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Toxic effect of biochemical and morphological changes on carp (*Cyprinus carpio*) exposed to cadmium chloride

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Abstract
The present work was designed to determine toxicity on carp *Cyprinus carpio* exposed to Cadmium Chloride and the effect of biochemical and morphological changes observed during both lethal and sublethal concentration. Acute toxicity LC_{50} of Cadmium Chloride detected 14mg/L and 1/5th, i.e. 2.8 mg/L of the acute toxicity selected as sub lethal for sub-acute test concentration. The present investigation to study the influence of Cadmium Chloride on certain aspects of Carbohydrate metabolism by estimating the levels of Blood glucose (BG), Glycogen (GLY), Lactate (LAC) and Pyruvate (PYR) in the organs of the fish, and the morphological changes like Caudal bending, Caudal region becomes thin, this was the main affect were studied during experimental tenures. The results were seen to be valuable tool that should be incorporated to a battery of biomarkers.

Keywords: carp, cadmium chloride, carbohydrate metabolism, caudal bending and biomarker

Introduction

The present studies determine toxicity on carp *Cyprinus carpio* exposed to Cadmium Chloride and the effect of Carbohydrate metabolism. 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) [18]. Carbohydrates of all the energy reserves are more rapidly utilized and undergo speedy reduction. 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 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 nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) for synthesis of adenosine triphosphate (ATP) molecules through electron transport system coupled by oxidative phosphorylation (Lehninger 1990) [25]. Glucose is the principal sugar in blood of fishes, serving the tissues as a major metabolic fuel. Besides yielding energy through glycolysis and tricarboxylic acid (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. There are evidences that in fish blood glucose level shows most striking alterations in response to the change in environmental factors (Umminger 1975; Hattinght 1977) [41, 20]. The levels of it may even be affected under toxic stress, which reflects the variations in the entire carbohydrate metabolism (Tewari *et al.* 1987) [40]. 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) [28]. 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) [40]. 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) [26] brought to light some fundamental but significant facts regarding glycolysis and glycolytic enzymes in fishes. Oxidation of pyruvate in Kreb's cycle under aerobic conditions produces more energy but it requires oxygen. If required oxygen is available, pyruvate enters the tricarboxylic acid (TCA) cycle after decarboxylation to Acetyl coenzyme A (Acetyl CoA), the reaction is catalyzed by pyruvate dehydrogenase complex in the presence of thiamine diphosphate. A number of reports are available on the presence of tricarboxylic acid (TCA) cycle enzymes in fish tissues (Anthony and Munro 1964; Tarr 1969; Hodson P.V 1976) [3, 39, 21]. The effects of heavy metals on the glucose, glycogen, pyruvate and lactate levels, rate of oxygen consumption in freshwater fishes. A decrease in pyruvate and an increase in lactate levels were observed in the muscle and liver of rainbow trout *Salmo gairdneri* on exposure to different concentrations of zinc and hypoxia (Burton *et al.* 1972) [7]. (Norbonne *et al.* 1975) [30] reported decrease in liver glycogen and an increase in blood glucose levels in *Cyprinus carpio* on exposure to lead nitrate. These Cadmium Chlorides (heavy metal) are available in the water and are further added into aquatic ecosystem as a result of direct input of atmospheric deposition, leaching of mineral

and soil erosion due to rain water which causes the hazardous effects on aquatic biota majorly fishes and other minute aquatic organisms. Organisms to adjust external and internal stimuli in order to meet the challenges of surviving in the changing environment criteria, whereas morphological changes are external changes caused due to distorted environment. Heavy metal pollution of aquatic ecosystem poses a serious environmental hazard because of their persistence and toxicity (Joshi 2011) [24]. This wide lacuna in the field of cadmium toxicity on freshwater fishes prompted to take up this investigation to access the toxic effect of Cadmium Chloride on Carbohydrate metabolism in freshwater fish, *Cyprinus carpio*. Since, *Cyprinus carpio* is sufficiently available in the freshwater tanks and ponds in and around tumakuru. Therefore the current investigation is carried on this species.

