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Effect of cadmium on glycogen content in muscle, liver, gill and kidney tissues of freshwater fish *Channa punctatus* (Bloch)

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Abstract
Aquatic environment gets polluted by heavy metals because of their environmental persistence and ability to bioaccumulate in aquatic organisms. Cadmium is a ubiquitous toxic heavy metal, biologically non-essential element, it is not biodegradable and has a very long biological half-life. The aim of the present study was to assess the glycogen content in muscle, liver, gill and kidney of *Channa punctatus* exposed to sublethal concentrations of cadmium chloride after 4, 7, 15 and 30 days of exposure. The results clearly showed significant decrease in the glycogen levels in the experimental fish *C. punctatus*. Decrease in muscle glycogen was found highly significant (P<0.001) after 30 days in both low concentration (36.823 mg/L) 6.12±0.41mg/g and in high concentration (73.646 mg/L) 4.04±0.32 mg/g in comparison to control. Decrease in liver glycogen content was found highly significant (P<0.001) after 30 days in high concentration 9.12±0.49 mg/g when compared with control. The decrease in gill glycogen content after 30 days exposure was found highly significant (P<0.001) 1.36±0.13 mg/g in low concentration and in high concentration 0.79±0.25 mg/g in comparison to control. Decrease in kidney glycogen content was found highly significant (P< 0.001) at 30 days in low concentration 3.92±0.05 mg/g and in high concentration 2.81±0.20 mg/g in comparison to control. The influence of toxicant cadmium chloride in selected tissues of fish was taken into account in evaluating fish response against stressor. Hence, we can use glycogen content as biomarker of cadmium stress in fish.

Keywords: Cadmium, *Channa punctatus*, Fish, Glycogen content, Heavy metal

INTRODUCTION

Pollution of water is an important dimension of environmental degradation. The contamination of aquatic ecosystem by toxic materials is closely connected with increased concentration of different types of pollutants. Intense activities in industrial and agricultural sectors has led to increased in the levels of heavy metals in natural waters (Gumgum *et al.,*1994; Nimmo *et al.,* 1998; Jordao *et al.,* 2002; Rauf *et al.,* 2009; Javed and Usmani, 2013). The contamination of water bodies with heavy metals has become a source of great concern not only because of their threat to aquatic life especially fishes (Opaluwa *et al.,* 2012; Bawuro, *et al.,* 2018; Ali *et al.,* 2019) but also due to the public health implications of such contaminations (Sim *et al.,* 2016; Baharom and Ishak, 2015).
Non degradable heavy metals are regarded as hazardous to aquatic ecosystem because of their environmental persistence and their tendency for bioaccumulation (Das *et al.,* 2001; Agrahari and Gopal, 2007). Cadmium (Cd) is considered as a nonessential element (Viarengo, 1985) that has gained great importance from the toxicological (USDHHS-ATSDR, 1993; Waisberg *et al.,* 2003; USEPA, 2016) and ecotoxicological (WHO, 1992) point of view. It has been listed in the “Black list” of European community (Mason, 1996). Cadmium occurs naturally in ores together with zinc, lead and copper. It is widely used in mining, metallurgical operations, electroplating industries and manufacturing vinyl plastics. Effluents from such activities are a source of cadmium into aquatic environment.
Fish, as they come into intimate contact with large amounts of polluted water can be used as early warning biological indicators of polluted environment (Viana and Lucena Frédou, 2014; Plessl *et al.,* 2017).

To understand the mode of action of toxicants on the aquatic animals biochemical investigations are done. Stress condition cause alterations in metabolic cycles, therefore it becomes necessary to understand the significance of these variations in the organic content of tissues. The present study was planned to investigate the effect of sublethal concentrations of Cd on glycogen content in muscle, liver, gill and kidney of *C. punctatus* after 4, 7, 15 and 30 days of Cd exposure.

MATERIALS AND METHODS

Live and healthy specimens of *C. punctatus,* a fresh water murrel, were procured from local resources having 15-18 cms length and 25±2gm weight and brought to the laboratory. Before introducing into the aquarium, fishes were treated with 0.1% $KMnO_4$ solution to avoid any cutaneous infection. Fishes were acclimated to laboratory conditions for a period of two weeks. During acclimation water was changed daily and fish were fed ad libitum. *Channa punctatus* was selected as the model organism for this study because of its availability, survival for longer period under laboratory conditions, easy handling etc. Fish were maintained following standard maintainence procedure (APHA, 2012) in glass aquaria.

