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Research Article

LEAD AND CHROMIUM INDUCED ALTERATIONS IN THE TOTAL LIPIDS AND LIPASE ACTIVITY IN THE TISSUES OF CATLA CATLA

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ABSTRACT

Lipids or fats are heterogenous group of complex molecules having high caloric value present in all biosystems. Lipids are generally stored in the liver, adipose tissue and muscles, and are one of the most important fish energy sources. They are mobilized when food intake cannot supply the energy demands of growth and maintenance. Lipase is a metabolic enzyme associated with the reactions of lipolysis and lipogenesis i.e. breakdown of lipids into fattyacids and glycerol resulting in the release of water and energy; and the synthesis of neutral lipids by its reverse reaction, respectively. In the present study fresh water fish *Catla catla* was exposd to lead and chromium heavy metals and study the total lipids and lipase activity in various tissues of the fish.

Total lipids were decreased in all the tissues irrespective of the toxicant, where as there was a increase in the lipase activity under the influence of lead and decrease under the influenced of the chromium.

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INTRODUCTION

Lipids or fats are heterogenous group of complex molecules having high caloric value present in all biosystems. Essentially lipids are esters of fattyacids or substances capable of forming esters which consist of fats, oils, phospholipids, triglycerides, glycerol, cholesterol, neutral lipids etc. Lipids constitute not only the architecture of cells but also form co-basis for the structure of some enzymes like Mg^{2+} ATPases. They offer full complementary structure to steroid hormones and also contribute for energy synthesis as an alternative to carbohydrates (Guyton, 1981; Sai Chandra, 2002). Lipids are generally stored in the liver, adipose tissue and muscles, and are one of the most important fish energy sources. They are mobilized when food intake cannot supply the energy demands of growth and maintenance (Moreira *et al.,* 2002).

The lipid source is contributed by the process of lipogenesis from carbohydrates in intermediary metabolism of fishes, that lipid accumulates extensively as an end product of carbohydrate metabolism. The incorporation of glucose carbon into lipid has been well reported in fish, however, it varies between tissues (Hoar and Randall, 1969). The lipid requirement of fishes varies with the medium in which they are cultured (Benitez and Gorriceta, 1983).

The concentration of metabolic enzymes vary in fishes according to their size and growth rate (Pelletier *et al.,* 1993) reproductive state (Kiessling *et al.,* 1995), feeding status (Kiessling *et al*., 1989), physical injuries (Grizzle *et al.,* 1992), and acclimation temperature (Seddon, 1997). Lipase is a metabolic enzyme associated with the reactions of lipolysis and lipogenesis i.e. breakdown of lipids into fattyacids and glycerol resulting in the release of water and energy; and the synthesis of neutral lipids by its reverse reaction, respectively. The physiological role of lipase in mobilization of lipids consists essentially in promoting the hydrolysis of fatty deposits and the synthesis of neutral fats by its reverse action. Earlier studies on lipase activity were directed towards correlating its activity to the general activity of the fish. In general, the lipase activity is higher in more active fish than in those forms which are sluggish. Fish muscle is known to contain a lipase capable of catalyzing the hydrolysis of short – chain triglycerides rather than the long-chain ones (George, 1962).

MATERIALS AND METHODS

Experimental fish species *Catla catla* belongs to the order cypriniformes and family cyprinidae (Jhingran, 1991). It is highly cultivable species and contributes their might in the

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provision of animal protein in India. In generally they are adaptable to healthy life in the laboratory, readily available in number and from a common source. Hence it is selected as the experimental species.

Catla catla (Hamilton) is an economically important edible fish having great commercial value, occurring abundant in the freshwater tanks and ponds and around Guntur. Besides its wide availability and commercial importance, this fish is known for its adaptability laboratory conditions and suitability to toxic studies (Sreenivasan and Swaminathan, 1967). Hence, *Catla catla* is selected as the experimental fish for the present investigation.

Test fish *Catla catla* weighing 5.55 ± 0.20g were procured from Nandivelugu, near Tenali, Guntur District, Andhra Pradesh, India. They were critically screened for symptoms of disease, stress, physical damage and mortality. The injured, severely diseased, abnormal and dead animal was discarded immediately. Then they were acclimated to laboratory conditions in large glass aquaria (90 × 45 × 45cm length, width and breadth respectively) containing non-chlorinated ground water for weeks prior to the experiment.

Abrupt changes in the physico-chemical properties of the holding water (colourless, clear, odorless with turbidity 8 silica units, total solids calcium 80mg/l, magnesium 40mg/l, total hardness as $CaCO_3$ 320mg/l, chlorides as Cl^- l40mg/l, fluorides as F 1.8mg/l, dissolved oxygen 6.0 - 8.0mg/l, pH 8.2, methyl orange alkalinity as $CaCO_3$ 472mg/l use throughout the experiment was avoided.