Materials and methods

Biological Species Collection, Maintenance and Acute Toxicity Test

The biological test system *Cyprinus carpio* weighing about 5 ± 2 g and measuring an average length of 4-5 cm were collected from the State Fisheries Department, Tumkur, 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 analyzed by following the international guideline method, American Public Health Association (APHA 2005) [1]. 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. Cadmium Chloride about 95% purity was procured from local market of Bangalore, Karnataka, India, under the trade name Thermo Fisher Scientific India Pvt Ltd., Supplied by Vasa Scientific Co., Bangalore, Karnataka, India. The Quantity of Cadmium Chloride at a concentration of (9 mg/L, 11mg/L, 14mg/L, 18 mg/L and 22 mg/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 Cadmium Chloride was found to be 14 mg/L. The LC_{50} value at (24h, 48h, 72h & 96 hours) was determined by following the method of (Finney 1971). Based on the results of LC_{50}, the fishes were exposed sublethal concentrations for (1, 5, 10 and 15 days) One fifth ($1/5^{th}$, i.e. 2.8 mg/L) of the acute toxicity value was selected as the sublethal concentration for subacute 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

Blood Glucose estimation

Glucose in the samples was determined by colorimetric method as described by (Nelson and Somogyi 1952) [29]. 0.1 ml of blood was collected, to it 3.9 ml of deproteinizing solution (5% zinc sulphate and 0.3 N sodium hydroxide in 1:1 ratio) was added and the mixture was centrifuged at 3000 rpm for 10 min. To 1 ml of the supernatant from each of these mixtures 1 ml of alkaline copper reagent was added, shaken vigorously and heated in a boiling water bath exactly for 20 min. Then it was cooled and 1 ml of arsenomolybdate colour reagent was added. Entire solution was made to 10 ml with distilled water and the absorbance was measured in a spectrophotometer at a wavelength of 540 nm. A blank and glucose standards were also run simultaneously. Glucose content was expressed as mg of glucose/100ml of blood.

Glycogen estimation

Glycogen content in the tissues of fish was estimated using the anthrone reagent method as described by (Caroll *et al.* 1956) [8]. Since glycogen concentration in tissues is known to vary in different regions of body (Amano *et al.* 1953; Fraser *et al.* 1966) [2, 15]. Hence, care was taken in dissecting out this sample from the same region of body of fishes i.e. the anterodorsolateral region of the trunk. The organs were digested with 3 ml of hot 30% potassium hydroxide (Hassid and Abraham 1957) [19]. The digest was cooled and 3.75 ml of absolute ethanol was added to it. The entire mixture was kept overnight in a refrigerator. Then the mixture was centrifuged for 15 min at 2500 rpm. Supernatant was decanted and 10 ml of warm distilled water was added to the residue to dissolve the precipitated glycogen. To 0.2 ml of this 1.8 ml of distilled water and 0.5 ml of 2% enthrone reagent dissolved in 72% concentrated sulphuric acid were added and heated in a boiling water bath exactly for 10 min. The mixture was cooled and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 620 nm. A blank and glucose standards were also run similarly. The glycogen content is expressed as mg of glycogen/g wet wt. of the organ.

