Open Access

CARAS

© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 05 (Sep-Oct 2023); pp 1598–1602

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Studies on the Behavioral Expressions and Histopathological Alterations in *Labeo rohita* due to Chromium Toxicity

Urmi Mitra*[1] and Sumit Homechaudhuri[2]

[1] Post-Graduate Department of Zoology, Bidhannagar College, Kolkata - 700 064, West Bengal, India
[2] Aquatic Bio-Resource Research Laboratory, Department of Zoology, University of Calcutta, 87/1, College Street, Kolkata - 700 073 West Bengal

Abstract

Heavy metals such as chromium persist in aquatic environments as a result of their resistance to biodegradation. *Labeo rohita* is a rich source of nutrient as well as widely distributed commercially interesting species. In this study, a total number of 180 fishes were randomly chosen and evenly distributed in 6 aquarium containing different concentrations of chromium including one test aquarium with no chromium. The current experiment was performed with the objective to determine the median lethal concentration (LC_{50}) of chromium to *Labeo rohita* and to investigate changes in their behavioral expressions and histopathological consequences in several vital organs. At the behavioral level, several abnormalities like hyperactivity, jerky movement, abnormal swimming behavior, loss of equilibrium and abnormal ventilatory function were found in fishes. At the histopathological level, several alterations in the gill tissues like degeneration of epithelium, curling of gill lamellae and hypertrophy, while in the liver there were necrosis, dilation of the vein, hepatocytes degeneration, pigmentation and hemorrhage. These findings are significant to monitor and potentially manage the consequences of heavy metal pollution in aquatic fauna.

***Key words*:** *Labeo rohita*, LC_{50}, Chromium toxicity, Histopathology, Behavioral expression

The chromium (Cr) is well-known as a toxicant to living organisms due to their bioaccumulation and non-biodegradable properties. It is the 6th most abundant metal in the earth's crust and their compounds are carcinogenic, mutagenic, genotoxic and poisonous to humans and animals [1]. The waste water generated by tanneries is a major source of chromium which contains Cr (VI) ranging from 40-25,000 mg/L [2]. According to Indian standards, the maximum tolerance of total Cr for public water supply is mg/L. Chromium (VI) salts have several applications in different industries and their indiscriminate use lead to their introduction into the aquatic ecosystem causing a serious threat to the growth and survival of the aquatic fauna including the fish populations. Chronic contamination of aquatic environment by heavy metals is a severe problem since these pollutants persist long-term in the environment. The natural aquatic systems are extensively being contaminated with heavy metals released from domestic, industrial and other man-made activities.

Cr (VI) compounds readily penetrate into cell membranes via anion transport systems and converted into its trivalent form resulting in genotoxic effects [3]. Fishes, being at the top of the aquatic food pyramid, may bio concentrate large quantity of such metals from water. Fish have been largely used as bioindicators for environmental pollutants and have been used to estimate the presence and accumulation of environmental pollutants [4]. Heavy metals accumulate in tissues of fish and may pose a health risk to those who frequently consume them. A toxicity test using aquatic organisms is very much significant for the development of proposals for environmental management and protection, especially for the aquaculture environment [5]. Lethal Concentration of 50% (LC_{50}) tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals. Heavy metals such as mercury and chromium are toxic to aquatic animals at very low concentrations and are detrimental to living beings [6].

The acute toxicity test is used to ascertain the concentration of a toxicant or the level of an agent that produces a harmful effect on a group of test organisms during a short-term exposure under controlled conditions. Majority of the studies concerning the effects of heavy metals on fish has been confined to the acute toxicity test with the death of fish as an end point. Hence, this particular study aimed at determining the acute effects of the toxicant on the behavioral expression of the test sample and their histopathological alteration at the tissue level.

MATERIALS AND METHODS

Received: 28 Jul 2023; Revised accepted: 11 Oct 2023; Published online: 18 Oct 2023

Correspondence to: Urmi Mitra, Post-Graduate Department of Zoology, Bidhannagar College, Kolkata-700064, West Bengal, India, Tel: +91 9051418422; E-mail: urmimitra@gmail.com

Citation: Mitra U, Homechaudhuri S. 2023. Studies on the behavioral expressions and histopathological alterations in *Labeo rohita* due to chromium toxicity. *Res. Jr. Agril. Sci*. 14(5): 1598-1602.

Sample collection, preparation and analysis

The test specimens *Labeo rohita* (average length 10±2 cm average weight 10±2 gm) havebeen collected from local fish Market, Naihati, West Bengal and were acclimatized in 1 feet × 2 feet glass tanks one week prior to the experiments. The study has been conducted from 2017-2022. During acclimatization the fishes were fed *ad libitum* with a prepared food.

Acute toxicity bioassay

LC_{50} value were determined for the freshwater carp *Labeo rohita* at different exposure period (24hours, 48hours, 72hours, 96 hours) for indicating the toxicity end point for Chromium.

