"The Sublethal Toxicity of Cadmium on Glycogen Levels in the liver and Muscle Tissues of fish Oreochromis mossambicus"

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Abstract

The level of glycogen (polysaccharide) reserves in liver and muscles tissues were studied in fish, **Oreochromis mossambicus** exposed to sublethal concentrations of heavy metal cadmium compared with the levels measured in the controlled groups. A decrease in glycogen content was observed in both the liver and muscle tissues, indicates the possible effect of cadmium toxicity in fishes, subjected to increasing concentration of cadmium and the time period of exposure to different sub-lethal concentrations.
KEYWORDS: Glycogen, **Oreochromis mossambicus**, liver, muscles, Cadmium.

Introduction

Heavy metal accumulation in fish is matter of considerable practical importance since, in many parts of the world pollutants containing heavy metals are being discharged in to water bodies which fishes are taken for consumption. The natural waters have a high potential risk for receiving metals from anthropogenic sources, such as water runoff, sewage treatment plants and domestic garbage dumps which eventually cause adverse on biota. The filtration of toxic heavy metals into aquatic ecosystems is on the increase due to natural factors such as anthropogenic and geochemical. In aquatic ecosystems the toxic metals are carried via the food chain to the top tropic level and create important ecological imbalances and problems. The cadmium is one of biologically a non- essential heavy metals, it has a cumulative polluting effect and could cause toxicity to aquatic organisms even in low concentration. The high accumulation of heavy metals in abiotic and biotic components can lead to serious ecological consequence. In polluted environments concentrations can be considerably higher (USEPA, 2001)[23].Aquatic organisms are affected by water hardness and some other characteristics of aquatic media. The toxic effect of cadmium in various aquatic animals, fishes are numerous like retardation of growth and development, pathological changes in organs such as liver and tissues. Lemaire, GS., Lmaire, P. 1992)[7]The heavy metals tend to accumulate in metabolically active tissues and organs like liver. The accumulation rate of heavy metals such as cadmium in various aquatic animals including fishes depending upon sex, size, age, feeding status of the organisms(Witeska, M.,Jezierska, B. Chaber J., 1995)[24].In fish tissues and liver could change when exposed to cadmium, is more sensitive to physiological and biochemical parameters.(Sastry. K. V., Rao D.R. 1984)[15].It has been found that cadmium could change glycogen reserve in fish by affecting liveractivity that have roles in the carbohydrate metabolism glycolysis and gluconeogenesis(Levesque, H. M., T. W.Campbell, P.G. C. Hoentela, A. 2002)[8].In fish muscles could be used as an indicator of heavy metal toxicity of cadmium on several biochemical parameters.(Togyani. A. Fauconneau. B., Boujard, T., Fostier., A.Kuhn, E. R.Mol. K.A. Baroiller. J. F. 1997)[21]. Due to heavy metal cadmium toxicity in an aquatic environment exert an extra stress on fish, there must be some changes in glycogen reserves in fish when exposed to cadmium indicates the status of fish.

The heavy metals enter into the hydrosphere via many pathways. The different aquatic environment like rivers, lakes, river streams, estuaries and even ocean may thus be affected by heavy metals like cadmium either by concentration and degradation. The high concentration have been found to cause toxic effects to aquatic animal like fishes, lobsters, crabs, etc. which are economically important as food for human consumption of cadmium creates ecological and physiological imbalance. The toxicity of cadmium produces toxic effect on a wide range of animals. The life span condition factors and health are all functions of metabolic activities in fish exposed to heavy metals. This study the aimed to demonstrate effect of glycogen reserves in liver and muscles of **Oreochromis mossambicus** which has economic value in Kalwa creek area and Thane city premises in Maharashtra.

