Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 71 (2017) 144-149 EISSN 2392-2192

Short-term Impact of Chromium on Erythrocytic Profiles of *Channa punctata* (Bloch, 1793)

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ABSTRACT

The tannery industries add chromium as one of the heavy metals to the aquatic environment and tannery waste waters continue to cause negative effects on the aquatic fauna. In the present study, the erythrocytic alterations produced on short-term exposure to sub-lethal concentration of chromium (20 mg/L) were investigated in fresh water air-breathing fish, *Channa punctata* (Bloch, 1793) for 24h, 48h and 72h respectively. The 96h LC_{50} of chromium salt, potassium dichromate was determined to be 33.125 mg/L. The results revealed statistically significant decrease in Total Erythrocytic Count (TEC), Haemoglobin (Hb) and Haematocrit (Hct %) in all experimental animals when compared to the control with an increase in exposure periods. The absolute corpuscular values like Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) also exhibited significant increase in fishes of experimental groups. However, the Mean Cell Volume (MCV) contrarily exhibited a fluctuating pattern. The depression of erythrocytic parameters clearly indicates that the fishes have become anaemic due to sublethal exposure of chromium.

***Keywords*:** heavy metals, chromium, *Channa punctata*, erythrocytic parameters, *Labeo rohita*

1. INTRODUCTION

Water pollution by heavy metals has become a health hazard in recent years. With industrialization, heavy metals are released into natural waters in large quantities. Due to their

(Received 05 April 2017; Accepted 01 May 2017; Date of Publication 03 May 2017)

high toxicity even in low concentrations, harmful effects may be produced in aquatic organisms, including teleosts. Chromium is extensively used in chrome plating, tanning and as a corrosion inhibitor in cooling tower operations. The wastewater generated by tanneries is a major source of chromium which contains Chromium (Cr^{6+}) and their indiscriminate introduction in the aquatic ecosystem pose a serious threat to the growth and survival of the fish population. A considerable amount of experimental data on chromium toxicity to aquatic life was reviewed (Irwin *et al.*, 1997) but the data on chromium toxicity to Indian teleosts are scarce and are mostly restricted to the effects on biochemical or enzymological profiles (Sastry & Tyagi, 1982; Vutukuru, 2003). The present work was undertaken to examine the short-term acute toxicity of this heavy metal to a freshwater murrel, *Channa punctata* and deals with alterations in the erythrocytic parameters viz.TEC (Total Erythrocytic Count), Hb (Haemoglobin), Hct% (Haematocrit) and absolute corpuscular values like Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC).

2. MATERIAL & METHODS

Live specimens of adult *Channa punctata* (Family: Channidae) of length 13.78 ±0.33 cm and 39.36 ±2.35 g weight, were procured from clean and unpolluted local freshwater sources of the fish market. They were acclimatized in laboratory glass aquaria in clean tap water for 5 days prior to experimentation. Water used in the aquaria had a pH: 7.2 ± 0.05, water temperature: 23 ±2 °C, DCO_2: 3.4 ± 0.05 mg/L, DO: 7.5 ±1 mg/ L. APHA (2005) method was followed for the water quality determination. Fish were fed, *ad libitum*, with live *Tubifex* sp. and commercial dry pellets during the acclimatization period only. Two-third of the water was renewed every day to avoid accumulation of unutilized food or metabolic waste products. Analytical grade Potassium dichromate ($K_2Cr_2O_7$) by BDH (India) was used as a metal toxicant throughout the experiment and for the determination of LC_{50} of chromium. The LC_{50} value was determined according to the arithmetic method of Karber as adopted by Dede & Kaglo (2001) using the daily renewal bioassay system. Five test concentrations of narrow range viz. 30, 35, 40, 45 and 50 mg/L respectively and a control (without chromium) were selected to find the 96 h LC_{50} value of the chromium. Eight fish were placed in each of the aquarium and duplicates were maintained for the each of the treatment as well as for control. In this experiment, no distinction was made between the sexes and no feeding was allowed. Dead fishes were removed from the aquaria immediately. The whole exposure medium was monitored on a regular basis with a view to maintain the desired concentration of chromium.

