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Studies on Changes in Biochemical Parameters Of Channa Punctatus Following Prolonged Hexavalent Chromium Exposure

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Abstract: *Over the last few decades the contamination of fresh water with a wide range of pollutants has become a matter of concern. Metal contamination accounts for potential threat for aquatic organisms, which are exposed to significant amounts of heavy metals as a consequence of Industrial, Agricultural and Anthropogenic activities. Heavy metals at high concentration can be hazardous to metabolic, physiological and biochemical system of fish, which in turn affects human beings. The aim of the present study is to investigate the changes in biochemical parameters of Channa Punctatus exposed to hexavalent chromium.*
Key Words: *Channa Punctatus, Hexavalent Chromium, Biochemical Parameters.*

I. Introduction

The survival of plants and animals is fully dependent on the freshwater, which includes lakes, ponds, streams and rivers. There is growing recognition that functionally intact and biologically complex freshwater ecosystems provide many economically valuable commodities and services to society. Kris-Etherton (2002) highlighted in his study that among the aquatic biota, fishes are given supreme importance as they contribute the cheap and best source of protein in human diet.

Ecological services are costly and often impossible to replace when aquatic ecosystems are degraded. Yet today, aquatic ecosystems are being severely altered or destroyed at a greater rate than at any other time in human history, and far faster than they are being restored [National Research Council 1992]. Usually the aquatic ecosystem is polluted by few natural sources present in it. However, most freshwater ecosystems are becoming unbalanced by factors due to human activities [Mona Zaki et al 2015].

In the past century, the problem of environmental pollution is increased due to pace of the industrialization, use of advanced technologies and modern life style products. All these practices cause damage to the freshwater ecosystems in one or other ways [Javed and Usmani 2013]. The aquatic environment is badly polluted by the waste released from industries or factories constructed near the water bodies which deteriorate not only the quality of water but also harm the aquatic animals [Javed and Usmani 2013].

Mostly high pollutants of different nature are produced in the form of effluents by the tannery industries [Praveena and Jayanth Rao 2013]. In addition to a large quantity of organic compounds, industrial effluents contains chromium, cadmium, lead, sulphides, ammonia, chlorides and other salts. These toxic compounds greatly disrupt biological processes when discharged into air, water and soil which get into food chain [Gupta 1998]. Chromium, a potential pollutant, is well known for its mutagenicity [Cheng and Dixon 1998], and carcinogenicity [Shumilla et al 1999] when extensively used in tanning industries have resulted in contamination of soil and ground water at production sites which pose a serious threat to human health, fish and other aquatic biodiversity [Turick et al 1996]. Accumulation of the toxic chemical pollutants are known to adversely affect the liver, kidney, muscles and other tissues of fish. In the present study, attempts have been made to investigate the quality of water collected from ambur (industrial area) and to study the changes in biochemical parameters of Channa punctuaus which is exposed to hexavalent chromium.

II. Materials And Method

Collection of Water Samples

For the assessment of surface water quality, water samples were collected in polyethylene bottles from water bodies distributed in Ambur and Thirumullaivoyal area and labelled as test sample (T) and control sample (C) respectively and was assessed in CPR Environment Education Centre, Chennai.

Analysis of Water Samples

Both the samples were preserved according to the standard methods Ref. Their pH, salinity and electrical conductivity were measured with a conductive meter. The samples were filtered and acidified with nitric acid to maintain the pH of the sample (2) for trace metal analysis using atomic absorption spectrophotometer. Chromium, sulphate, phosphate calcium, manganese, magnesium, calcium chloride, iron, fluoride and nitrate were analysed by gravimetric methods.[Thangarajan 1999, Vosyliene 1996, Witeska 1999].

Fish Sample Collection And Maintenance

Channa punctatus were used for toxicity tests. The fish samples were collected from the same area (Ambur and Thirumullaivoyal) and were labelled as test (fish from ambur) and control (fish from Thirumullaivoyal) and was authenticated by Dr. Rema Devi 'E' Scientist Zoological Survey of India. They were collected in oxygenated polyethylene bags and transported to the laboratory. The fish samples were immediately transferred into glass aquaria of 100- litre capacity containing well aerated ground water. They were allowed to accimilate for 15 days before the commencement of the experiment. They were fed with rice bran during the accimilation period. The fishes were subsequently transferred to 50-litre glass aquaria for easy handling during the experiment [Donald and Grosell 2006, Nriagu 1998, Palm et al 1992, Yang et al 2003]. Only fishes which were healthy and showed active movements used for the experiment. No differentiation was made between the sexes.

III. Blood Sample Collection

Ten fishes from each group (test & control) were removed and blotted. The blood samples were collected by heart puncture method [Neill et al 1998, Singh and Reddy 1990]. Three replicates of blood were drawn and transferred into sterilized test tubes containing EDTA and maintained at room temperature for coagulation. After coagulation, the coagulated and uncoagulated part were separated and centrifuged for 10 minutes at 3000 rpm [Heath 1987, Vosyliene and Suecevicius 1997]. The supernatant containing plasma and serum respectively were used for analyzing glucose, urea and enzymes such as ALT, AST, ALP, LDH, CPK and α-amylase by chemo auto analyzer [Canli 1996, Markovich and James 1999, Vosyliene 1999].

