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PROTECTIVE EFFICACY OF L-ASCORBIC ACID AGAINST THE TOXICITY OF MERCURY IN *LABEO ROHITA* (HAMILTON)

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Mercury

L-Ascorbic acid

Protein

Carbohydrate

Lipid.

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ABSTRACT

The common edible fish *Labeo rohita* (Hamilton) was exposed to different concentrations of $HgCl_2$ separately and in combination with identical concentrations of L-Ascorbic acid for 24, 48, 72 and 96 hr and the muscle was studied for biochemical changes. In the first set of experiment, the maximum decrease in the protein, lipid and carbohydrate content were found to be 76.32%, 82.97% and 88.88% at 96 hr of exposure at 0.08 ppm of mercury alone respectively. When the fishes were exposed to different concentrations of $HgCl_2$ in combination with L-Ascorbic acid at different interval time; the biochemical parameters were found to be increased over mercury treated test species.The maximum increase in protein, lipid and carbohydrate content were found to be 162.29% at 96 hr, 165.48% at 72 hr, and 134.04% at 48 hr. of exposure at 0.08 ppm of mercury along with 300 ppm of L-Ascorbic acid respectively. A parallel behavioural and morphological study also revealed that the same trend and it may be concluded that mercury is highly toxic and L-Ascorbic acid modifies the abnormal behavioural and morphological characteristics as well as protects the reduction of various biochemical parameters in *Labeo rohita*.

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INTRODUCTION

Mercury enters the tissues by direct uptake through gill, skin and by ingestion of contaminated food. As a result of human activities, mercury levels have increased four fold in river sediment from lake and estuaries (Das *et al.,* 1982). During the past 100 years, it has been estimated that more than 5, 00,000 metric tons of mercury entered the atmosphere, hydrosphere and surface soil with eventual deposition in surface soil and sediments (Das *et al.,* 1982). It comes from weathering process of earth crust, industrial discharge, pest and disease control agent applied to plants, surface runoff, mining, soil erosion, sewage effluent (Mitchel, 1972). Das *et al.,* (1988) have reported accumulation of mercury in different tissues of fishes of river Ib. The trend of accumulation in order is gill > kidney > liver (Das and Kaviraj, 1992). Many researchers have found geographic variability in mercury concentration among various commercially important fish and shellfish species. For example, various tuna species caught from Atlantic, Pacific and Mediterranean Oceans have mercury residues (Adams, 2004). Signs of chronic mercury poisoning in fish include emaciation due to appetite loss, brain lesions, cataract, diminished response to change in light intensity, inability to capture food, abnormal motor coordination and various erratic behaviour (Hawryshyn *et al.,* 1982). Mercury at comparatively low concentration adversely affects reproduction, growth, behaviour, metabolism, blood chemistry, osmoregulation and oxygen exchange of marine and fresh water organisms. The paper reports on the behavioral and morphological studies in test fishes exposed to different sub-lethal concentrations of mercury in respect to biochemical changes (total protein, lipid and carbohydrate content).The possible protective role of L – Ascorbic acid against the toxicity of mercury in fish has also been studied.