Lead

Lead is a naturally occurring heavy metal which has been used in various ways including mining, smelting, refining, gasoline, battery manufacturing, electrical wiring, soldering, painting, ceramic glazing, and making of stained glass. Due to its non-degradable nature, it gets into the environment and eventually enters the human and animal's blood stream.

Chromium

Chromium is one of the essential trace metal. It is widely used in chromeplating and in the manufacture of alloy steels (Partington, 1966) and tanning industry (Sastry, 1986). The two most common, stable and biologically and environmentally significant forms of the element are hexavalent (Cr^{6+}) and trivalent (Cr^{3+}) chromium. Hexavalent chromium was reported to be more toxic than trivalent chromium (Mc Kee and Woolf, 1963). Hexavalent chromium is very soluble in natural water and readily penetrates biological membranes. Stock solutions of experimental concentrations of chromium were prepared from analytical grade potassium dichromate ($K_2Cr_2O_7$) salts using glass distilled water (Vincent, 1992).

Total lipids were estimated separately in the gills, brain, liver and muscle using the method of Folch *et al*. (1957). Estimation of Lipase activity (Glycerolester hydrolase) was estimated separately in the gill, brain, liver and muscle using the procedure described by Colowick and Caplan (1955).

RESULTS

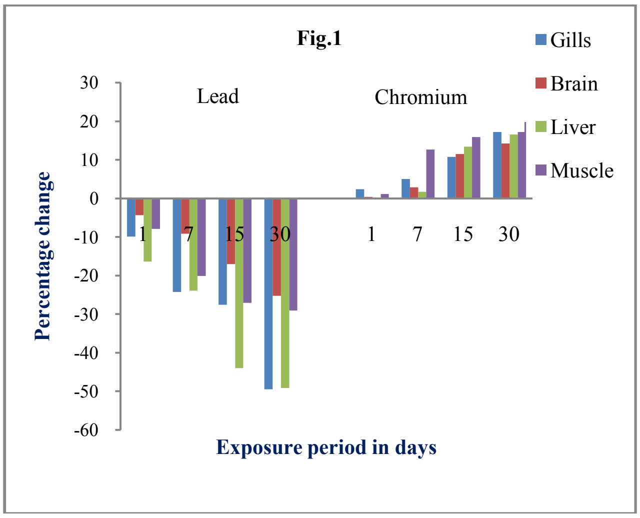
The data on the levels of total lipids, free fattyacids, phospholipids and cholesterol (mg/g wet wt) and the activity of lipase (lipase units/g wet wt) in gills, brain, liver and muscle of *Catla catla* at 1, 7, 15 and 30 days on exposure to the sublethal concentrations of lead and chromium, besides controls, are presented in tables 1 - 2. For comparison, the differences obtained in the levels of total lipids, free fattyacids, phospholipids, cholesterol and lipase activity in each organ of the fingerlings between the controls and the respective exposure periods in the sublethal concentration of lead and chromium were converted as percentages of the corresponding control level / activity and these values are also given in the same tables and plotted against exposure periods in figures 1 - 2.

Relative to controls the level of total lipids recorded a decrease in gills, brain, liver and muscle of fingerlings subjected to the sublethal concentration of lead. The decrease in total lipids was significant (P<0.05) in almost all organs of the fingerlings at all the exposure periods, except in the brain at day 1. In the sublethal concentration of chromium the organs of fingerlings showed a gradual increase in total lipid level at all the exposure periods and this increase was mostly insignificant (P>0.05) at both 1 and 7 days.

Table 1 Total lipids (mg/g wet wt) in the organs of *Catla catla* at different periods of exposure to sublethal concentrations of lead and chromium.

Organ	Control	Exposure period in days							
		Lead				Chromium			
		1	7	15	30	1	7	15	30
Gills	41.28	37.20	31.25	29.90	20.85	42.26*	43.36*	45.72	49.15
S.D.±		1.23	0.98	0.62	0.50	1.00	1.10	1.07	0.93
%		(-9.88)	(-24.29)	(-27.56)	(-49.49)	(+2.37)	(+5.03)	(+10.75)	(+19.06)
Brain	83.84	80.18*	76.15	69.54	52.65	84.19*	86.26*	93.48	95.81
S.D.±		2.59	2.04	1.89	1.32	2.62	2.03	2.00	0.72
%		(-4.36)	(-9.17)	(-17.05)	(-25.27)	(+1.41)	(+2.88)	(+11.49)	(+14.27)
Liver	75.48	63.11	57.44	42.25	38.39	75.61*	76.75*	85.63	87.96
S.D.±		1.45	0.86	0.64	0.50	2.95	2.07	0.71	0.45
%		(-16.38)	(-23.90)	(-44.02)	(-49.13)	(+0.17)	(+1.68)	(+13.44)	(+16.53)
Muscle	27.69	25.50*	22.12	20.19	19.63	28.00*	31.20	32.09	33.18
S.D.±		0.49	0.40	0.39	0.35	0.48	0.36	0.49	0.37
%		(-7.90)	(-20.11)	(-27.08)	(-29.10)	(+1.11)	(+12.67)	(+15.89)	(+19.82)