Materials and methods:-

For the present experiment the fish **Oreochromis mossambicus** have been selected as a model test species for the present experiment. With the help of local fisherman's the active and healthy specimen of fishes collected from Kalwa creek, commonly named as Thane creek in Maharashtra and brought to a controlled laboratory conditions in aquaria measures 20cm in width , 30cm in length and 15cm height. The mean weights, lengths of the fishes used in experiments. Because metabolic activities changes with size and in the experiments individuals of similar size and length were used and effects the parameters to be measured Canli, M., Furness, R. W. (1993) [1]. To avoid any fungal infection they were washed with 0.1% $KMnO_4$ solution and examined for any pathological symptoms. The fishes were controlled laboratory environment and acclimatized to the laboratory condition for about four weeks to laboratory conditions in aquariabe for being used for experiment. Fishes were regularly fed with dried tubifex worms and chironomus larvae. Feeding was stopped two days before being used for experiments. Chlorine free tap-water was used throughout the course of the experiment. Some of the physicochemical parameters of the cadmium free test water used in the experiment such as total alkalinity, total acidity, total alkalinity, total hardness, pH, and Temperature, were regularly reported and listed in table no.1.

Table 1: Physiochemical parameters of test water

Temperature	27 0C
PH	7.3
Do	5.9mg/litre
Free chlorine	Nil
Total acidity	3.5mg/litre
Total alkalinity	44mg/litre
Total hardness (as $CaCO_3$)	31mg/litre

After a general selection for healthy and same group of fishes (4.2±0.5) cm. and (0.850±0.5) in weight, they were transferred to glass aquaria containing de-chlorinated tap-water. After acclimatization, the healthy fishes were selected for experimental purpose without sex-discrimination. 10 such fishes were transferred to glass aquaria of 50 litres capacity, each aquarium containing 20 litre of de-chlorinated tap-water.one of the aquaria which was designated as a control, were used to conduct the experiments. For the preparation of stock solutions cadmium chloride $CdCl_{2}.H_{2}o$ salt was utilized.

For selecting sub-lethal concentration of the toxicant to which fishes could be exposed, earlier information of acute toxicity data was made use of. This reveals that the toxicity of heavy metals cadmium does not increase with the time of exposure. Therefore the toxicant doses selected were close to their 96hrs. LC_{50}values of heavy metal cadmium to **Oriochromis mossambicus** was found to be 1.00mg/litre, 1/3rd, 1/5th and 1/10th of 96hrs. LC50 values were selected for sub-lethal concentration of cadmium listed in table no.2

Table 2: Selected sub-lethal concentration of cadmium for toxicological studies (mg/litre)

Toxicant	Concentrations		
Cadmium	0.1(1/10th)	0.2(1/5th)	0.34(1/3rd)

To maintain these concentrations constant throughout the experimental period, and to avoid the accumulation of metabolic waste, the entire water was changed every alternate day. The water is replenished to keep the metal concentrations constant.

The **Oreochromis mossambicus** kept separately in separate aquaria. At the end of the experiment the fish were separately sacrificed carefully. The estimation of glycogen in muscles and liver tissues to analysed for biochemical estimation. Weighed tissues (liver and muscles 100mg taken in pool from both the control and treated fish) were digested to 5 ml of 30% KOH solution. This was diluted to 20 ml with distilled water. The diluted solution was used for the assay of glycogen content. According to the Anthron method as describe by Siefer et al. (1950) [16] the estimation of glycogen is carried out.

Result and discussion:

Cadmium exposed tissues of **Oriochromis mossambicus** show glycogen content of mg/gm wet weight for muscle and liver as cited in table no.3 and 4. Table 5 Glycogen content in liver of fish when exposed to sublethal doses of cadmium for a period of 4weeks.

Table-3

Liver glycogen in mg/gm. wet weight tissues of fish, **Oreochromis mossambicus** during chronic cadmium exposure for period of 4 weeks.