MATERIAL AND METHODS

Fish, *Labeo rohita* (Rohu) having the length of 10-15 cm and weight of 10-12 g were collected from the nearest commercial fish breeding farm at Chiplima and they were acclimatized to the laboratory condition for 10 days. The physiochemical parameters of the water were analyzed and the values were DO 10mg/ L, temperature 17.9^{0}C, pH 7.36, conductivity 1.61 mmho/cm and TDS 0.671 mg/L. To maintain the proper oxygen concentration, water was aerated continuously and also hydrilla plants were put in the aquarium to maintain the natural habitat. The fish were fed with commercially available food. The water along with excess food and faecal materials were removed from the aquarium in every four days. The 96 hr LC_{50} of Mercuric chloride, following the standard protocol (Brown, 1972) was determined by graphical method and the value was 0.18 ppm. After determining the 96 hour LC_{50} dose; irrespective of sex the fishes were divided into 11 groups of 20 each with an average weight of 10-12 gms. One of the these groups was maintained as control where as the rest groups were exposed separately to 0.01, 0.02, 0.04 and 0.08 ppm of Hg in water as $HgCl_2$ and 200, 300 ppm of L-Ascorbic acid and identical concentration of L-Ascorbic acid in combination with 0.01, 0.02, 0.04 and 0.08 ppm of mercury for 24, 48, 72 and 96 hr. Fishes were fed regularly during the tenure of the experiment. Three replications of each dose were maintained for the estimation of Protein content in white muscles by Lowry *et al.,* (1951), Lipid content in white muscles by Folch *et al.,* (1957) and Carbohydrate content in white muscle by Anthrone method (Samseifter *et al.,* 1949).In the mean time the fishes were observed regularly for the study of behavioral and morphological changes.

RESULTS

Behavioural and Morphological Changes

To study the behavioural and morphological changes, the fishes were exposed to 0.01, 0.02, 0.04 and 0.08 ppm of mercury for an exposure period of 24, 48, 72 and 96 hr. Following treatment the fishes showed exciting and agonistic behaviour and symptoms of restlessness. The opercular movement became erratic, which may be due to difficulties in normal respiration. They released air bubbles through

Table 1: showing percent increase over zero concentration in Protein, Lipid and Carbohydrate content (gm/gm dry muscle tissue) of *Labeo rohita* exposed to different concentrations of L-Ascorbic acid in different exposure periods

Exposure duration in hr	Different concentrations of L-Ascorbic acid in ppm 0			200			300		
	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate
24	0.74	0.41	0.33	0.741	0.413	0.343	0.744	0.416	0.35
	±0.13	±0.5	±0.07	±0.14	±0.08	±0.013	±0.08	±0.008	±0.21
				0.135%	0.73%	3.93%	0.540%	1.46%	6.06%
48	0.75	0.429	0.34	0.754	0.444	0.348	0.756	0.51	0.351
	±0.019	±0.08	±0.02	±0.2	±0.15	±0.008	±0.01	±0.02	±0.09
				0.533%	3.49%	2.35%	0.8%	18.88%	3.23%
72	0.754	0.43	0.34	0.774	0.452	0.35	0.80	0.536	0.37
	±0.17	±0.06	±0.28	±0.31	±0.01	±0.08	±0.45	±0.008	±0.09
				2.652%	5.11%	2.94%	6.1%	24.65%	8.82%
96	0.771	0.453	0.36	0.787	0.478	0.36	0.813	0.563	0.39
	±0.012	±0.25	±0.014	±0.017	±0.01	±0.014	±0.37	±0.01	±0.05
				2.075%	5.96%	0%	5.44%	24.28%	8.33%

Table 2: showing percent decrease over zero concentration in protein, Lipid and Carbohydrate content (gm/gm dry muscle tissue) of *Labeo rohita* exposed to different sub lethal concentrations of mercury in different exposure periods