Lactate estimation

Lactate in the organs was estimated using the method of (Barker and Summerson 1941) [5] as modified by (Huckabee 1961). A 5% homogenate (w/v) was prepared in cold 10% trichloroacetic acid and centrifuged at 3000 rpm for 15 min. The supernatant was used for the estimation of lactate. To 1.0 ml of supernatant, 1.0 ml of 20% copper sulphate was added and the mixture was made to 10 ml with distilled water. Then 1 g of powdered calcium hydroxide was added, shaken vigorously and kept it for an hour at room temperature with intermittent shaking. Later the contents were centrifuged at 3000 rpm for 10 min and to 1.0 ml of the supernatant 0.5 ml of 4% copper sulphate was added followed by 6.0 ml of concentrated sulphuric acid. The contents were mixed by lateral shaking, kept in boiling water bath for exactly 6.5 min and cooled. When the contents were sufficiently cooled, 0.1 ml of 1.5% p-hydrophenyl (prepared in 5% of sodium hydroxide) was added and the precipitate formed was kept at laboratory temperature for 30 min. Then the contents were placed in boiling water bath for 1.5 min, cooled and the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 560 nm against reagent blank. Lactate standards were prepared alongside for comparison. The lactate content is expressed as mg lactate/g wet wt. of the organ.

Pyruvate estimation

Pyruvate in the organs of fish was estimated using the method of (Friedman and Haugen 1942) [16]. A 5% homogenates (w/v) were prepared in 10% trichloroacetic acid and centrifuged at 3000 rpm for 15 min. The supernatant was used for the estimation of pyruvate. 1 ml of supernatant was taken and to it 1.0 ml 0.001 M 2, 4-dinitrophenyl hydrazine and 3 ml of 0.4 N sodium hydroxide were added. After 10 min the optical density of the colour developed was measured in a spectrophotometer at a wavelength of 540 nm against the reagent blanks. Pyruvate standards were prepared alongside for comparison. The pyruvate content in the organs is expressed as mg pyruvate/g wet wt. of the organ.

Morphological deformities observed

In this experiment sublethal concentration of Cadmium Chloride exposed to fish after long periods of study 1, 5, 10 and 15 days, in these periods of time interval we observed morphological deformities on fish in caudal region.

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

Blood glucose (BG):

From the data presented in table 1 and it is seen that under median lethal concentration of Cadmium Chloride relative to control, blood glucose level increased in all exposure periods. Minimum increase over control was observed on day 1 and day 4 witnessed maximum percent increase (58.385%), in the order 1 < 2 < 3 < 4 days. Among the sublethal concentration the percent change in the level of blood glucose level showed an increasing trend up to day 10, with maximum percent increase of over control on day 10. While day 15 showed declines in blood glucose level when compared to day 10. Day 1 and 15 under sublethal exposure witnessed least glucose concentration. Mean percent change was observed under sublethal concentrations of Cadmium Chloride.

Glycogen (GLY):

Glycogen level in all the three tissues (Gill, Kidney and Liver) showed continuous decrease under median lethal concentration of Cadmium Chloride at all the periods of exposure. Lowest glycogen level in the fish was observed on 4th day of exposure in gill tissue, while highest quantity of glycogen (27.545 mg/g wet wt.) was observed in liver on day 1.From the data presented in table 2 and a decrease in the concentration of glycogen was observed in the gill, kidney and liver of fish exposed to sublethal concentration of Cadmium Chloride. The decrease was continuous up to day 10, while day 15 onwards a recovery trend was observed.

Lactate (LAC):

From the data presented in table 3 and it is seen that the lactate level significantly (P > 0.05) increased in the gill, kidney and liver, at all the exposure periods studied in median lethal concentration of Cadmium Chloride. These levels also increased in the organs of fish exposed to the sublethal concentration of Cadmium Chloride but the degree of increase was less compared to the increase in the median lethal concentration. Among the exposure periods the increase in lactate in the tissues of fish exposed to the lethal concentration the lactate level increased over time of exposure in the order 1 < 2 < 3 < 4 days. Whereas in sublethal concentrations the increase in lactate level at day 1 progressed up to 15 days in the order 1 < 5 < 10 > 15 days.

Pyruvate (PYR):

From the data presented in table 4 and it is seen that the pyruvate level decreased significantly in the gill, kidney and liver of the fish in ail the exposure periods in the median lethal concentration. Pyruvate level decreased in the organs of fish at all the periods of exposure in the sublethal concentration also. But, this decrease was relatively less compared to the decrease in the median lethal concentration. Among the exposure periods the decrease in pyruvate level in the organs of the fish exposed to the sublethal concentration, the decrease in pyruvate level increased over the time of exposure in the order 1 > 5 > 10 > 15 days.