Days of exposure	Control	Concentrations of Cadmium mg/litre		
Initial		0.001	0.002	0.003
0 Days	2.51	2.36	2.12	2.07
SD		0.075	0.195	0.22
PV		6.1601	16.8467	19.214
7 Days	2.56	2.45	2.36	2.03

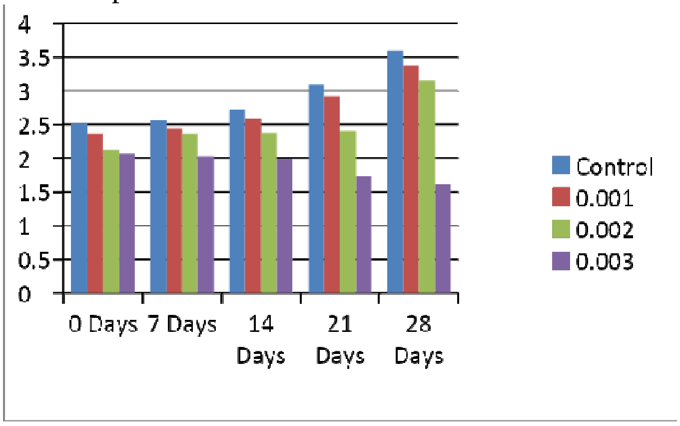
SD		0.055	0.1	0.275
PV		4.3912	8.1300	23.0937
14 Days	2.72	2.58	2.37	1.99
SD		0.08	0.175	0.365
PV		5.2830	13.7525	30.9979
21 Days	3.09	2.92	2.40	1.72
SD		0.49	0.75	1.09
PV		5.6572	25.1366	56.9647
28 Days	3.60	3.37	3.14	1.62
SD		0.115	0.23	0.99
PV		6.5997	13.6499	75.8621

SD=Standard deviation, PV=Percentage variation

Range of SD	**Confidence Level**
Less than 1	68.3%
Up to 1.645	90%
Up to 1.960	95%
Up to 2.576	99%
Up to 3.291	99.9%
Up to 3.891	99.99%
Up to 4.417	99.999%
Up to 4.892	99.9999%

Graph-1(Fig 1)

Liver glycogen in mg/gm. wet weight tissues of fish, **Oreochromis mossambicus** during chronic Cadmium exposure for period of 4 weeks.



Physico-chemical parameters of water used for toxicological study.

Temperature	27°C
PH	7.3
DO	5.9mg|Litre
Free Chlorine	Nil
Total Acidity	3.5mg/Litre
Total Alkalinity	44 mg/Litre
Total Hardness as $CaCO_3$	31 mg/Litre
Length of fish	4.2 ± 0.5cm
Weight of Fish	2.5 ± 0.5gm

Table-4

Muscle glycogen in mg/gm. wet weight tissues of fish ***Oreocromis mossambicus*** during chronic cadmium exposure for period of 4 weeks.

Days of exposure	**Control**	**Concentrations of Cadmium mg/litre**		
Initial		0.015	0.03	0.05
0 Days	3.98	3.75	3.34	2.99
SD		0.115	0.32	0.495
PV		5.9508	17.4863	28.4075
7 Days	4.50	3.63	3.07	2.62
SD		0.435	0.715	0.94

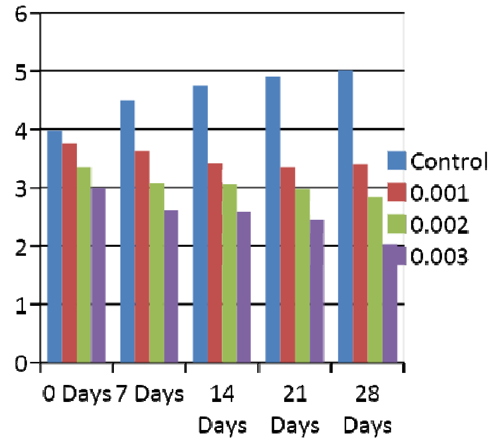
PV		21.4022	37.7807	52.809
14 Days	4.75	3.42	3.05	2.58
SD		0.665	0.85	1.085
PV		32.5581	43.5897	59.2087
21 Days	4.91	3.34	2.97	2.45
SD		0.785	0.97	1.23
PV		38.0606	49.2386	66.8478
28 Days	5.01	3.40	2.84	2.03
SD		0.805	1.085	1.49
PV		38.2878	55.2866	84.6591

SD=Standard deviation, PV=Percentage variation

Range of SD	**Confidence Level**
Less than 1	68.3%
Up to 1.645	90%
Up to 1.960	95%
Up to 2.576	99%
Up to 3.291	99.9%
Up to 3.891	99.99%
Up to 4.417	99.999%
Up to 4.892	99.9999%

Graph-2

Muscle glycogen in mg/gm. wet weight tissues of fish, ***Oreocromis mossambicus*** during chronic Cadmium exposure for period of 4 weeks.