IV. Analysis Of Biochemical Parameters

In the test and control samples the following biochemical parameters were analysed.

S.No	Parameters	Method	Reference
1	Estimation of glucose	Glucose oxidase –Peroxidase method	Trinder 1969
2	Estimation of urea	Diacetyl monoxime method	Shivananda Nayak 2005
3	Analysis of Alanine transaminase	Splittstoesser method	Splittstoesser 1976
4	Analysis of Aspartate transaminase	Bergmeyer and Bernt method	Bergmeyer and Bernt 1974
5	Analysis of Alkaline phosphatise	Balasubaramanian method	Balasubaramanian 1983
6	Analysis of Lactate dehydrogenase	King method	King 1965
7	Analysis of Creatine phosphokinase	NAC method	Oliver 1985
8	Analysis of α-amylase	Kaplan method	Sadasivam 1996

V. Statistical Analysis

The values are given as Mean ± S.D.

VI. Results And Discussion

In the present study, the biochemical parameters of Channa punctatus surviving in water contaminated with industrial effluent was compared with same species surviving in fresh water system.

Assessment of Water Quality

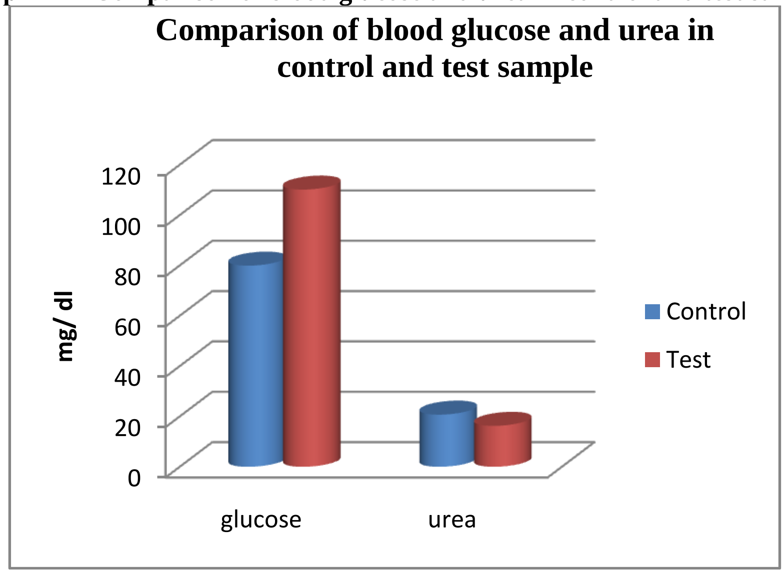
Table 2 describes the water quality parameters of control and test sample which showed the physicochemical characteristics and concentration of heavy metals. The heavy metal chromium was present in high concentrations in the test sample.

Table 2: Assessment Of Water Quality

S.No	Parameter	Unit	Control Sample	Test Sample
1	Nature of the sample	-	Ground water	Effluent
2	Colour	Hazen	Nil	Dark ash
3	Odour	-	Nil	Sewage smell
4	Turbidity	-	Nil	7.0
5	pH	µS/cm	7.3	8.3
6	Conductivity	mg/L	1663.00	19240.00
7	Total dissolved solids	mg/L	1081.00	12506.00
8	Total alkalinity as $CaCo_3$	mg/L	380.00	1340.00
9	Chloride	mg/L	100.00	300.00
10	Total hardness as $CaCo_3$	mg/L	600.00	1140.00
11	Calcium	mg/L	128.00	256.00
12	Magnesium	mg/L	68.00	122.00
13	Sulphate	mg/L	112.00	375.00
14	Phosphate	mg/L	1.50	35.00
15	Nitrate	mg/L	83.00	95.00
16	Fluoride	mg/L	1.50	8.10
17	Iron	mg/L	0.07	1.96
18	Manganese	mg/L	0.08	3.30
19	**Chromium (hexavalent)**	**mg/L**	**Nil**	**1.20**

Graph 1 shows the level of glucose and urea in control and test sample of Channa punctatus. It is clear from the graph there is an appreciable increase in blood glucose level. The change in blood glucose might be related to renal injury, liver damage and lack of nutrition. This was supported by the work of Canli that showed the glucose level in fresh water fish increased significantly by chromium [Canli 1995] and he also concluded in his studies that glycogen level in fish living under environmental stress might be depleted as a result of extra energy demand in the metabolism that may also reflect on increased glucose level [Canli 1996]. In another study it was reported that metals alter the carbohydrate metabolism, and increase in glucose content may be occurred as a result of glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids [Zikic et al 2001].