Exposure duration in hr.	Different concentrations of Mercury in ppm 0			0.01			0.02			0.04			0.08		
	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate
24	0.743	0.42	0.333	0.663	0.38	0.256	0.563	0.363	0.22	0.3873	0.30	0.116	0.286	0.243	0.08
	±0.012	±0.008	±0.017	±0.26	±0.008	±0.008	±0.008	±0.006	±0.09	±0.005	±0.008	±0.014	±0.006	±0.006	±0.016
				10.76%	9.52%	24.92%	24.22%	13.57%	33.93%	48.45%	28.57%	65.16%	61.5%	42.14%	75.97%
48	0.746	0.44	0.34	0.606	0.37	0.24	0.512	0.34	0.197	0.38	0.28	0.099	0.277	0.17	0.047
	±0.013	±0.006	±0.024	±0.19	±0.006	±0.016	±0.016	±0.008	±0.06	±0.012	±0.21	±0.006	±0.08	±0.008	±0.016
				18.76%	15.9%	29.41%	31.36%	22.72%	42.05%	49.06%	36.36%	70.88%	62.86%	61.36%	86.17%
72	0.753	0.46	0.34	0.573	0.303	0.176	0.413	0.30	0.12	0.32	0.23	0.07	0.213	0.113	0.04
	±0.017	±0.014	±0.008	±0.05	±0.006	±0.016	±0.013	±0.006	±0.18	±0.01	±0.33	±0.008	±0.08	±0.008	±0.016
				23.9%	34.13%	48.23%	45.15%	34.78%	64.70%	57.1%	50%	79.41%	71.71%	75.43%	86.47%
96	0.773	0.47	0.36	0.53	0.26	0.15	0.39	0.236	0.09	0.26	0.20	0.06	0.183	0.08	0.04
	0±0.014	±0.008	±0.014	±0.013	±0.008	±0.02	±0.09	±0.008	±0.41	±0.016	±0.008	±0.006	±0.008	±0.016	±0.006
				31.43%	44.68%	58.33%	49.54%	49.78%	75%	65.2%	57.44%	83.33%	76.32%	82.97%	88.88%

Table 3: showing the effect of L-Ascorbic acid in Protein, Lipid and Carbohydrate content (g/g dry muscle tissue) of *Labeo rohita* exposed to different sublethal concentrations of mercury in different exposure periods

Exposure duration in hr.	Different conc. of mercury and L-Ascorbic acid in ppm											
	Hg (0.01) + vitamin C (300)			Hg (0.02) + vitamin C (300)			Hg (0.04) + vitamin C (300)			Hg (0.08) + vitamin C (300)		
	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate
24	0.67	0.39	0.256	0.576	0.37	0.223	0.39	0.31	0.14	0.316	0.29	0.096
	±0.06	±0.009	±0.14	±0.09	±0.006	0±0.008	±0.008	±0.008	±0.008	±0.008	±0.005	±0.008
48	0.61	0.40	0.24	0.653	0.37	0.20	0.403	0.32	0.20	0.34	0.213	0.11
	±0.008	±0.008	±0.04	±0.013	±0.19	±0.016	±0.07	±0.01	±0.01	±0.12	±0.005	±0.014
72	0.636	0.426	0.22	0.60	0.38	0.19	0.44	0.32	0.113	0.397	0.30	0.05
	±0.05	±0.014	±0.016	±0.008	±0.13	±0.006	±0.014	±0.008	±0.57	±0.01	±0.01	±0.19
96	0.68	0.443	0.173	0.60	0.406	0.183	0.503	0.403	0.11	0.48	0.20	0.047
	±0.008	±0.06	±0.02	±0.008	±0.05	±0.09	±0.01	±0.01	±0.016	±0.02	±0.008	±0.21

mouth. Their hyperactivity was more pronounced in higher concentrations that too in earlier phase of exposure. The swimming pattern of the treated fishes also became erratic. A large scale pigmental disturbance leading to colour change was observed. The colour seen was usually pale and dull. There was excessive mucous secretion, which formed a thick coating over the body of the fishes even at lower concentrations.

During the second phase of experiment when the fishes were exposed to 0.01, 0.02, 0.04 and 0.08 ppm of mercury along with 300 ppm of L-Ascorbic acid for different exposure period of 24, 48, 72 and 96 hr, their behavioural and morphological changes were markedly reduced to some extent. There hyperactivity was noticed during later period of exposure *i*.e. at 72 to 96 hr. The swimming patterns of the fishes were found to be normal. Also there was normal opercular movement and pigmentation.