Each value is a mean of six replicates. Percent change over the respective control is given in parenthesis.
The differences between control and experimental are statistically significant (P<0.05)
*denotes not significant with control (P>0.05)



Percentage change over control in the lipid content in the organs of Catla catla at different period of exposure to the sublethal concentration of lead and chromium

The increase observed in total lipid level at 1 and 7 days in the organs of fingerlings exposed to the sublethal concentration of chromium was very less. (P>0.05). In the sublethal concentration of chromium the increase in total lipid levels was greater in degree in the muscle less in brain in the order muscle>gill>liver>brain. However, the differences among the organs were mostly insignificant (P>0.05).

Lipase Activity as A Function of Lead and Chromium Toxicity

Corresponding to the changes in total lipids and free fatty acids the activity of lipase recorded an increase relative to controls in the gills, brain, liver and muscle of fingerlings subjected to sublethal concentration of lead. The increase observed in lipase activity was mostly significant (P<0.05) at all the exposure periods studied. In sublethal concentration of chromium the lipase activity showed a significant decrease in all the organs of fingerlings at all the exposure periods except at day 1 in gills, brain and muscle and at day 7 in liver. However, this decrease was insignificant (P>0.05) at those days of exposure (Table 2 and Figure. 2).

Among the exposure periods the degree of increase in lipase activity in all the organs of fingerlings was less at 1 day and more at 30 days on exposure to the sublethal concentration of lead, and it was in the order 1<7<15<30 days. In the sublethal concentration of chromium the decrease in the lipase activity followed the above order in all the organs of fingerlings.

Table 2 Lipase activity (Lipase units/g wet wt) in the organs of *Catla catla* at different periods of exposure to sublethal concentrations of lead and chromium

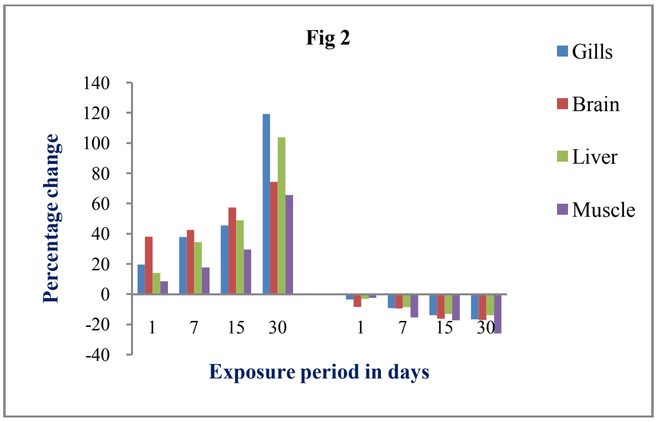
Organ	Control	Exposure period in days							
		Lead				Chromium			
		1	7	15	30	1	7	15	30
Gills	145.2	173.5	200.2	211.2	318.2	140.2*	131.6	125.0	120.9
S.D.±		5.32	6.30	5.09	5.81	6.39	5.72	5.71	5.98
%		(+19.49)	(+37.87)	(+45.45)	(+119.14)	(-3.44)	(-9.36)	(-13.91)	(-16.73)
Brain	133.80	184.70	190.70	210.50	233.30	122.6*	121.20	115.70	110.90
S.D.±		6.01	4.91	4.93	7.11	6.70	5.10	5.78	5.60
%		(+38.04)	(+42.52)	(+57.32)	(+74.36)	(-8.37)	(-9.41)	(-16.36)	(-17.11)
Liver	351.80	401.30	472.80	523.70	716.70	340.6*	321.8*	305.60	302.70
S.D.±		3.80	7.91	6.11	6.40	6.71	4.67	4.93	4.20
%		(+14.07)	(+34.39)	(+48.86)	(+103.72)	(-3.18)	(-8.52)	(-13.13)	(-13.96)
Muscle	83.50	90.60*	98.30	108.20	138.30	81.50*	70.60	69.00	61.80
S.D.±		7.32	7.73	4.92	5.43	2.50	3.51	1.69	0.98
%		(+8.50)	(+17.72)	(+29.58)	(+65.62)	(-2.39)	(-15.44)	(-17.36)	(-25.98)

