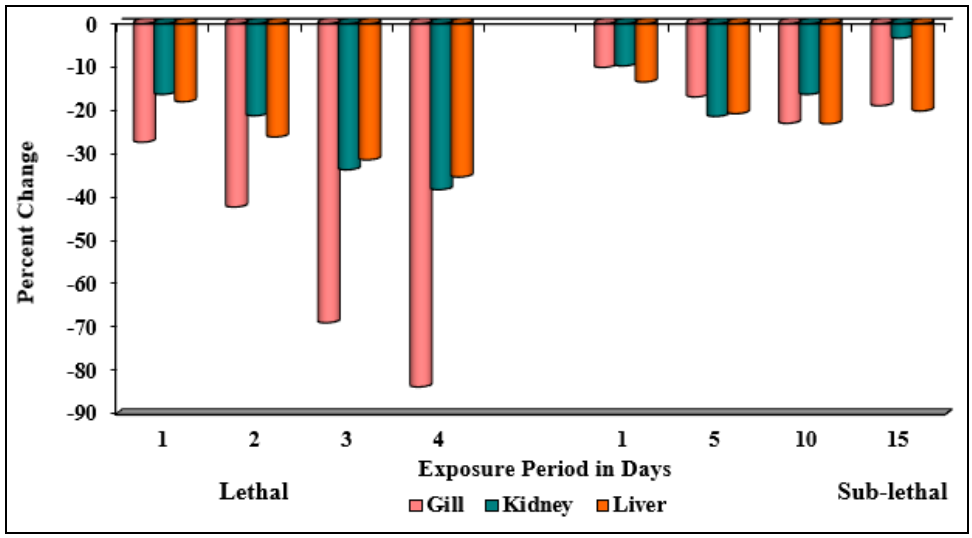

Fig 3: Per cent change over control in succinate dehydrogenase activity (µM formazon/mg protein/h) in the tissues of fish, *Cirrhinus mrigala* on exposure to the lethal and sub-lethal concentrations of deltamethrin.

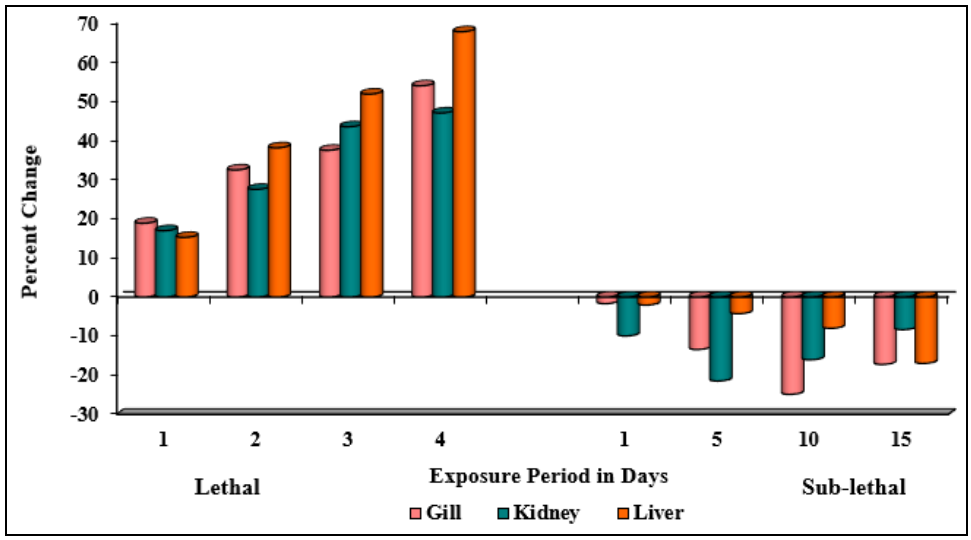

Fig 4: Per cent change over control in lactate dehydrogenase activity (µM formazon/mg protein/h) in the tissues of fish, *Cirrhinus mrigala* on exposure to the lethal and sub-lethal concentrations of deltamethrin.