Table 1: Levels of blood glucose (mg/100 ml) in the blood of fish, *Cyprinus carpio* on exposure to the lethal and sublethal concentrations of Cadmium Chloride.

Estimations	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Liver	71.030 I	84.510 D	89.560 C	97.890 B	112.501 A	74.650 H	79.158 F	81.101 E	77.701 G
± SD	0.0258	0.654	0.087	0.089	0.0974	0.0036	0.096	0.425	0.826
% Change	----	18.977	26.087	37.815	58.385	5.096	11.443	14.178	9.391

Means are ± SD (n = 6) for a parameter in a row followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's multiple range test.

Table 2: Glycogen levels (mg/g wet wt.) in the tissues of fish, *Cyprinus carpio* on exposure to the lethal and sublethal concentrations of Cadmium Chloride.

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	1.898 A	1.871 E	1.844 G	1.812 H	1.781 I	1.912 B	1.899 D	1.853 F	1.879 C
± SD	0.0991	0.0654	0.0555	0.4564	0.0004	0.0225	0.0039	0.044	0.0068

% Change	----	-1.422	-2.845	-4.531	-6.164	0.737	0.052	-2.370	-1.001
Kidney	5.548 A	5.261 E	4.873 G	4.384 H	3.780 I	5.501 B	5.389 D	5.164 F	5.423 C
± SD	0.0004	0.0003	0.0005	0.0006	0.0004	0.069	0.0253	0.0562	0.0004
% Change	----	-5.173	-12.166	-20.980	-31.867	-0.847	-2.865	-6.921	-2.253
Liver	30.874 A	27.545 F	25.891 G	21.997 H	19.412 i	30.121 B	29.801 D	27.987 E	29.681 C
± SD	0.078	0.0254	0.039	0.0036	0.0555	0.0874	0.0925	0.0689	0.0258
% Change	----	-10.782	-16.139	-28.752	-37.125	-2.438	-3.475	-9.350	-3.864

Means are ± SD (n = 6) for a parameter in a row followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan's multiple range test.

Table 3: Lactate levels (mg/g wet wt.) in the tissues of fish, *Cyprinus carpio* on exposure to the lethal and sublethal concentrations of Cadmium Chloride.

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	3.008 I	3.789 G	4.649 E	4.738 D	5.550 A	3.238 H	4.009 F	4.748 C	5.007 B
± SD	0.098	0.874	0.546	0.369	0.26	0.028	0.814	0.098	0.36
% Change	----	25.964	54.554	57.513	84.507	7.646	33.277	57.845	66.456
Kidney	7.748 I	8.110 G	8.950 E	9.784 B	9.987 A	8.038 H	8.896 F	9.201 D	9.611 C
± SD	0.03	0.099	0.075	0.096	0.001	0.556	0.701	0.256	0.084
% Change	----	4.672	15.513	26.277	28.897	3.742	14.816	18.753	24.044
Liver	11.350 I	12.970 G	14.850 C	15.874 B	17.018 A	12.030 H	12.971 F	13.489 E	14.518 D
± SD	0.001	0.006	0.069	0.258	0.036	0.087	0.058	0.032	0.254
% Change	----	14.273	30.837	39.859	49.938	5.991	14.281	18.845	27.911

Means are ± SD (n = 6) for a parameter in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

Table 4: Pyruvate levels (mg/g wet wt.) in the tissues of fish, *Cyprinus carpio* on exposure to the lethal and sublethal concentrations of Cadmium Chloride.