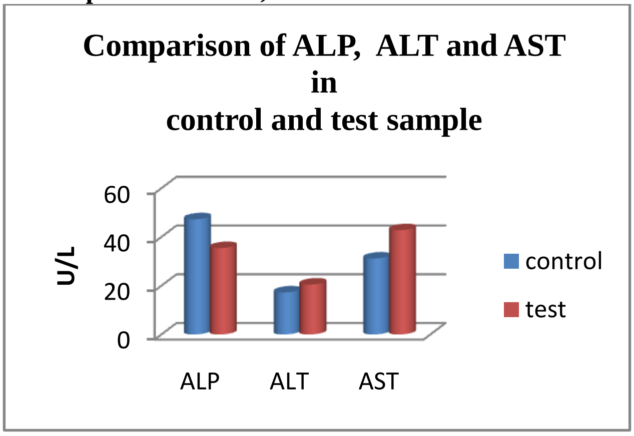
"Graph 1" – Comparison of blood glucose and urea in control and test sample.



The result also shows reduced urea concentration in the test sample compared to that of control sample, which is similar to the study done by Muazzez Oner et al (2008). The bioaccumulation of heavy metals triggers the oxidative stress in liver cells by the generation of reactive oxygen species. The defensive surface proteins antagonize the toxic radicals resulting in the elimination of protein from the liver cells. A valuable indicator in the diagonosis of fish toxicity is the lowered level of blood protein in plasma, muscle and liver The present findings are in good agreement with previous reports of decreased level of soluble protein and thereby decreased urea level.

Graph 2 shows the level of alkaline phosphatase, alanine transaminase and aspartate transaminase in control and test sample of Channa punctatus.

"Graph 2" – Comparison of ALP, ALT and AST in control and test sample.

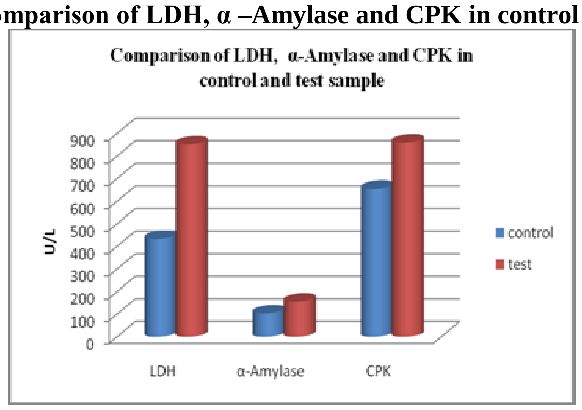


Alkaline phosphatase is a polyfunctional enzyme acts as transphosphorylase at alkaline pH and plays an important role in mineralization of the skeleton of aquatic animals and in membrane transport activities [Bernt et al 2001, Lan et al 1995]. The reduced alkaline phosphatase activity was found in test sample compared to control sample. Similar report of decreased alkaline phophatase activity was shown in Salmo trutta which are attributed to disturbance of the mitochondrial transport system [Bernt et al 2001]. In an another study Jiraungkoorskul et al (2003) showed that the change in ALP activity was a result of physiological and functional alteration in metal exposed fish.

It is clear from the results, the activity of aspartate transaminase and alanine transaminase was increased in test sample than control sample. Transaminases like AST and ALT plays a significant role in protein and amino acid metabolism and is released into plasma following tissue damage and dysfunction. Singh and Reddy demonstrated that 0.25 mg Cu/L caused significant increase in serum AST and ALT activities in Heteropneustes fossilis with increased exposure period [Singh and Reddy 1990]. Zikic et al showed increased plasma AST and ALT activities in cadmium exposed fish Carassius auratus gibelio [Zikic et al 2001].

Graph 3 shows the activity of lactate dehydrogenase, α-amylase and creatine phosphokinase in control and test sample of Channa punctatus. The result reveals increased LDH activity in test sample which is comparable to the study done by Shiffman et al (1959) where the elevation of lactate dehydrogenase in muscle and liver of fresh water fish after the exposure of hexavalent chromium in Salano gairdneri [Shiffman et al]. LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in Embden Meyerhoff pathway. This enzyme shows an increased activity during strenuous exercise. The increased level may be due to an alternative pathway in conversion of lactate to pyruvate for the production of glucose, which is a major source of energy during stress induced by heavy metals [Vagilo and Vandriscina 1999].

"Graph 3" – Comparison of LDH, α –Amylase and CPK in control and test sample



α-amylase and CPK activity is also increased in test sample. Acute pancreatitis can be diagnosed by determining serum amylase activity [Lott and Lu 1991] which demonstrates that hyperamylasemia might be caused by cancer-destructed excretory function, inflammation and gall stones which leads to excretion of enzymes in blood [Yang et al 2005]. CPK is found in high concentration in skeletal muscle, myocardium and brain which appears to be sensitive measure of myocardial infarction and muscle diseases, but remains normal in liver diseases [De La Torre et al 2000].

VII. Conclusion

Metal concentration in the aquatic environment is seen as potential threat for aquatic organisms that are exposed to significant amount of heavy metals as a consequence of industrial, agricultural and anthropogenic activities. The results of the present study shows that the water in the Ambur area is contaminated with hexavalent chromium, which causes metabolic, physiological and biochemical alteration in fishes thereby affecting human population.

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