Temperature	27°C
PH	7.3
DO	5.9mg|Litre
Free Chlorine	Nil
Total Acidity	3.4mg/Litre
Total Alkalinity	44 mg/Litre
Total Hardness as $CaCO_3$	31 mg/Litre
Length of fish	4.2 ± 0.5cm
Weight of Fish	2.5 ± 0.5gm

Physico-chemical parameters of water used for toxicological study.

Fig.1: Glycogen content in the liver of fish ***Oreochromis mossambicus*** when exposed to sub-lethal doses of cadmium for 4 weeks. **Table 3 :**Glycogen content in the muscle of fish ***Oreochromis mossambicus*** when exposed to sub-lethal doses of cadmium for period of 4 weeks. Table No.3 and 4 shows that level of glycogen decreased in the muscle and liver when exposed to cadmium.

In every living creature glycogen is the immediate and main source of energy .Dangeet.al., (1984) [2] suggested that to release glucose in to the circulatory system to meet the energy requirement The reduction in the glycogen content may be due to rapid breakdown of glycogen. Gaikwad (1981) [3] reported that in Tilapia mosambica chronically exposed to Thiodon, There is decrease in the glycogen content observed may be due to tremendous increase in energy demand. Soman (1987) [19] and Gopi (1992) [4] proved that in liver and muscle decline in the glycogen content in the fish Colisafasciata and Cyprinus carpio respectively. Ramalingam (1986) [11] studied the effect of DDT and Malathion on the carbohydrate metabolism of the Sarotherodon sp. He has suggested that a possible shift from aerobic to anaerobic metabolism, in which sugar were converted in to lactate via Pyruvate. Swaminathan et.al., (1990) [20] observed decline in tissue glycogen content of the liver and muscle and related it to the hypoxia condition under which stored glycogen might have been utilized by the fish Tilapia mossambica exposed to Thiodon. Mukhopadhyaya and Dehadrai (1980) [10] suggested that the increased glycogenolysis decreases glycogen content in the liver of Clarius batrachus exposed to Malathion. This view has been suggested by other workers. Reddy and Yellumma (1991) [13] monitored perturbation in the carbohydrate metabolism during Cypermethrin toxicity in Tilapia mossambica. They observed that decline in the glycogen content of the tissues which had been related to the enhanced oxidation through HMP- pathway. Ravindra Kumar (2000) [12] stated that in all tissues reduction in the carbohydrate content may be due to fast depletion of stored glycogen to provide energy for Mystusgulio under stress. Decline in the body muscle glycogen caused severe anaerobic stress resulting in the breakdown of tissue glycogen (McLeay and Brown, 1979) [9].Tripathiet.,al. (2003) [22] proved that depletion of glycogen may be due to direct utilization of energy generation, a demand caused by pyretheroid induced hypoxia. Sastryet.,al. (1984) [15] suggested that carbohydrate reserve depleted to meet energy demand in stress condition. The decrease in the glycogen and glucose suggested the possibility of active glycogenolysis and operation of glycolytic pathway as reported.

Conclusion

In the present studies observed that in **Oriochromis mossambicus** treated with cadmium altered the carbohydrate metabolism, the decrease in the glycogen content in both the liver and muscles, may be due to glycogenolysis in order to meet energy demand under the heavy metal cadmium toxicity stress as per the view expressed by other workers. The changes in the glycogen reserves of muscles and liver tissues under the effect of cadmium might result in impairments in energy requiring vital process. Hence give an idea about health status of the fish population.

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