Experimental design: A total of 108 fishes were used for the experiment. Fish were maintained in static renewal condition. They were divided into three groups of thirty six fishes each, which served as replicates. Each group was further subdivided into three subgroups having twelve fishes in each aquaria. The fish in the subgroup I and subgroup II were treated with $1/5^{th}$ LC_{50} (73.646mg/L) and $1/10^{th}$of (LC_{50} 36.823mg/L) respectively. Subgroup III served as control. Control group was not treated with Cd. The aquarium of both control and exposed groups were cleansed on every two days one hour after feeding period to reduce contamination with food remains. All experimental waters were completely renewed on every two days.

Three fishes from each subgroup were taken after 4, 7, 15 and 30 days of metal exposure and were sacrificed and tissues like liver, muscle, kidney and gill were excised rapidly and processed for biochemical estimations. The glycogen content in the tissues was estimated by the method of Nicholas *et al.* (1956). Values were compared using student 't' test.

RESULTS

After exposure to sublethal concentrations of Cd, the level of total glycogen content was altered in muscle, liver, gill and kidney of the fish, *C. punctatus*.

Muscle glycogen: Total glycogen content in muscle of control fish and fishes exposed to lower and higher concentrations were recorded as 7.84mg/g, 7.50mg/g and 7.15mg/g respectively after 4 days. At the end of 7 days of exposure, glycogen was noticed 7.98mg/g in control fish, 7.35mg/g in lower and 6.98mg/g in higher concentrations exposed fishes. Similarly at the end of 15 days of exposure, it was found to be 8.16mg/g in control, 7.10mg/g in lower concentration and 6.25mg/g in higher concentration exposed fishes. Whereas at the end of 30 days of exposure, it was noticed 8.32mg/g in control, 6.12mg/g in lower concentration and 4.04mg/g in fishes exposed to higher concentration. Decrease in muscle glycogen was found highly significant ($P<0.001$) in comparison to control after 15 days in high concentration and 30 days in both low (36.823 mg/L) and high concentrations(73.646 mg/L) (Table 1). These observations revealed that the decline in total glycogen content in muscle was directly proportional to the concentration of Cd and duration of exposure.

Liver glycogen: Total glycogen content in liver of control fish and fishes exposed to lower and higher concentrations were recorded as 12.05mg/g, 12.02mg/g and 11.52mg/g respectively after 4 days. At the end of 7 days of exposure it was noticed 12.20mg/g in control fish, 12.0mg/g in lower and 11.36mg/g in higher concentrations exposed fishes. Similarly at the end of 15 days of exposure, it was found to be 12.42mg/g in control, 11.15mg/g in lower concentration and 10.26mg/g in higher concentration exposed fishes. Whereas at the end of 30 days of exposure, it was noticed 12.56mg/g in control, 10.08mg/g in lower concentration and 9.12mg/g in fishes exposed to higher concentration.

The decrease in glycogen content was found slight significant ($P<0.01$) in low concentration (36.823 mg/L) exposed fishes at 7 days. At 15 days in low concentration it was highly significant ($P<0.001$). The decrease was slight significant ($P<0.01$) in low concentration (36.823 mg/L) and highly significant ($P<0.001$) in high concentration (73.646 mg/L) at 30 days of exposure (Table 2). The observations revealed that like muscle, decline in total glycogen content in liver was directly proportional to the concentration of Cd and duration of exposure.

Gill glycogen: Total glycogen content in gills of control fish and fishes exposed to lower and higher concentrations were recorded as 2.30mg/g, 2.23mg/g and 2.10mg/g respectively after 4 days. At the end of 7 days of exposure it was noticed 2.52mg/g in control fish, 2.40mg/g in lower and 2.14mg/g in higher concentrations exposed fishes. Similarly at the end of 15 days of exposure, it was found to be 2.56mg/g in control, 2.12mg/g in lower concentration and 1.82mg/g in higher concentration exposed fishes. Whereas at the end of 30 days of exposure, it was noticed 2.65mg/g in control, 1.36mg/g in lower concentration and 0.79mg/g in fishes exposed to higher concentration.

