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The Effect of Chromium on Glucose Content of Freshwater Fish, *Heteropneustes fossilis*

Tahmina Hoq[1,*], Rakibul Hasan[1], Nazmul Haque[2], Md. Ashrafuzzaman[3]

[1]Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka-1205
[2]Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202
[3]Food Instructor, Department of Food Technology, Gopalganj Polytechnic Institute, Gopalgonj
*Corresponding author: tahminahoq_ami@yahoo.com

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Abstract Heavy metal contamination have devastating effects on the ecological balance of the recipient environment and on diversity of aquatic organisms. Heavy metal like Chromium as an environmental stress or may alter the glucose content in fishes. Alternation of blood glucose level is the primary metabolic symptoms that can be noticed in organisms subjected to stressful situations. In the present investigation the effect of a heavy metal Potassium Di-Chromate on the blood glucose level of freshwater major freshwater fish, Heteropneustes fossilis have been studied at different concentration for three days. A group of ten fish were subjected to serial dilutions of the stock solution of $k_2Cr_2O_7$ ranging from 20 ppm to 600 ppm in twelve large plastic bowls of 25 liter capacity by the semi-static (renewal) method. At the end of the exposure period, blood samples were taken from the control and experimental fish. Blood glucose level was monitored. The study showed that the values of blood glucose have a positive correlation with concentrations of this metal. The glucose levels were found higher in the exposed fish when compared to the control. In conclusion, the changes observed indicate that glucose level can be used as an indicator of chromium related stress in fish.

Keywords: *Potassium Di-Chromate ($k_2Cr_2O_7$), Chromium, Heteropneustes fossilis, toxicity, Blood glucose level*

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1. Introduction

Heavy metals are introduced into the environment by both natural sources and anthropogenic ones including industrial wastes as well as a leakage. Some of these metals including lead, nickel, cadmium, mercury are toxic to living organisms even at quite low concentrations, while others such as copper, iron, zinc and manganese are biologically essential and natural constituents of the aquatic ecosystems and become toxic only at very high concentrations [1,2,3]. Another heavy metal, Chromium is considered as pollutant as well as an essential micronutrient. Waste water pollution by chromium originating from electroplating, dyeing, tannery, hard-alloy steel and stainless steel manufacture, has affected the life on earth. Chromium is also used as a catalyst and coating material [4]. Welding, grinding and polishing of stainless steel are among principal ways of introducing chromium into the land environment [5]. This pollution could affect all ecosystems and human health directly or through food chain [6]. Fishes are often at the top of the aquatic food chain and may concentrate large amounts of certain metals from water [7]. Since, heavy metals are non-biodegradable; they can be bio-accumulated by fish, either from the surrounding water or by ingestion of food [8,9]. In addition, [10] indicates that when metals reach sufficiently high concentrations in body cells they alter the physiological function in the fish. Changes in physiological and biochemical blood indices induced by environmental conditions and presence of contaminants have investigated here by studying the glucose level. Thereby, the blood glucose level in fish are valid for physio-pathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant damage [11,12,13].

2. Materials and Methods

Adult live fish, *Heteropneustes fossilis* were collected from the local market and brought to laboratory. Only healthy fishes were acclimatized in 12 plastic aquariums with twenty–five liters capacity each for 15 days and were fed with artificial feed and ground shrimps obtained locally to avoid possible effects of starvation. Fish of both sexes were used without discrimination. The length of the fish varied from 12.2 to 13.9 cm and the weight 8.6 -13.44 g. The mean of both length and weight are shown in Table 1. Water in the aquaria was replaced by fresh water at every 24 hrs to maintain the standard quality for acclimatization and the physico-chemical conditions of water was viz. dissolved oxygen : 6.75 mg/l, free CO_2:

43.6 mg/l, pH: 6.4, alkalinity: 145 ppm, hardness: 100 ppm, nitrate: 0.60 ppm. Stock solution of Potassium Di-Chromate was prepared by dissolving appropriate amount of $k_2Cr_2O_7$ as Cr salt in distilled water. The fish *Heteropneustes fossilis* were exposed to Cr (as $k_2Cr_2O_7$) to know the acute toxicity at 24, 48 and 72 hrs. For selection of test concentration, some pilot tests were carried out. The range of concentration was selected between 0 to 100% mortality shown in Table 2. The mortality rate of *Heteropneustes fossilis* was recorded at 24, 48 and 72 exposure to the heavy metal. The percentage for corrected mortality was calculated using the Abbott's formula (1952) in Table 3.

Table 1. The length and weight of the fish.

Parameters	Range	Mean
Length (cm)	12.2 -13.9	13.05
Weight (g)	8.6 -13.44	11.02

The fish were divided into 2 groups of 12 fish each and treated as follows: Group 1 (control) was exposed to tap water only; while Groups 2 (the length and weight of them shows in Table 1) was exposed to 25 ppm to 500 ppm $k_2Cr_2O_7$. The test was performed by the semi-static (renewal) method. The parameters of the diluting water used to reach the desired concentration of test chemical were determined by standard methods.