Discussion

Carbohydrates of all the energy reserves are most rapidly utilized and thus undergo speedy depletion. The main product of carbohydrate, digestion is glucose and its role in the intermediate metabolism of fishes has been reviewed (Holcombe *et al.*, 1982) [21]. Changes in carbohydrate metabolism are to meet the changing energy demands which can be expected in animals exposed to stress. The result obtained in the present study clearly indicates that the carbohydrate metabolism is disturbed when fish, *Cirrhinus mrigala* is exposed to deltamethrin. The hyperglycemic condition observed in fish exposed to fenvalerate may also be due to the stimulation of a variety of chemical substances released from the neuro-endocrine system. There are reports on the occurrence of hyperglycemic condition in animals exposed to a variety of toxicants by the stimulation. Hyperglycemic hormone which increase the degradation of glycogen by the activation of phosphorylase enzymes (Dhavale and Masurekar, 1986) [10]. In addition, the hyperglycemic condition can also be attributed to serve as anaerobic stress on the fish imposed by the metal (Samuel and Sastry, 1989) [43].

The significant increase in glucose level and depletion in glycogen content of the fish due to glycogenolysis, either through the hormonal imbalance and/or the other influencing factors results in primarily the depletion of carbohydrate energy reserves of *Cirrhinus mrigala* on exposure to acute concentrations of deltamethrin. Further, increased depletion of the carbohydrate energy reserves, over time of exposure from day 1 to day 4, is a clear indication of the high metabolic imbalance and failure of metabolic homeostasis. This is possible in tissues. wherein exposure might have impaired greatly the neuro-endocrinal coordinative centers thereby leading to a continuous breakdown of glycogen reserves in liver, Kidney and gill of the fish by improper stimulation of phosphorylase enzymatic machinery.

In sub-lethal concentrations also the fish initially exhibited significant glycogenolysis, which indicates that cadmium even at sub-acute concentrations could affect the energy reserves with the influence of factors responsible for it. However, the lesser degree of glycogenolysis compared to that observed in the lethal concentrations suggests that the rate of glycogenolysis is concentration dependent. Further, the decrease in glucose level along with the decrease in glycogen content and increase in the activities of phosphorylase and glucose-6-phosphatase in the fish suggest the diversion of

glucose for energy requirements more or less proportionate to the breakdown of glycogen content. Thus the utilization of glucose seems to be more at 1 day of exposure as energy is required to face the chronic toxic stress. Later, the gradual regression in utilization of glucose and in the breakdown of glycogen indicates the development of resistance in the fish under sub-lethal toxic stress.

The raise in the glycogen phosphorylase and glueose-6-phosphatase activities in the fish, *Cirrhinus mrigala* might have been accorded by the inhibitory effect of the pesticide on the release of insulin, are due to the direct effect on pancreas and this reported the cypermethrin induced significant increase in the activities of phosphorylase activity. It is well documented that, during stress conditions fish secrete and catecholamines (Nakato and Tomlinson, 1967) [37] in large amounts which cause rapid hyperglycemia. Numerous results observed in fish (Srivastava and Mishra, 1982) [47] models during metal toxicosis indicate glycogenolysis and hyperglycemia by activating the phosphorylase enzyme system. Elevation of phosphorylase can be taken as a meaningful biochemical indicator of metal toxicosis, as elevation was also observed in several animal models. (Koundinya and Ramamurthi, 1979) [23] reported hyperglycemia accompanied by decrease in the level of glycogen in liver and kidney of the fish, *Sarotherodon mosambica* exposed to sumithion and suggested that hepatic glycogen is the main source of blood glucose, have observed the depletion of liver and kidney glycogen and increase in blood sugar due to methyl parathion consequent to the increased secretion of catecholamines in Indian cat fish, *Heteropneustes fossilis*. It is possible that the increased glycogenolytic activity and/or gluconeogenesis may be indicated not only through hormonal change but also through neuromuscular changes (Lowejinde and Nimmi, 1984) [29].

