



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 7(E), pp. 28042-28045, July, 2018

**International Journal of
Recent Scientific
Research**

DOI: 10.24327/IJRSR

Research Article

GLYCOGEN LEVELS AND GLYCOGEN PHOSPHOTYLASE ACTIVITY IN LIVER AND MUSCLE OF CATLA CATLA UNDER THE TOXICITY OF LEAD AND CHROMMIUM

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DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0907.2381>

ARTICLE INFO

Article History:

Received 4th April, 2018

Received in revised form 25th May, 2018

Accepted 23rd June, 2018

Published online 28th July, 2018

Key Words:

Carbohydrates, Liver, Muscle, Toxicity, Lead, Chromium, CatlaCatla

ABSTRACT

Most of the living organisms derive energy by the metabolic breakdown of carbohydrates. The chief carbohydrate in the tissue is glycogen, while glucose is utilizable sugar found in the tissues and body fluids. The oxidation of glucose is mediated by catabolic pathways viz., glycolysis, Krebs's cycle, electron transport system and hexose mono-phosphate shunt, which constitute the major segments of carbohydrate metabolism. Thus carbohydrate metabolism gained importance in physiology of animals. In the present study fresh water carp, *Catla catla* was exposed to heavy metals lead and chromium to study the impact of these metals on the carbohydrate metabolism of the fish. Study revealed that there was a clear deterioration of carbohydrate metabolism under the toxicity of the heavy metals lead and chromium. It was clearly observed that liver and muscle glycogen levels were decreased where as the activity of glycogen phosphorylase was increased in both the tissues studied.

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INTRODUCTION

Most the living organisms derive energy by the metabolic breakdown of carbohydrates. The chief carbohydrate in the tissue is glycogen, while glucose is utilizable sugar found in the tissues and body fluids.

The oxidation of glucose is mediated by catabolic pathways viz., glycolysis, Krebs's cycle, electron transport system and hexose mono-phosphate shunt, which constitute the major segments of carbohydrate metabolism. Thus carbohydrate metabolism gained importance in physiology of animals. Carbohydrate metabolism in fishes is almost similar to the other vertebrates including mammals (Umminger,1970; Prosser, 1973;Dange and Ajit,1986;Naga Bhasker,2005). However, they have a greater capability to adapt themselves to the environmental variations.

Blood glucose level has been reported as a reliable and sensitive indicator of environmental stress in fishes (Silbergeld, 1974; Saravanan et al., 2000; Jenkins et al., 2003).

Alternations in liver and muscle glycogen under situations of stress have been reported, and a significant depletion in tissue glycogen is said to reflect a state of strenuous activity on the part of the fish (Tewari et al., 1987; Vijayaram et al., 1989).

The muscle fibres play main role in maintaining the blood glucose level and that muscle glycogen is utilized after depiction of liver glycogen (Nagabhaskar et al., 2005).

Glycogen phosphorylase is an enzyme that concerned with the metabolic breakdown of glycogen, assumes considerable importance in studies involving liver and muscle glycogen levels. Increase in glycogen phosphorylase activity confirms glycogen breakdown, a cause for the lowered glycogen level, which may even be caused by the decrease in its synthetic rate.

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 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

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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 ± 0.20 g 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 ($90 \times 45 \times 45$ cm 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 140mg/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.

Table 1 Liver glycogen (mg/gm wet wt) and liver glycogen phosphorylase ($\mu\text{M pi}$ formed/mg/protein/h) in *Catla catla* at different periods of exposure to sublethal concentrations of lead and chromium

Estimation	Control	Exposure period in days							
		Lead				Chromium			
		1	7	15	30	1	7	15	30
Liver glycogen	24.17	18.01	16.28	15.29	13.12	11.03	17.3	22.52*	25.74*
S.D.±	1.72	1.02	0.96	0.73	0.65	0.58	0.62	2.32	1.79
%		(-25.48)	(-32.64)	(-36.73)	(-45.71)	(-56.36)	(-28.42)	(-6.82)	(+6.49)
Liver glycogen phosphorylase	4.48	5.55	6.00	6.27	6.56	5.42	5.66	5.01*	4.03*
S.D.±	0.002	0.001	0.006	0.004	0.005	0.005	0.004	0.003	0.003
%		(+23.88)	(+33.92)	(+39.95)	(+46.42)	(+20.98)	(26.33)	(+11.83)	(-10.04)

Each value is a mean of six replicates

Percent change over the respective control is given in parenthesis

S.D.±: Standard deviation P: Level of significance

The differences between control and experimental are statistically significant ($P < 0.05$)

*denotes not significant with control ($P > 0.05$)

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.