Tissue	Control	Exposure periods in days							
		Lethal				Sublethal			
		1	2	3	4	1	5	10	15
Gill	0.193 A	0.152 D	0.111 G	0.121 H	0.092 I	0.192 B	0.188 C	0.147 E	0.119 F
± SD	0.098	0.055	0.546	0.369	0.222	0.021	0.014	0.085	0.074
% Change	----	-21.243	-42.487	-37.305	-52.331	-0.518	-2.590	-23.834	-38.341
Kidney	0.117 A	0.107 C	0.091 E	0.079 H	0.049 I	0.113 B	0.101 D	0.091 F	0.0845 G
± SD	0.047	0.089	0.069	0.08	0.015	0.569	0.789	0.256	0.074
% Change	----	-8.547	-22.222	-32.478	-58.119	-3.418	-13.675	-22.222	-27.777
Liver	0.180 A	0.165 B	0.155 E	0.131 H	0.122 I	0.131 C	0.161 D	0.138 F	0.142 G
± SD	0.001	0.006	0.005	0.003	0.003	0.002	0.003	0.002	0.002
% Change	----	-8.333	-13.888	-27.222	-32.222	-27.222	-10.555	-23.333	-21.111

Means are ± SD (n = 6) for a parameter in a row followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

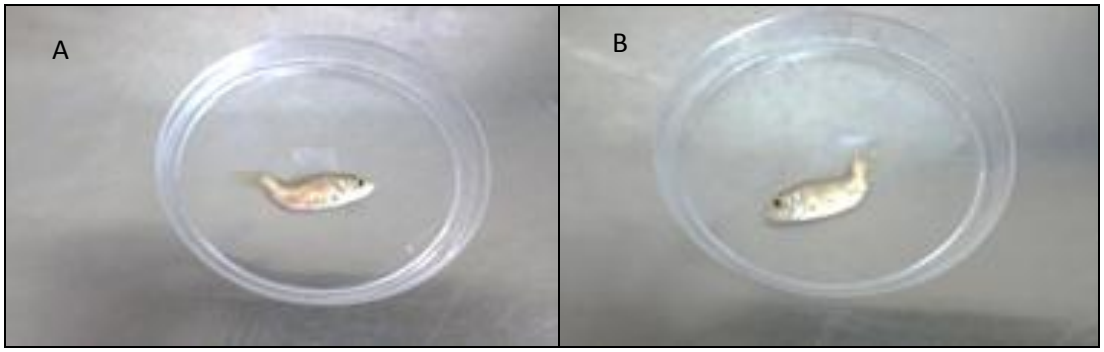

Fig 1: Morphological deformities: **A:** Caudal bending, **B:** Caudal region becomes thin

Caudal bending and Caudal region becomes thin; fig. 1(a) & (b) indicates and it was noticed in the toxicant concentration with time and persisted even under recovery tenure, which heavily retarded the normal swimming pattern. Thus Cadmium Chloride reduced instinctive behavioural response and affected morphological features and being under stress in

sublethal exposure periods was observed.

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) [22]. 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, *Cyprinus carpio* is exposed to Cadmium Chloride. The blood glucose level varies in fishes from one species to another in proportion to the levels of activity and hence to their levels of metabolism (Vernberg and Gray 1953) [42]. Sluggish fish like *Laphius discatorlus* with low rate of metabolism have low level of blood glucose, whereas more active fish with high rate of metabolism have high level of blood glucose (Fukaka 1958) [17]. This has made to assume that the blood glucose level indicates the level of carbohydrate metabolism in fish. The elevation in blood glucose level in fish, *Cyprinus carpio* exposed to lethal and sublethal concentration of Cadmium Chloride indicated the stepping up of carbohydrate metabolism during toxic stress. This hyperglycemic condition may be due to stepping up of glycogenolysis or gluconeogenesis or both. The 1 stepping up of glycogenolysis am evident by the fact that the increase blood glucose is accompanied by a decrease in glycogen content in *Cyprinus carpio*.

