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Cadmium exposure on serum glucose level and glycogen reserve in the liver and muscle tissue of Indian Cat fish *Heteropneustes fossilis* (Bloch)

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Abstract: The Indian Cat fish *Heteropneustes fossilis* (Bloch) was exposed to sub lethal concentration (Lc50) of cadmium (3.21ppm) for 10 days. The level of serum glucose and glycogen reserves in liver and muscle tissues were calculated in both fish treated and untreated to Cd. The level of glycogen reserve in liver and muscle tissue were significantly ($P < 0.05$) decreases in fish exposed to sublethal concentration of Cd in comparison to the level of the same in the control group. This depletion of glycogen level in both liver and muscle tissue under the highest metal concentration (1.0 mgl-1) were 26% and 31% respectively. The blood serum glucose level of fish exposed to Cd were significantly ($P < 0.05$) increases compare with that of level measured in control group. Perhaps this increase was correlated with the increase in water Cd concentration.

Keywords: Liver, Muscle, Glycogen, Serum Glucose, *H.fossilis*.

INTRODUCTION

The accumulation of toxic heavy metal in the aquatic ecosystem is going to increase day by day due to natural geochemical and anthropogenic factors. The toxic heavy metals are carried to the natural ecosystem by means of food chain and contaminate the upper tropic level and automatically cover hazardous problems.[1,2]

Being the nonessential and non-biodegradable nature of the cadmium it shows cumulative polluting effect and could cause toxic effect on the flora and fauna of the aquatic ecosystem even in the trace amount Cadmium has adverse effect like (i) Interrupting growth and development (ii) Preventing calcium uptake through gills (iii) disturbing liver function (iv) Skeletal deformations and (v) Pathological change in vital tissue and organs.[3,4]

Since the heavy metal excretion, deposition, and detoxification in fish are not capable of handling heavy metal in short time frames so these are accumulated specially in metabolically active tissues and organs. The accumulation rate varies species to species even it is influenced by the age, size, feeding status and sex.[5,6]

From review of literature it is evident that biochemical parameter in fish blood and tissue could change when exposed to heavy metal and that these parameters are extremely sensitive to these elements. Cadmium could change glycogen reserves and serum glucose level in fish by affecting the activities of liver enzymes that have role in carbohydrate metabolism such as gluconeogenesis and glycolysis.[7] As we know that

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glycogen reserve in liver and muscles tissue acts as an emergency energy supply under the stress, so change in the glycogen level in these tissues could indicate the health, status of the fish population.[8] It is supposed that Cd might change glycogen reserve in fish via endocrine system, because the life span, reproduction and health are all functions of metabolic events in fish exposed to the heavy metals.[9] This study aimed to be demonstrate the effect of sublethal concentration of Cd on the level of glucose in serum and glycogen reserves in the liver and muscle tissue of Indian cat fish *Heteropneustes fossilis* which has economic value in India specially in Koshi zone as these fishes are found abundantly in the vicinity of this water logged area .

MATERIALS AND METHODS

The fishes were collected from the adjoining Koshi area with the help of local fisherman and was kept in laboratory environment and acclimatized for a fortnight to the laboratory conditions, in an aquarium measuring 40x60x80 cms in width, depth and length respectively. The initial mean weight and length of the fish were 50.25 + 0.5 gms and 12.3 + 1.02 cms respectively. The room temperature and photoperiod during experiment were 25 + 1^0C and 12L:12D respectively. Five aquaria 1 of which was designated as control and 4 for treatment. $CdCl_2$ salt was used for the preparation of stock solution.

The chemical parameter of Cd-free tap water used in the experiment were as follows .

pH - 7.7 + 0.5

Total hardness- 201+ 0.8mgl-1

Total alkalinity- 270+ 3.15mgl-1

Four aquaria were filled with tap water and Cd stock solution was added to each aquarium to make the final concentration 0.05, 0.1, 0.5 and 1.0 mgl-1 Cd. The fifth aquarium was treated as control. Six fish were transferred to each of the aquarium and the effect of Cd concentration on glycogen reserve and serum glucose level in liver and muscle tissue were investigated after 10 days. The fish were kept starved during the experiment. The aquria were well aerated and dissolved oxygen levels were kept at around 7.5 + 1.02 mgl-1 throughout the experiment. Every 2 days, the water in each aquarium was replenished to keep the metal concentration constant. The fish were anaesthetised with MS 222 (tricane methane sulfonate, sandoz) Blood sample were collected in heparinized vials with the help of 1 ml disposable syringe equipped with 2 gauge hypodermic needle by puncturing the ventral aorta. These blood samples were then centrifuged at 3500 rpm for 10 min. to obtain serum samples for glucose analysis. The glucose level in the serum sample were analyzed using the O-toluidine method. For this 50 µl serum samples were added to glass tubes and 3.5 ml. of O-toluidine reagent was added to each tube and then all the tubes were kept in a hot water bath (100ºC) for 10 min. The glucose level in cooled sample were measured spectrophotometrically.