The decrease in glycogen content was found

slight significant (P<0.01) at 15 days in high concentration exposed fishes. After 30 days the decline was found highly significant (P<0.001) in fishes exposed to lower (36.823 mg/L) and higher concentration (73.646 mg/L) of Cd in comparison to control (Table 3).The observations revealed that the decline in total glycogen content in gill was directly proportional to the concentration of Cd and duration of exposure, though it was not so significant in the short duration exposures.

Kidney glycogen: Total glycogen content in kidney of control group and fishes exposed to lower and higher concentrations were recorded as 4.80mg/g, 4.68mg/g and 4.51mg/g respectively after 4 days. At the end of 7 days of exposure it was noticed 4.83mg/g in control fish, 4.50mg/g in lower and 4.26mg/g in higher concentrations exposed fishes. Similarly at the end of 15 days of exposure, it was found to be 4.91mg/g in control, 4.42mg/g in lower concentration and 3.84mg/g in higher concentration exposed fishes. Whereas at the end of 30 days of exposure, it was noticed 4.98mg/g in control, 3.92mg/g in lower concentration and 2.81mg/g in fishes exposed to higher concentration (Table 4).

The decrease in glycogen content was found slightly significant (P<0.01) at 15 days in high concentration (73.646 mg/L) exposed fishes. At 30 days in low (36.823 mg/L) and high concentration (73.646 mg/L) exposed fishes the decline was found highly significant (P<0.001). It is clear from the results that fishes exposed to lower and higher concentration of Cd showed decrease in glycogen content of kidney from the control values after 4, 7, 15 and 30 days of exposure, with maximum decrease after 30 days.

DISCUSSION

In the present study, glycogen content was depleted significantly in muscle, liver, gill and kidney tissues of *C. punctatus* after Cd exposure These findings are well supported by the observations of earlier workers who have exposed various experimental models for Cd for different durations (Sastry and Sunita, 1982; Reddy *et al*., 1989; Kamaraju and Ramasamy, 2011; Prabhahar *et al.,* 2012; Veeraiah *et al.,* 2013; Goswami *et al.,* 2016). The various experimental models used were *C. punctatus*, fresh water field crab *Barytelphusa guerini,* fish *Hypopthalmichthys molitrix, Cirrhinus mrigala* and *Tilapia mossibica.*

Sastry and Subhadra (1982) reported decrease in glycogen content of muscle and liver in *Heteropneustes fossilis* after 15 and 30 days Cd exposure however they found increased glycogen content after 60 days exposure in both tissues. Similar observations have been reported by Malik *et al,* (1998) after zinc exposure in *C. punctatus*, Jagadeesan (1990) after mercuric chloride exposure in *Anabas testudineus* fingerlings and Karuppasamy (2000) after phenyl mercuric acetate exposure in *C. punctatus*.

Same findings were reported by Cicik and Engin (2005) in *Cyprinus carpio,* Sobha *et al.* (2007) in *Catla catla* and Kamaraju and Ramasamy (2011) in *H. molitrix* in various tissues after exposure to Cd for different durations.

Sujata (2015) reported significant decrease in protein and glycogen levels in reproductive organs of freshwater fish *C. punctatus* exposed to sub lethal concentrations of Cd for 30 days.

Metal intoxication in fishes usually results in glyco-

Table 1. Alteration in total glycogen content in muscles (mg/g) of *C. punctatus* after exposure to different concentrations of Cd.

Duration of exposure (Days)	Control group Not treated with Cd	Experimental groups	
		Low concentration (36.823 mg/L)	High concentration (73.646 mg/L)
4	7.84±0.21	7.50±0.38^{NS}	7.15±0.31^{NS}
7	7.98±0.28	7.35±0.34^{NS}	6.98±0.23*
15	8.16±0.36	7.10±0.20*	6.25±0.16***
30	8.32±0.15	6.12±0.41***	4.04±0.32***

Values are Mean ± S.E., N=6; N= Number of observations for each value;*P<0.05 and ***P<0.001 (in comparison to control); NS=non significant

Table 2. Alteration in total glycogen content in liver (mg/g) of *C. punctatus* after exposure to different concentrations of Cd.