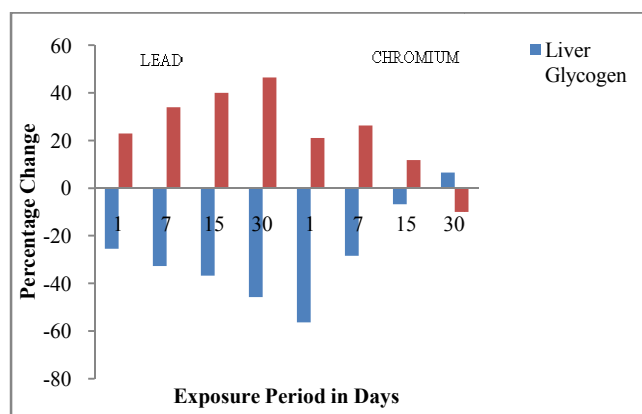


Figure 1 Percentage changeover control in the glycogen level and glycogen phosphorylase activity in the liver of *Catla catla* at different period of exposure to the sublethal concentrations of lead and chromium

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 ($\text{K}_2\text{Cr}_2\text{O}_7$) salts using glass distilled water (Vincent, 1992).

Estimation of Glycogen content in the liver and muscle was estimated using the anthrone reagent method as described by Carroll *et al.* (1956) and Estimation Of Glycogen Phosphorylase activity in the muscle was estimated using the method described by Cori *et al.* (1955).

RESULTS

The data on the levels of blood glucose (mg/100ml) in liver and muscle glycogen (mg/g wet wt), and the activity of liver and the muscle glycogen phosphorylase ($\mu\text{M pi}$ /mg protein/h) of the fish at 1, 7, 15, and 30 days on exposure to the sublethal concentrations of lead and chromium, besides controls, are presented in the tables 1 and 2.

In accordance to the changes in the blood glucose level, the liver glycogen level decreased at all the four exposure periods in the fish exposed to both lead and chromium, expect an insignificant increase observed at day 30 in the fish exposed to chromium. The decrease in the liver glycogen in the lead intoxicated fish was relatively greater than the decrease observed in the chromium exposed fish, and it was significant at all the four days of exposure which was in the order: day 1 < 7 < 15 < 30. The decrease, however, was significant only at days 1 and 7 in the fish exposed to chromium, and it was in the order: day 1 > 7 > 15. Thus the decrease increased with exposure period in the lead intoxicated fish where as the initial decrease in the liver glycogen level restored to normalcy at day 30 in chromium exposed fish (Table 1 and Figure 1).

Corresponding to the changes in the liver glycogen level, the glycogen phosphorylase activity in lead exposed fish

significantly increased at all the four exposure periods in the order: 1<7<15<30. But it was increased at days 1, 7 and 15 followed by an insignificant decrease at day 30 in the fish exposed to chromium toxicity. The increase observed was also insignificant at day 15 and it was in the order: day 1<7>15. Thus the increase in the enzyme activity was greater in the lead exposed fish, as against to the changes observed in chromium-exposed fish where in the activity restored to normalcy at day 30 (Table 6 and Figure 4).

In the lead exposed fish in the additions to the liver glycogen the muscle glycogen level decreased at all the four exposure periods in the order: day 1<7<15<30 and it was insignificant at day 1. It was also decreased at days 1 and 7, in the order: day 1<7, in the fish exposed to chromium followed by an increase of it at days 15 and 30 in the order: 15<30. The decrease of it at day 1 and the increase at day 15, however, were insignificant (Table 2 and Figure 2).

Corresponding to changes observed in muscle glycogen level the muscle glycogen phosphorylase activity in the lead intoxicated fish progressively increased from day 1 to 30 in the order: day 1<7<15<30. The increase was significant at all the four days of exposure. Where as in the chromium exposed one, the muscle glycogen phosphorylase activity increased initially at days 1 and 7 followed by the decrease of it at days 15 and 30. The increase was significant at both the days of exposure and was in the order of: day 1<7; the decrease was insignificant at day 15 and it was in the order of: day 15<30 (Table 2 and Figure 2).