The muscle and liver tissue to analyzed for glycogen level were first wet weighed and then placed into centrifuge tubes containing 3 ml. of KOH Soln. (30%). The centrufuge tube were kept in a hot water bath for 20 min. Then 0.5 ml. of saturated Na_2So_4 and 3 ml. of ethyl alcohol (95% pure) were added, followed by boiling for a further 15min. After being cooled, all samples were centrifuged at 3500 rpm and the supernatants were discarded. The precipitate in the tube were dissolved in 2ml. of distilled water followed by the addition of 2.5 ml. of ethyl alcohol (95% pure). The tubes were then centrifuged at 3500rpm for a further 10min. and again the supernatant were discarded. The final precipitation in the tubes free form lipid and protein were then dissolved in 2ml. Hcl (5M) and neutralized with 0.5M NaOH followed by dilution to 50ml. with distilled water before analysis the glycogen level in the sample were determined by the anthrone method.

RESULT

The effects of Cd on glycogen reserves and serum glucose level in the muscle and liver tissue of *H.fossilis* are shown in the table and figure respectively.

The glycogen level in the liver and muscle tissue of fish exposed to predetermined concentrations of Cd were significantly ($P < 0.05$) lower compared with the level found in the control fish. The highest concentration (1.0mgl-1Cd) tested in this study decreased the glycogen level in the liver and muscle tissue by 26% and 31% respectively. Although the decrease in the liver glycogen level of *H.fossilis* differed ($P > 0.05$) between all the Cd concentration tested, there was no significant ($P > 0.05$) difference between those in muscle tissue of fish exposed

to 0.05 and 0.1 mgl-1 concentration Cd (Table). There was significant difference ($P < 0.05$) for higher concentrations.

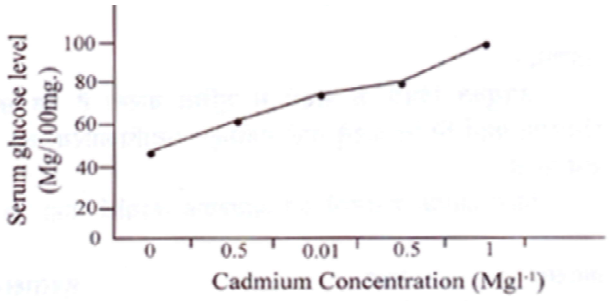
The serum glucose levels of fish increased in all in Cd concentration tested. However, this increment was significantly different in all Cd concentrations except for 0.05 and 0.1 kgl-1($P < 0.05$) Fig.

DISCUSSION

The result of the present study exhibited that predetermined concentration of Cd significantly altered the carbohydrate metabolism of *H.fossilis* after 10 days exposer.[10]

The glycogen reserve in the muscle and liver tissues of *H.fossilis* exposed to Cd decreased significantly in this study compared with the glycogen reserved measured in the control groups. The decreasing trend found in the muscle and liver glycogen reserves of *H.fossilis* in this study might in fact be the result of Cd stimulating the activities of enzymes that work in glycogenolysis.[12]

Glycogen stored in the fish tissue and organs like the muscle and liver in order to supply the energy need during hypoxic conditions intensive stocking and starvation period. The carbohydrate metabolism of the fish used in the present experiment might also have been affected due the lack of food since they were not fed during the experiments.[16]



Graph :- Showing glucose level in ***H.fossilis*** after 10 days of exposure to different concentration of Cd.

Table :- Showing the level of glycogen reserve in the muscles and liver tissue of *H.fossilis* after 10 days exposure to different Cd. Concentrations.

Tissues	Cadmium Concentrations(mgl^{-1})				
	Control	0.05	0.1	0.5	1
Liver	44.0 ± 2.32	33.24 ±1.2	26.2 ± 1.28	19.09 ± 1.7	10.5 ± 1.25
Muscle	4.8 ±0.22	3.27 ± 0.15	2.98 ± 0.12	2.05 ± 0.20	1.18 ± 0.33

Some investigations also reported that Cd decrease the glycogen reserve in *Channa punctatus* by stimulating glycolytic enzymes like lactate dehydrogenase, Pyruvate dehydrogenase and succinate dehydrogenase.[13]

Prolonged environmental stress in fish makes adaptation difficult and creates weakness resulting in depletion of liver glycogen and serum cholesterol level, which subsequently create a series of alternations in the metabolism and shorten the life span of fish.[14]

The decrease in glycogen reserves in the muscle and liver tissue of fish under acute exposure of heavy metal has been demonstrated to change with species. This change might stem from the metabolic difference between species and the environmental concentrations of heavy metals and durations which the fish are exposed to Serum glucose level of *H.fossilis* exposed to sub lethal concentration of Cd for 10 days increased with increasing concentration of Cd.[15]

Conclusively we can draw inference that present study showed that Cd altered the carbohydrate metabolism in *H.fossilis* by affecting the levels of glucose in serum and glycogen reserve in both muscle and liver of fishes. Such change in the glycogen reserve of muscle and liver tissue and serum glucose level under the effect of Cd stress might result in impairments in energy requiring vital processed, and hence give an idea about the health status of the fish population.

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