Effect of L-Ascorbic acid in Protein, Lipid and Carbohydrate content of *Labeo rohita*

In order to find out the effective dose of L-Ascorbic acid in protein, lipid and carbohydrate content the fishes were treated with 200 and 300 ppm of L-Ascorbic acid for different exposure periods and then the biochemical parameters were estimated.

From the result it was observed that the percent increase in protein content (g/g dry muscle tissue) over zero concentration of L-Ascorbic acid at 200 and 300 ppm was 0.135% and 0.540% for 24 hr. of exposure. After 48 hr. of observation the protein content increased upto 0.533% and 0.8%. After 72 hr. of exposure the increase was 2.652% and 6.1% and at 96 hour the increase was recorded as 2.075% and 5.44% for 200 and 300 ppm of L-Ascorbic acid respectively. (Table 1). When the data were analyzed by Two-way analysis of variance they are found to be significantly difference for exposure duration ($F_1 = 11.11$) and there was no significant difference in concentrations ($F_2 = 4.66$) at $p \leq 0.05$ level of significance.

The percent increase in lipid content (g/g dry muscle tissue) at 200 and 300 ppm of L-Ascorbic acid over zero concentration was 0.73% and 1.46% for 24 hr. of exposure. Similarly after 48 hr the increase was found to be 3.49% and 18.88%, after 72 hr. the lipid content was increased to 5.11% and 24.65% and finally after 96 hr. of exposure duration the percent increase was found to be 5.96% and 24.28% respectively (Table 1). When the data were subjected to Two way analysis of variance they were found to be statistically significant ($F_1 = 5.83$, $F_2 = 9.74$) at $p \leq 0.05$ level.

The percent increase in carbohydrate content (gm/gm dry muscle tissue) at 200 and 300 ppm of L-Ascorbic acid over zero concentration was found to be 3.93% and 6.06% for 24 hr.of observation. After 48 hr. the percent increase was found to be 2.35% and 3.23%, at 72 hr. observation the increase was 2.94% and 8.82% and after 96 hr the percent increase was recorded as 0% and 8.33% respectively (Table-

1). Two-way analysis of variance test revealed a significantly difference between concentration as well as hours of exposure ($F_1 = 10.71$, $F_2 = 12.01$) at $p \leq 0.05$ level.

The fishes exposed to various sublethal concentrations of mercury were sacrificed and white muscles were collected to estimate the total protein, lipid and carbohydrate content at different hours of exposure duration. In the second phase the fishes were then exposed to different sublethal concentrations of mercury along with 300 ppm of L-Ascorbic acid and protein, lipid, carbohydrate content were estimated to know the protective efficacy of L-Ascorbic acid, against the toxic effect of mercury, if any.

In the first set of experiment a drastic reduction was found in the protein content of *Labeo rohita*. The protein content (g/g dry muscle tissue) of *Labeo rohita* at zero concentration was 0.74 ± 0.012, 0.74 ± 0.013, 0.75 ± 0.017 and 0.77 ± 0.014 during 24, 48, 72 and 96 hr of observation. At 0.01, 0.02, 0.04 and 0.08 ppm, there was a decrease of about 10.76%, 24.22%, 48.45% and 61.5% for 24 hr of exposure. Similarly, for the same concentrations after 48 hr of exposure the percent reduction was found to be 18.76%, 31.36%, 49.06% and 62.88%. After 72 hr the decrease was observed to be 23.9%, 45.15%, 57.1% and 71.71%. Finally after 96 hr. of exposure the decrease was 31.43%, 49.54%, 65.2% and 76.32% for 0.01, 0.02, 0.04 and 0.08 ppm of mercury respectively (Table 2). Two way ANOVA test showed a significant difference between the exposure duration and different concentrations of mercury at $p \leq 0.05$ level ($F_1 = 7.63$, $F_2 = 124.76$).