Earlier (Samuel and Sastry 1989) [33] showed hyperglycemic condition and decrease in the glycogen content of liver and Kidney tissue in *Channa punctatus* under monocrotophos toxicity. Similar increase in blood glucose level was also reported under sublethal concentration of diazinon in the same species (Sharma 1978). Cadmium is known to increase glucose concentration in the haemolymph of honey bees (Bounias *et al.* 1985). (Mohamed 1988) reported that small to moderate increase in blood glucose at the end of the treatment period with fenvalerate and decamethrin respectively, in albino rats. Decrease in the utilization of glucose for energy yielding purpose may also elevate its level in the blood. This is well manifest in the fish exposed to lethal and sublethal concentrations of Cadmium Chloride in the present investigation. High level of blood glucose may also be due to channeling of the glucose from tissue to blood. Numerous researchers using fish as models (Sastry and Siddiqui 1982) during metal toxicity, observed glycogenolysis and hyperglycemia by activating the phosphorylase enzyme system (Bakthavathsalam 1980). Hence the elevation of blood glucose level in the fish exposed to fenvalerate may be mostly due to the imbalance in pancreatic hormones involved in carbohydrate metabolism as suggested by (Diwan *et al.* 1979). 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). 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) [33]. In the present study the decrement of the glycogen was observed in different tissues of fish, *Cirrhinus mrigala* exposed to lethal and sublethal concentration of fenvalerate. In support of this, (Sivaprasada Rao and Ramana Rao 1979) [36] reported a decrease in glycogen content in the tissues of fish, *Tilapia mossambica* exposed to methyl parathion. (Sridevi 1991) [37] also observed the decrement of glycogen with corresponding increase in blood glucose level in the fish, *Labeo rohita* under cypermethrin stress. The decrease in glycogen content in the tissue of experimental fish suggests its utilization by anaerobic glycolysis, perhaps, to meet the energy warranted by the toxic environment. (Edwards 1973) [13] has reported that the synthesis and utilization of glycogen is altered during pesticide stress. Depletion of glycogen might be due to prevalence of hypoxic or anoxic conditions which normally increase glycogen use (Dezwan 1971) [9]. (Srinivasa Moorthy 1982) [38] reported anoxic conditions in Kidney exposed to methyl parathion.

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 cadmium. 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 sublethal concentrations also the fish initially exhibited significant glycogenolysis, which indicates that cadmium even at subacute 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 sublethal toxic stress.

In the present study the fish could not utilise the release of energy due to the inhibition of oxidative metabolism, hence, glucose utilization decreases thereby the hyperglycaemic condition resulted in the fish under acute cadmium stress. Pyruvate mobilization during methyl parathion stress. Several investigations linked enzymes to the changes in integrity of mitochondria as a consequence of methyl parathion exposure. 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 and 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 benzene hexa chloride (BHC), (Philip *et al.* 1991) [31]. An excess secretion of mucous in fish forms a non-specific response against toxicants, thereby probably reducing toxicant contact. It also forms a barrier between the body and the toxic medium, so as to minimize its irritating effect, or to scavenge it through epidermal mucous. Similar observations were made by (Rao 2006) [32] following Remotely Piloted Research Vehicle (RPR-V) exposure to euryhaline fish, *Oreochromis mossambicus.* Leaning of fish indicates reduced feeding behaviour and diversion of fish metabolism towards adaptability to the toxic media. Feeding preferences were affected and consumption of food in fish was impaired and reduced. This was noticeable even under recovery tenure. For these species, it might be profitable to decrease their food uptake under toxic environmental conditions to lower the energetic costs of digestion. A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations.

Conclusion

In this present investigation to study the influence of Cadmium Chloride on certain aspects of Carbohydrate metabolism by estimating the levels of Blood Glucose, Glycogen, Lactate and Pyruvate 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 sublethal pollution in the aquatic environment. It is evident from the results that the $CdCl_2$ can be rated as extremely toxic to the aquatic species and terribly affected responses of *Cyprinus carpio* fishes in both lethal & sublethal concentrations. Therefore clearly suggests the species response to Cadmium Chloride and it is an evidence to carry the experiment further.

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