Each value is a mean of six replicates. Percent change over the respective control is given in parenthesis.
The differences between control and experimental are statistically significant (P<0.05)
*denotes not significant with control (P>0.05)

Among the exposure periods the decrease observed in the levels of total lipids in the organs of fingerlings exposed to the sublethal concentration of lead differed in degree. It was more pronounced at 30 days and less at 1 day in the order 1<7<15<30 days. The differences between 1 and 30 days were statistically significant (P<0.05). In the organs of fingerlings exposed to the sublethal concentration of chromium the increase in lipid level though greater at 7 days than at 1 day, the differences in them were not significant (P>0.05). But the increase was greater at 30 days than at 15 days with significant (P<0.05) differences between these two exposure periods (table 20; figure.18).

Among the organs studied the magnitude of decrease in total lipid levels in the organs of fingerlings exposed to the sublethal concentration of lead was in the order gills>liver>muscle>brain. However, the differences between liver and gills, brain and muscle were mostly insignificant

Among the organs, in lead exposed animals, the increase in lipase activity was in the order: gills>liver>brain>muscle, with significant differences between gills and muscle, and brain and liver. Whereas in the fish exposed to the sublethal concentration of chromium the decrease in lipase activity was in the order muscle>brain>gills>liver and these differences were mostly significant.



Percentage change over control in the lipase activity in the organs of Catla catla at different period of exposure to the sublethal concentration of lead and chromium

DISCUSSION

In the present study the decrease in total lipids in the gills, brain, liver and muscle of fingerlings of *Catla catla* exposed to the sublethal concentration of lead at the exposure periods, 1, 7, 15 and 30 indicates the breakdown of these biomolecules due to subacute toxicity stress of the metal. However, the degree of breakdown is dependent on the organ of the fish and length of exposure period. The decrease in the total lipid content might be due to the utilization of it to meet the energy demand associated with the situation of stress (Rao and Rao, 1981; Ganesan *et al.,* 1989; Sai Chandra, 2002; Maharajan *et al*., 2012; Aruldoss *et al*., 2014).

Lipase activity is known to present in organs of fishes (Patton *et al.,* 1975; Urmila Devi, 1993), however, there is little information on its role during toxic stress. Increase in lipase activity in the organs of fingerlings on exposure to the sublethel concentration of lead could be taken to indicate the lipid breakdown, the lipolysis. This is clearly evident by the decrease in total lipids and increase in the levels of free fattyacids. The induction of severe breakdown of lipids could cause the disintegration in the structural organization of the fish, which in turn could lead to the imbalance in its homeostatic mechanism and the failure of metabolic compensation and regulation (Sivaramakrishna *et al.,* 1992). As the animals are continuously exposed to sublethal concentration of lead for a long period of study the intensity of lypolysis increased over time of exposure i.e.from day 1 to day 30. Similar changes in lipase activity were also observed in fishes over a length of exposure to toxicants, especially pesticides (Rath and Misra, 1980; Manoharan and Subbaiah, 1982; Ganesan *et al.,* 1989; Urmila Devi, 1993; Virk and Sharma, 2003; Pandey *et al*., 2008).

In the present study the greater decrease in the total lipids, phospholipids and the increase in lipase activity, free fattyacids and cholesterol level in the gills, brain, liver and muscle of fish on exposure to sublethal concentration of lead could be due to the higher rate of accumulation of metal ions. Probably the intensity of lypolysis is dependent on the rate of accumulation. In lead exposed fish, increased rate of accumulation of metal overtime of exposure might have severely affected the organs of them. Furthermore the internal defensive mechanisms and the immunochemical process might have failed to dominate the metal effect. Therefore the degree of lypolysis is greater in the organs of lead exposed fish. But in chromium exposed fish, the greater activation of lipid synthetic potential were observed, than the lead exposed ones. It is possible if the concentration of chromium accumulated within the organs of fish is in the limits of detoxification. For the activation of such detoxification mechanisms, lipids, probably, are more useful for structural rigidity to prevent the entry of toxic ions. The decrease in cholesterol level, in the organs of chromium exposed fish, than the fish exposed to lead could be due to the partial divertion of it to lipogenesis. The overall shifts in lipid metabolism, however, indicate that the sublethal concentration of lead is adversely effective to the fish, but they can resist to the sublethal concentration of chromium.

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