Table 2 Muscle glycogen (mg/gm wet wt) and muscle glycogen phosphorylase (μ Mpi formed/mg/protein/h) in *Catla catla* at different periods of exposure to sublethal concentrations of lead and chromium

Estimation	Control	Exposure period in days							
		Lead				Chromium			
		1	7	15	30	1	7	15	30
Muscle glycogen	1.68	1.65*	1.40	1.32	1.26	1.56*	1.23	1.75*	2.03
S.D.±	0.009	0.010	0.008	0.011	0.009	0.010	0.008	0.010	0.012
%		(-1.78)	(-16.66)	(-21.42)	(-25.00)	(-7.14)	(-26.87)	(+4.16)	(+51.47)
Muscle glycogen phosphorylase	2.89	3.42	3.45	3.69	3.84	3.46	3.98	2.77*	2.33
S.D.±	0.007	0.009	0.006	0.005	0.007	0.003	0.002	0.005	0.003
%		(+18.33)	(+19.37)	(+27.68)	(+32.87)	(+19.72)	(+37.71)	(-4.15)	(-19.73)

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

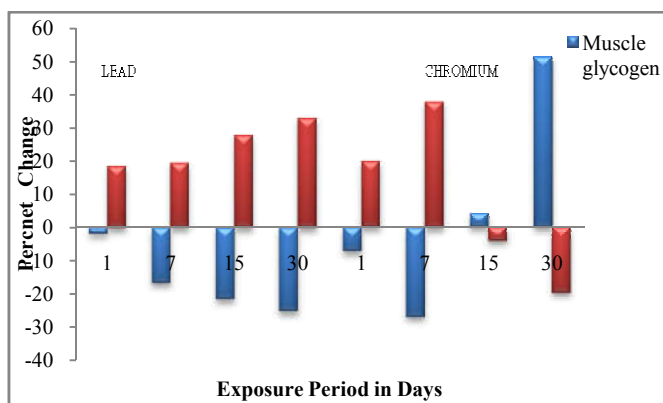


Figure 2 Percentage change over control in the glycogen level and glycogen phosphorylase activity in the muscle of *Catla catla* at different period of exposure to the sublethal concentrations of lead and chromium

DISCUSSION

Blood is a patho-physiological reflector of the whole body so, blood parameters are important in diagnosing the structural and functional status of the animal exposed to the toxicant (Jenkins *et al.*, 2003). It is well established that the toxicants including heavy metals influence many biochemical changes in fish proceeding cellular and systematic dysfunctions (Gill and Pant, 1981; Christopher, 2003; Sobha *et al.*, 2007; Kawade and Khillare, 2012; Chaudhari *et al.*, 2015). Hence changes in carbohydrates to meet the changing energy demands can be expected in these animals subjected to heavy metal level could have resulted by the breakdown of liver glycogen through the activation of liver glycogen phosphorylase activity.

In fishes, glycolysis and glycolytic enzymes are dominant in muscle (Hazel and Prosser, 1974; Suresh *et al.*, 2013c; Cyril Arun kumar *et al.*, 2013). Hence, there are more breaks-down of glycogen in muscle and also greater elevation in LDH activity leading to more accumulation of lactate in the fish exposed to lead. In chromium exposed fish, the stimulated functioning of oxidative cycle along with the glycolytic activity could indicate enhanced energy demands corresponding to the improvement in muscular activity of this effector organ.

On the whole, the decrease in oxidative cycle and dependency on only anaerobic glycolysis to meet the energy requirement in the lead exposed fish indicate that compensation through metabolic adaptability is not possible on prolonged exposure of the fish to lead intoxication.

The elevation in SDH activity, even from 7 days of exposure, in the organs of fish exposed to chromium signifies high adaptive ability of it to the subacute concentrations of chromium. Thus the adaptive ability of the fish *Catla catla* to sublethal stress is metal dependent; it is sensitive and susceptible to lead but resistant to chromium in sublethal concentrations.

Acknowledgements

When I present this work on the completion of my research, I remember with gratitude the source of inspiration, materials, assistance and guidance that lead to this achievement of goal. The only way left for me to express my gratitude to them is to make them feel proud of me.

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How to cite this article:

Suresh B et al. 2018, Glycogen Levels and Glycogen Phosphotylase Activity in Liver and Muscle of Catla Catla Under The Toxicity of Lead and Chrommium. *Int J Recent Sci Res.* 9(7), pp. 28042-28045.
DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0907.2381>