Duration of exposure (Days)	Control group Not treated with Cd	Experimental groups	
		Low concentration (36.823 mg/L)	High concentration (73.646 mg/L)
4	12.05±0.10	12.02±0.26^{NS}	11.52±0.38^{NS}
7	12.20±0.34	12.0±0.28**	11.36±0.45^{NS}
15	12.42±1.10	11.15±0.21***	10.26±0.20^{NS}
30	12.56±0.23	10.08±0.60**	9.12±0.49***

Values are Mean ± S.E., N=6; N= Number of observations for each value;**P<0.01 and ***P<0.001 (in comparison to control); NS=non significant

Table 3. Alteration in total glycogen content in gill (mg/g) of *C. punctatus* after exposure to different concentrations of Cd.

Duration of exposure (Days)	Control group Not treated with Cd	Experimental groups	
		Low concentration (36.823 mg/L)	High concentration (73.646 mg/L)
4	2.30±0.14	2.23±0.12 ^{NS}	2.10±0.35 ^{NS}
7	2.52±0.13	2.40±0.21 ^{NS}	2.14±0.24 ^{NS}
15	2.56±0.11	2.12±0.18 ^{NS}	1.82±0.19**
30	2.65±0.20	1.36±0.13***	0.79±0.25***

Values are Mean ± S.E., N=6; N= Number of observations for each value; **P<0.01 and ***P<0.001 (in comparison to control); NS=non significant

Table 4. Alteration in total glycogen content in kidney (mg/g) of *C. punctatus* after exposure to different concentrations of Cd.

Duration of exposure (Days)	Control group Not treated with Cd	Experimental groups	
		Low concentration (36.823 mg/L)	High concentration (73.646 mg/L)
4	4.80±0.13	4.68±0.04 ^{NS}	4.51±0.12 ^{NS}
7	4.83±0.18	4.50±0.02 ^{NS}	4.26±0.31 ^{NS}
15	4.91±0.24	4.42±0.29 ^{NS}	3.84±0.14**
30	4.98±0.12	3.92±0.05***	2.81±0.20***

Values are Mean ± S.E., N=6; N= Number of observations for each value; **P<0.01 and ***P<0.001 (in comparison to control); NS=non significant

gen depletion and is reported in several species of fishes such as *H. fossilis* (Qayyum and Shaffi, 1977), *Sarotheradon mossambicus* (Akhilendra Naidu, 1982), *C. punctatus* (Sastry and Sunita, 1983), *Labeo rohita* (Bengery and Patil, 1986), *L. rohita* (Radhakrishnaih *et al.,* 1992), *Perca flavescens* (Levesque *et al.,* 2002), *C. mrigala* (Bhilave *et al.,* 2008).

It is considered that protein and carbohydrate stores are mobilized to a varying degree as a compensatory mechanism in response to energy stress during acute Cd exposure (Xuan *et al.,* 2011). Most of the investigators have found that heavy metals cause glycogen depletion but the glycogenolytic response by different species of fish varies.

Like all other vertebrates, fish also store glucose in the form of glycogen in the liver, skeletal muscle, myocardium and brain (Leibson and Plisetskaya, 1968). When required, the glycogen from these stores is broken down (Glycogenolysis) and transported to the muscle as glucose. On reaching the muscle, the glucose may be used at once or reconverted into glycogen.

A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant. Some workers have also suggested that heavy metals could decrease the glycogen reserve in fish (Levesque *et al.,* 2002) by affecting the activities of enzymes that play a role in carbohydrate metabolism. Sastry and Subhadra (1982) reported decrease in glycogen reserve of *H. fossilis* by stimulating the glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase.

Decrease in carbohydrates is probably due to glycogenolysis and utilization of glucose to meet increased metabolic cost as suggested by Viswarajan and Muthukrishnan (1988) in *Oreochromis mossambicus* under the stress of tannic acid. Several other reasons have been suggested for the decreased glycogen level in fishes after exposure to metals such as acute hypoxia (Heath and Pritchard, 1965) and neuroendocrine stimulation of fish under stress of metal exposure which in turn causes disturbances in carbohydrate metabolism (Mazeand *et al.,* 1977). The duration taken in the present study of 4, 7, 15 and 30 days of Cd exposure to determine the glycogen content of *C. punctatus* makes this work different from others.

Conclusion

In the present investigation, the depletion of glycogen in muscle, liver, gill and kidney tissues of *C. punctatus* was directly proportional to the concentration of Cd and duration of exposure which clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to Cd. Glycogen content can be used as a biomarker of Cd stress in fish. There is need to focus on harmful influences of heavy metals on biochemical activities of aquatic organisms and on the environment at large.

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