In the present study corresponding to the decrease in the rate of oxygen consumption, the decrease in SDH activity in the gills, kidney and liver of fish exposed to lethal concentrations of fenvalerate suggests the suppression of oxidative phosphorylation of the animal (Akhilender Naidu *et al*., 1984) [1]. Though the reserve carbohydrates are mobilised, the fish could not utilise the release of energy due to the inhibition of oxidative metabolism, hence, glucose utilization decreases thereby the hyperglycemic condition resulted in the fish under acute cadmium stress and reported decreased SDH activity in tissues of rats administered to parathion. This could be attributed to decreased oxygen uptake (Moorthy *et al.*, 1985) [35] and pyruvate mobilization during methyl parathion stress. Several investigations linked enzymes to the changes in integrity of mitochondria as a consequence of methyl parathion exposure. Interestingly the increase in LDH activity in the organs of fish exposed to lethal concentration of fenvalerate indicate that the fish under fenvalerate stress might have relieved on anaerobic glycolysis in meeting the energy requirements. Similar observations have been reported by (Rangaswamy and Padmanabha, 1999) [42] in *Tilapia mossambica* under endosulfan toxicity. (Asztalos and Nemcsok, 1985) [4] correlated the magnitude of increase in LDH activity in the carp due to the tissue damage and organ necrosis. Similar reasons have been reported by (Margarat *et al.*, 2001) [32] in mice treated with penicillamine and turine on mercury poisoned mice. Elevated activity of LDH was also reported in workers occupationally exposed to DDT, (Kossman and Wartal Sha, 1984) [22] and in rat liver LDH activity increased upon dieldrin treatment. (Usha Rani and Ramamurthi, 1987) [51] Reported that sub-lethal concentration of cadmium increased the LDH activity, the decrease in SDH and elevation in LDH activities could lead to the accumulation or pyruvate and lactate in the organs or fish. However, the increase in lactate level and decrease in pyruvate level could indicate the activation of pyruvate. So a part of pyruvate accumulated might have been converted into lactate by anaerobic glycolysis for energy requirements as evidenced by the increased LDH activities and lactate levels. The decreased levels of pyruvate and increased levels of lactate indicate the predominance of anaerobic glycolysis under heavy metals stress. These imply its utilization through Kreb's cycle in energy requirement and also indicate reduction in further oxidation of pyruvate in the citric acid cycle. Similar trend has been reported in mice treated with BHC (Philip *et al.*, 1991) [39].

In contrary to the changes observed in lethal concentrations starting with an initial decrease in the whole animal oxygen consumption and SDH activity at day 1, elevation in these parameters at 5, 10, 15 days in the organs of fish exposed to sub-lethal concentration of deltamethrin indicate their activity in overcoming the sub-acute toxic stress. It is possible that the less concentration of deltamethrin accumulated in the organs of fish could not competitively inhibit the enzyme activity. As more energy is required for elimination and/or for enhanced protein synthetic potentials. The oxidative metabolic cycle is elevated in the fish on prolonged exposure to sub-lethal concentration, which is also evidenced from the increased glycogenolysis and decreased glucose level. The initial suppression of oxidative metabolism and subsequent elevation as observed in anaerobic glycolysis May be in order to compensate the energy demands. However, with the raise in oxidative metabolism the anaerobic glycolysis slowly regressed and reached normalcy by 15 days. This type of adaptation and acclimation was observed in the fishes on exposure to the sub-lethal concentrations of metals (Buckley, 1982) [6], Vijayaram *et, al.*, 1989) [52].

Conclusion

In this present investigation to study the influence of deltamethrin on certain aspects of Carbohydrate metabolism by estimating the levels of activities of glycogen phosphorylase, glucose-6-phosphatase, LDH and SDH in the fish organs like gills, liver and kidney of the *Cirrhinus mrigala*. With the following results were seen to be valuable tool that should be incorporated to a battery of biomarkers to maximize the confidence with which ecotoxicologists and environmental toxicologists assess impacts of sub-lethal pollution in the aquatic environment. It is evident from the results that the deltamethrin can be rated as extremely toxic to the aquatic species and terribly affected responses of *Cirrhinus mrigala* fishes in both lethal & sub-lethal concentrations. Therefore clearly suggests the species response to deltamethrin and it is an evidence to carry the experiment further.

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