The percentage of mortality was recorded at 96 h interval for each of the test concentrations which was used to estimate LC_{50} value of the $K_2Cr_2O_7$ at 96h. The fishes were divided into four groups each containing 8 individuals, one group was taken as control, with no chromium added to the water, and other 3 groups were exposed to chosen sublethal dose of chromium (20 mg/L) for 24, 48 and 72 h respectively after determining LC_{50} value at 96h. During the exposure, mortality of the fish, if any, was monitored. Different blood parameters viz., TEC, Hb and Hct were measured following the methods earlier described (Hesser, 1960). Some modifications were made following the methods of Blaxhall & Daisley (1973), Dacie & Lewis (1984). After stipulated exposure periods, the control and experimental fishes were wiped dry before collecting the blood samples. Free flowing blood was collected by severing the caudal peduncle of fish without using anesthesia. TEC and TLC were analyzed following

by standard clinical method, with the help of improved Neubaur double haemocytometer (Fein-OPTIK, Blankenburg, G.D.R.) using Hayem's solution as diluting fluid. The haemoglobin content (g %) of blood was determined with the help of Sahli's Haemometer. The Hct and Lct values were estimated by microhaematocrit method (Wintrobe, 1967) using microhaematocrit capillary tubes and a microhaematocrit centrifuge (3000 g for 25 min). The values of MCV, MCH and MCHC were calculated from the red cell count (TEC), haemoglobin (Hb) and haematocrit (Hct %) values using standard formulae. The results were presented as mean and standard error (mean ± SE). Independent sample t-test was used to distinguish between means of significant differences. Treatments were taken to be significantly different where $P < 0.05$ and highly significant where $P < 0.01$.

3. RESULTS

The 96h LC_{50} of chromium salt, $K_2Cr_2O_7$ for *C. punctata* was determined to be 33.125 mg/L. The fish exposed to sublethal dose of chromium (i.e. 20 mg/L) exhibited erratic swimming and loss of equilibrium gradually. The exposure of *C. punctata* to sublethal concentration of chromium for 24h, 48h and 72h caused remarkable and significant alterations in erythrocytic parameters as represented in Tables 1 and 2. As shown in Table 1, the values of TEC ($\times 10^6$ /cmm) and haemoglobin concentration (g %) were found to be significantly decreased ($p < 0.01$) especially after 48 h and 72 h exposures of chromium when compared with control. A sharp decline ($p < 0.01$) of haematocrit count (Hct %) was observed from the first day onwards compared to control (Table 1).

Table 1. Changes in few erythrocytic parameters (TEC, Hb and Hct) of control and experimental freshwater fish, *Channa punctata* exposed to sublethal dose of chromium (20 mg/L)

Erythrocytic parameters	Control	Experimental fish groups		
		24h	48h	72h
TEC ($\times 10^6$) /cmm	3.07 ± 0.11	2.66 ± 0.41	2.15 ± 0.03 **	1.43 ± 0.13**
Hb (g %)	11.3 ± 0.35	10.12 ± 0.47	8.63 ± 0.46**	7.5 ± 0.11**
Hct (%)	24.43 ± 0.04	15 ± 0.73**	14 ± 0.57**	14.25 ± 0.65**

Values are expressed as Mean ± S.E., n = 6. ** = significant at p < 0.01 level.

In Table 2, MCV values were found to be significantly low ($p < 0.05$) after initial exposure but gradually increased later on. MCH were found to be significantly different in chromium exposed groups (72h) when compared with control. MCHC values were significantly high ($p < 0.01$) in treated fish groups especially during 24h and 48h of exposure.

Table 2. Changes in absolute corpuscular values (MCV, MCH and MCHC) of control and experimental freshwater fish, *Channa punctata* exposed to sublethal dose of chromium (20 mg/L)

Erythrocytic parameters	Control	Experimental fish groups		
		24h	48h	72h
MCV (fl)	79.91 ± 2.25	63.21 ± 3.02*	65.16 ± 9.02	102.88 ± 7.58*
MCH (pg)	37.09 ± 2.08	45.53 ± 6.86	40.22 ± 2.47	54.92 ± 5.41*
MCHC (g/L)	46.28 ± 1.42	68.55 ± 5.0**	61.81 ± 2.79**	53.24 ± 2.81

Values are expressed as Mean ± S.E., n = 6. * = significant at $p < 0.05$ level, ** = significant at $p < 0.01$ level.

4. DISCUSSION & CONCLUSIONS

Haematology is used as an index of fish health status in a number of fish species to detect different stress conditions like disease, hypoxia, and exposure to metals and pollutants etc (Blaxhall, 1972). In general, the results of the present study revealed that hexavalent chromium induces effects on erythrocytic parameters of the fish. The significant reduction in TEC coupled with low Haemoglobin content may be due to destructive action of heavy metal on erythrocytes which was also reported by earlier workers (Karuppasamy, 2000; Bela Zutshi *et al.*, 2010). The decreased Haemoglobin count indicates the inability to provide sufficient oxygen to the vital organs in fish.