In the second phase of the experiment, the fishes were treated with 0.01, 0.02, 0.04 and 0.08 ppm of mercury along with 300 ppm of L-Ascorbic acid and biochemical parameters were observed. From the observation it was found that the protein content at 0.01, 0.02, 0.04 and 0.08 ppm of mercury along with 300 ppm of L-Ascorbic acid increased to 1.05%, 2.30%, 1.82% and 10.48% from their respective control set. Similarly after 48 hr. it showed an increase of 0.66%, 27.53%, 6.05% and 22.74%. After 72 hr of exposure the percent increase was noticed as 10.99%, 45.27%, 36.22% and 86.38% and after 96 hr. it was found to be 28.30%, 53.84%, 93.46% and 162.29% from their control set (Table 3). Two-way analysis of variance showed no significant difference for positive control (0.01 ppm of mercury) and experimental set (0.01 ppm of mercury + 300 ppm of L-Ascorbic acid) ($F_1 = 0.79$, $F_2 = 2.68$). Similarly for 0.02 ppm ($F_1 = 1.00$, $F_2 = 9.80$), 0.04 ppm ($F_1 = 1.00$, $F_2 = 9.80$) and 0.08 ppm ($F_1 = 0.05$, $F_2 = 5.44$) along with 300 ppm L-Ascorbic acid no significant difference were observed at p d" 0.05 level.

When the muscle were subjected for the estimation of lipid (g/g dry muscle tissue) at various sublethal concentrations for different exposure period a gradual decrease was noticed. From the observation the lipid content of *Labeo rohita* at zero concentration was found to be 0.42 ± 0.008, 0.44 ± 0.006, 0.46 ± 0.014, 0.47 ± 0.008 for 24, 48, 72 and 96 hr. of exposure duration. At 0.01, 0.02, 0.04 and 0.08 ppm the percent decrease in lipid content were found to be 9.52%, 13.57%, 28.57% and 42.14% for 24 hour of exposure. Similarly for the same concentrations after 48 hour the decrease was 15.9%, 22.72%, 35.36% and 61.36%. After 72 hr. of exposure duration the decrease was noticed as 34.13%, 34.78%, 50% and 75.43%. From the observation after 96 hr. the decrease in lipid content was found to be 44.68%, 49.78%, 57.44% and 82.97% respectively (Table 2). Two-way analysis of variance showed significant difference at p d" 0.05 level ($F_1 = 5.81$, $F_2 = 33.78$).

After 24 hr. of exposure duration the percent increase in lipid content were recorded as 2.63%, 1.92%, 3.33% and 19.34% from their respective control set. For the same concentrations after a period of 48 hr. the increase was noticed as 8.1%, 8.82%, 14.28% and 25.29%. After 72 hr of observation the increase were found to be 40.59%, 26.66%, 39.13% and 165.48% and finally at 96 hour of exposure duration the percent increase in lipid content were 70.38%, 72.03%, 101.5% and 150% from their respective control set (Table 3). There was no significant difference between exposure periods where as significant difference was observed for concentrations for positive control (0.01 ppm of mercury) and experimental set (mercury + 300 ppm of L-Ascorbic acid) ($F_1 = 3.45$, $F_2 = 10.98$). Similarly for 0.02 ppm ($F_1 = 0.29$, $F_2 = 3.94$), 0.04 ppm ($F_1 = 0.10$, $F_2 = 4.08$) and 0.08 ppm ($F_1 = 2.31$, $F_2 = 8.42$) no