The exposure period lasted 96 hours, after which blood samples were taken from the control and experimental fish. The blood samples were taken by puncturing the caudal vessels, using EDTA (ethylene-diamine-tetra-acetate) as anticoagulant and then centrifuged at 3500 rpm for 10 min to obtain serum samples for the analysis of blood glucose. The glucose levels in the serum samples were analyzed using 'One tech ultra^{TM}-2' device. To use it, 10 µl serum samples were added to sticks and inserted to the machine. The reading was recorded every time.

3. Results and Discussion

To test the toxicity of $k_2Cr_2O_7$, two trials were done. It was done in the range of concentration of $k_2Cr_2O_7$ within 20 ppm to 800 ppm (Table 2). From the Table 2, the cumulative mortality rate was calculated which presents that the fish can be killed by the dose above 50 ppm.

Table 2. Range of LC_{50} values for $K_2Cr_2O_7$ at 24h, 48h and 72h

Conc. of $k_2Cr_2O_7$ (ppm)	No of total fish	No of dead fish (24h)	No of dead fish (48h)	No of dead fish (72h)
Control (0)	10	0	0	0
20.00	10	0	0	0
50.00	10	0	0	0
100.00	10	0	01	01
150.00	10	02	02	03
200.00	10	02	03	04
300.00	10	03	04	04
400.00	10	04	06	08
600.00	10	08	08	09
700.00	10	10	10	10
800.00	10	10	10	10

Table 3. Range of LC50 values for $K_2Cr_2O_7$ at 24h, 48h and 72h(Trial 1, n=50)

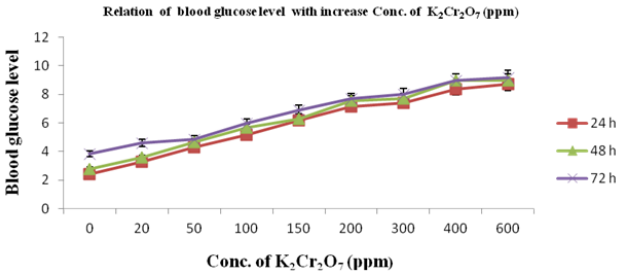
Conc. Of $k_2Cr_2O_7$ (ppm)	Cumulative Mortality Rate (%)		
	24h	48h	72h
Control (0)	0	0	0
20.00	0	0	0
50.00	0	0	0
100.00	0	10	10
150.00	20	20	30
200.00	20	30	40
300.00	30	40	40
400.00	40	60	80
600.00	80	80	90
700.00	100	100	100
800	100	100	100

Each time the blood glucose level was recorded. The records are shown in the following data and also in graph.

Table 4. Blood glucose level with increasing $K_2Cr_2O_7$

Conc. of $K_2Cr_2O_7$ (ppm)	24 h	48 h	72 h
Control (0)	2.39	2.78	3.83
20	3.27	3.58	4.6
50	4.3	4.67	4.88
100	5.15	5.65	5.96
150	6.18	6.3	6.88
200	7.15	7.53	7.69
300	7.39	7.68	8.03
400	8.35	8.98	8.98
600	8.7	8.96	9.2

This data is shown graphically to denote the increasing amount of glucose in blood.



Graph 1. Relation of Blood glucose level with increase Conc. Of $K_2Cr_2O_7$

The present study showed gradually increasing concentrations of blood glucose level of the treated fish

after (24, 48, 72,) hrs. for different concentration. This increasing is due to glycolsis and this took place as response to stress and transformation to glucose for energy requirement, by fish. This was supported by [14] showed that the increasing of glucose level is due to high secretion of hormones like catecholamines, glucocorticords and that lead to increasing of glycolysis resulting to high glucose level in blood. Fish, like other vertebrates, respond to a stressor by eliciting a generalized physiological response, which is characterized by an increase in stress hormones and consequent changes that help maintaining the animal's normal or homeostatic state [15,16]. This response includes, for example, increases in plasma cortisol, catecholamines and glucose levels, increases in branchial blood flow and increases in muscular activity [17]. A change of plasma glucose was observed in this study. It was found to be insignificant at low concentrations of chromium but acts according to time. This interesting finding is supporeted by [18]. Though. However at high concentrations, it exerts adverse effects by accruing structural damage, which affects the growth, development and survival of the fish. Thereby blood glucose has been employed as an indicator to environmental stress [19]. In conclusion, the changes in the blood glucose indicate that they can be used as indicators of chromium related stress in fish on exposure to elevated chromium levels in the water.

4. Conclusion

The exposure of *Heteropneustes fossilis* to water contaminated with heavy metal cromium was found to be toxic to the fish. This is worrisome as the water bodies of Bangladesh were found to contain considerably high concentrations of cromium ions. Though this contamination may have very severe negative consequences for the fish population and in turn may affect the source of animal proteins for human populations, it is relieving to check out the toxicity of this metal ion by elevated blood glucose level. Further studies are required to appreciate better this observed effect of the ion with a view to exploiting it in environmental biotechnology, with respect to the remediation of heavy metal polluted fresh water bodies.

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