The erythrocytic fragility following exposure to heavy metals might explain the initial anaemic condition observed in this short-term study. Prolonged reduction in haemoglobin content is harmful to oxygen transport and degeneration of the erythrocytes could be described as pathological condition in fishes exposed to heavy metals (Pamila *et al.*, 1991). The anemic condition of this experimental fish groups were further detected by haematocrit study. A distinct decrease in the level of Haemoglobin and Hct % after exposure to heavy metal chromium clearly suggests a haemodilution mechanism possibly due to gill damage or impaired osmoregulation. This study is in line with Smit *et al.* (1979) that heavy metal exposure exerts the decrease in RBC count, Hb and Hct values and that may be due to the impaired intestinal absorption of iron. Significant changes were recorded in the mean MCV, MCH, MCHC values and similar results have been reported in *Labeo rohita*, exposed to chromium (Venkatachalam & Natarajan, 2014). Cells released from the affected spleen, an erythropoietic organ in fish, would have lowered MCV values initially. A similar observation was reported in *C. carpio* after cadmium exposure (Koyama & Ozaki, 2002).

In conclusion, the present study clearly indicates that chromium in the form of $K_2Cr_2O_7$, a toxic heavy metal, experimentally introduced into aquatic environments induces severe anaemia and alterations in erythrocytic indices in the fresh water air-breathing fish, *Channa punctatus* at short-term exposures.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Subir C. Dasgupta, Head of the Department of Zoology, Maulana Azad College for providing infrastructural facilities.

References

[1] American Public Health Association. 2005. Standard methods for the Examination and Water and Wastewater, 21st ed., Washington DC.

[2] Bela Zutshi, S., Raghu Prasad, G. & Nagaraja, R. 2010. Alteration in Hematology of Labeo rohita under stress of pollution from lakes of Bangalore, Karnataka, India. *Environmental Monitoring & Assessment*, 168: 11-19

[3] Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. *Journal of Fish Biology*, 4: 593-605.

[4] Blaxhall, P.C. & Daisley, K.W. 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5: 771-781

[5] Dacie, J.V. & Lewis, S.N. 1984. Practical Haematology, 6th Edition. Edinburg, Churchill Livingstone, pp. 112.

[6] Dede, E.B. & Kaglo, H.D. 2001. Aqua-toxicological effects of water soluble fractions (WSF) of diesel fuel on O. niloticus fingerlings. *Journal of Applied Sciences & Environmental Management*, 5: 93-96

[7] Hesser, E.F. 1960. Methods for routine fish haematology. *The Progressive Fish-Culturist*, 22: 164-171

[8] Irwin, R.J., Van Mouwerik, M., Stevens, L., Seese, M.D. & Basham, W. 1997. ChromiumVI (Hexavalent chromium). Environmental Contaminants Encyclopedia. National Park Service, Water Resources Division, Fort Collins, Colorado, 43 pp.

[9] Karuppasamy R. 2000. Impact of Phenyl mercuric acetate on the bimodal respiration in an air-breathing fish C. punctatus. *Journal of Environmental Pollution*, 7: 287-293

[10] Koyama, J. & Ozaki, H. 2002. Haematological changes of fish exposed to low concentrations of cadmium in the water. *Bulletin of the Japanese Society for the Science of Fish*, 50: 199-203

[11] Pamila, D. & Subbaiyan, P.A., Ramaswamy, M. 1991. Toxic effect of chromium and cobalt on Sartherodon mossambicus (Peters). *Indian Journal of Environmental Health*, 33: 218-224.

[12] Sastry, K. V. & Tyagi, S. 1982. Toxic effects of chromium in a freshwater teleost fish, Channa punctatus. *Toxicology Letters*, 11: 17-21

[13] Smit, G. L., Hatting, J. & Burger, A. P. 1979. Haematological assessment of the effects of the anaesthetic MS222 in natural and neutralized form in three fresh water fish species: Interspecies differences. *Journal of Fish Biology*, 15: 633-643

[14] Venkatachalam, T. & Natarajan, A. V. 2014. Haematological investigation on freshwater teleost Labeo rohita (Ham.) following aquatic toxicities of Cr (III) and Cr (VI). *International Journal of Research in Biosciences* 3(3): 1-14.

[15] Vutukuru, S.S. 2003. Chromium induced alterations in some biochemical profile of the Indian major carp Labeo rohita (Hamilton). *Bulletin of Environmental Contamination & Toxicology*, 70: 118-123

[16] Wintrobe, M. M. 1967. Clinical haematology. 6th Edition, Lea and Febiger, Philadelphia, Library of Congress, Print USA.