significant difference were observed at $p \leq 0.05$ level of significance so far as exposure periods and concentrations is concerned. There was a decrease in carbohydrate content (g/g dry muscle tissue) exposed to various sublethal concentrations of mercury over zero concentration in different exposure periods. The carbohydrate content of *Labeo rohita* at zero concentration was found to be 0.33 ± 0.017, 0.34 ± 0.024, 0.34 ± 0.008 and 0.36 ± 0.014 for 24, 48, 72 and 96 hr of observation. At 0.01, 0.02, 0.04 and 0.08 ppm the percent decrease was recorded as 24.92%, 33.93%, 65.16% and 75.97% respectively for 24 hour of exposure. For the same concentrations after 48 hour of exposure the decrease was noticed as 29.49%, 42.05%, 70.88% and 86.17%. Similarly the decrease was found to be 48.23%, 64.70%, 79.41% and 86.07% after 72 hr. of exposure period and after 96 hr the percent decrease was 58.33%, 75%, 83.33% and 88.88% respectively (Table 2).Two-way analysis of variance reveals that the difference in carbohydrate content was significant between concentration and exposure time at d″ 0.05 level ($F_1 = 4.88$, $F_2 = 63.27$).

After 24 hr. of observation the carbohydrate content was increased to 2.4%, 1.36%, 20.68% and 20% from their respective control set. For the same concentrations after 48 hr. of exposure the increase was 0%, 1.52%, 102.02% and 134.04%. Similarly after 72 hr. of duration the percent increase was 25%, 58.33%, 61.42% and 25% and after 96 hr.the increase was observed to be 15.33%, 103.3%, 83.33% and 17.5% from their respective control set (Table 3). Two-way analysis of variance showed a significant difference between exposure period ($F_1 = 17.81$) where as there was no significant difference between concentration ($F_2 = 3.41$) at 0.01 ppm Hg. Similarly for 0.02 ppm there was no significant difference ($F_1 = 2.84$, $F_2 = 3.33$) for both exposure period and concentration, for 0.04 ppm concentration the trend followed was similar to that of 0.01 ppm concentration of mercury and L-Ascorbic acid ($F_1 = 3.45$, $F_2 = 10.9$). Finally at 0.08 ppm no significant difference was observed ($F_1 = 3.02$, $F_2 = 3.33$) at d″ 0.05 level of significance.

DISCUSSION

96 Hour LC_{50}

The death of fishes occurred during acute toxicity test may be due to protein denaturation, impaired glucose metabolism, alteration in membrane permeability and active transport due to toxic effect of metal. The LC_{50} value can be influenced by many factors. The factors may be as follows: Fishes are poikilothermic animals. Hence change in temperature may change the degree of toxicity (Mallat, 1985). The pH of the medium also plays an important role in determining the rate of toxicity of toxicants to fishes. Zinc found to be most toxic to *Tilapia mossambicus* at pH 7 (Mukhopadhyaya and Konar, 1988). Species variation is an important factor. It is an established fact that sensitivity to toxicants differs from species to species. Hardness of water also influences the lethal toxicity in fishes. Some toxicants are highly toxic to fish in distilled water and soft water, where as some toxicant are much less toxic in hard water and seawater.

Behavioural and Morphological Changes

Behavioural and morphological alterations as noted in the course of investigation were also noticed by many workers under heavy metals exposure (Bengtsson, 1974). The violent movement and loss of equilibrium may be due to accumulation of acetylcholine at nerve ends and neuromast organ causing disruption in synthetic transmission of nerve impulsed as observed by Ghosh (1990). Pandey *et al.*, (2003) were of the opinion that the excess secretion of mucous and body dispigmentation are due to change in number and area of mucous gland and chromatophores caused by the dysfunctions of the endocrine gland. Bengeri and Patel (1986) have also reported excessive mucous secretion due to various toxic chemicals. The fishes exposed to sublethal concentrations of mercury showed an increased opercular movement followed by a sharp fall during the course of investigation, which may be due to hypoxic stress along with gradual inhibitory influence on respiratory system. Jha (1991) was of the opinion that mucous clogs the gill resulting in the death of fish.

But in the second phase of experiment when the fishes were supplemented with L-Ascorbic acid the

abnormal behavioural and morphological changes were reduced to some extent. This may be due to the protective role of L-Ascorbic acid because L-Ascorbic acid increases the tolerance to environmental stress in fish (Fish and wildlife service, 1979).

Biochemical Changes

In the present investigation, the biochemical parameters like total protein, total lipid and total carbohydrate contents of muscle tissue in *Labeo rohita* showed decline following the exposure to various sublethal concentrations of mercuric chloride suggesting the increased proteolysis, the possible utilization of the products and their degradation for metabolic purposes. They may enter the TCA cycle through aminotransferase system to cope with excess demand of energy during toxic stress. In the muscle, the protein content of *Labeo rohita* exposed to different sublethal concentrations of mercury declined considerably from their respective control set. However it is not possible to establish the precise mechanism due to lack of detailed experimental evidences. The reduction in protein content may be due to the impairment in protein synthesis and altered relationship between ribosome and membrane of endoplasmic reticulum as suggested by Revathi *et al.,* (2005). Verma *et al.,* (1989) observed decrease in protein content of liver, intestine and muscle of fish, *Rasbora daniconicus* exposed to pulp and paper mill effluents.

Unlike the polysaccharides and proteins, lipids are not polymers and are mostly small molecules. They are of great importance to the body as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. The function of lipid as a precursor for energy production is limited due to their oxygen dependent degradation inside the mitochondria. A metabolic pathway that demand relatively larger amount of oxygen may not be preferred under such conditions. The decrease in lipid content may be attributed with the fact that lipid being an important fuel reserve gets mobilized during stress conditions to meet the energy needs. As a result of changes in carbohydrate metabolism during stress conditions there was change in lipid content of the animal. Depletion in lipid content may be due to lipolysis or the mitochondrial injury, which impaired the function of TCA cycle and the fatty acid oxidation mechanism (Revathi *et al.,* 2005).

Carbohydrate requires least oxygen for its oxidation. It forms the immediate source of energy in both normal and stress condition. During stress conditions, fishes mobilize the stored energy to meet the energy demands of body through glycogenolysis and gluconeogenesis. The reduction in carbohydrate content is an agreement with earlier findings, which suggest that carbohydrate metabolism is disturbed when animals are subjected to toxic pollutants.The decrease in carbohydrate content may indicate an immediate utilization to meet the excess demand of energy. This is perhaps achieved by rapid glycogenolysis through activation of glycogen transferase respectively as observed by Revathi *et al.,* (2005). Heavy metal due to its strong affinity for legands like phosphate can bind with carrier protein molecule, which may lead to reduction of carbohydrate content due to inhibition in liver metabolism (Sastry *et al.,* 1982).

But in the second phase when the fishes were exposed to both mercury along with L-Ascorbic acid, then the decline in the biochemical parameters were reduced to some extent. It may be due to the effect of L-Ascorbic acid as it increases the resistance to metal toxicity (Papp *et al.,* 1997) as well as positive effect on growth, bone formation, reproduction, wound healing and immune responses as observed by Lee and Dabrowski (2004). The effect of L-Ascorbic acid was high in earlier period of exposure, but got reduced in later. This may be due to the fact that L-Ascorbic acid acts in the water soluble compartment and has a sparing effect on vitamin E by regenerating reduced form of vitamin E (Tanaka *et al.,* 1997). Irrespective of mechanism involved it may be due to the immediate utilization of L-Ascorbic acid to avoid the stress condition and also may be due to variation in environmental factors as it plays an important role in regulating the physiological conditions of an organism.

CONCLUSION

In the present investigation the test chemical, mercury was found to be highly toxic in *Labeo rohita* as

there was a drastic change in behavioural and morphological characteristics and reduction in biochemical parameters.There were some positive modifications in behavioral and morphological activities when the fishes were supplemented with L-Ascorbic acid. Although the differences were not statistically significant in all cases but the results showed some protective role of L-Ascorbic acid in the reduction of total protein, lipid and carbohydrate content and some modifications of abnormal behavioural and morphological characteristics of the organism when exposed to various sublethal concentrations of mercury along with L-Ascorbic acid, irrespective of mechanisms involved.

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