Acute static renewal bioassays were performed according to static bioassay procedures of APHA (2005). Running tap water (Temperature 28°C ± 2_C, pH 6.77 ± 0.5, DO 6.15 ± 0.2 mg/L, TDS 0.3 mg/l) was used as the test medium. Toxicity tests were conducted in 20-L glass aquaria, each containing 10 L of water and 10 fishes each. There were three replicates for each of the concentration of Cr tested. Potassium dichromate (Merck, India) had been procured to prepare the stock solution of 1000 mg/L by dissolving weighed amount of K2Cr2O7 in distilled water. The different working test solutions have been prepared by dissolving the stock solution in water as 30 mg/L (T_1), 35 mg/L (T_2), 40 mg/L (T_3), 45 mg/L(T_4), 50 mg/L (T_5) and the negative control (T_0) sample was prepared with water. No food was supplied to the fish samples during the toxicity test to avoid additional toxicity caused by interaction of excretory products of the fish with Cr. Bioassays were continued for 96 hours. Mortality was recorded at 24, 48, 72 and 96 hours and the dead fishes were removed from the test medium. The 96 hours LC_{50} value and 95% confidence limit have been calculated from the actual mortality data using EPA-Probit Analysis Program Version 1.5 statistical software based on Probit Analysis [7].

Study of behavioral responses

The behavioral changes of the test fish were investigated during the exposure of chromium for 24–96 hours period. The behavioral alterations are associated with the biochemical and physiological responses which indicate their stress level. The behavioral abnormalities of fish such as hyperactivity, jerky movement, abnormal swimming behavior, loss of equilibrium, abnormal ventilatory function, mucus secretion and abnormal skin pigmentations have been monitored through visual inspection and photography (OECD 2019). The behavioral abnormalities were categorized as 'mild', 'moderate' and 'severe' where 'mild' indicated 30 to 35% of individuals showed the abnormalities; 'moderate' indicated 45 to 50% of individuals showed the abnormalities) and 'severe' indicated 60 to 70% of individuals showed the behavioral abnormalities, respectively [8].

Study of histopathological responses

At the end of chromium exposure period, tissues were dissected from moribund fish resulting from the exposure of LC_{50} dose only and processed for histopathological examination. Control tissues were dissected from the live fish of control aquarium. Samples from the liver and gills were fixed in10% neutral buffer formalin and w×ere stained with hematoxylin and eosin [9]. The slides were examined and the histopathological alterations of hepatocytes, sinusoids, hepatic portal vein and red blood cells (RBCs) for the liver and epithelial cells, primary lamellae and secondary lamellae for the gill tissues were captured through light microscope at 40X magnification (Carl Zeiss Model- Axioscope A1).

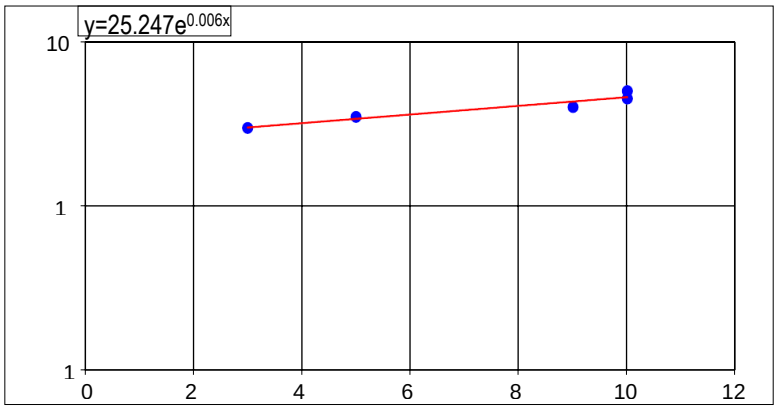
RESULTS AND DISCUSSION

Median lethal concentration (LC_{50}) for 96 h exposure to chromium through dose-response test

During the dose-response test no mortality has been recorded in control (0 mg/L) during 96-h exposure period. Percentage mortality of test fish in different concentrations of chromium has been represented in the following table. The resulting 96-h LC_{50} value for chromium was 33.4 mg/L which resulted in 50% mortality of the test fish (Table 1).

Table 1 Median lethal concentration (LC_{50}) of chromium at 96 hours exposure period

Exposure Conc. (mg/l)	Total number of test fish	Number of dead fish replicate			Total number of dead fish	% mortality	LC50 (mg/l)
		1	2	3			
0	30	0	0	0	0	0	
30	30	3	3	3	9	30	
35	30	4	5	6	15	50	33.427
40	30	9	9	9	27	90	
45	30	10	10	10	30	100	
50	30	10	10	10	30	100	



x	Y	ln(y)
30	30	3.401197382
50	35	3.555348061
90	40	3.688879454
100	45	3.80666249
100	50	3.912023005
	slope	0.006001664
	intercept	25.24678798

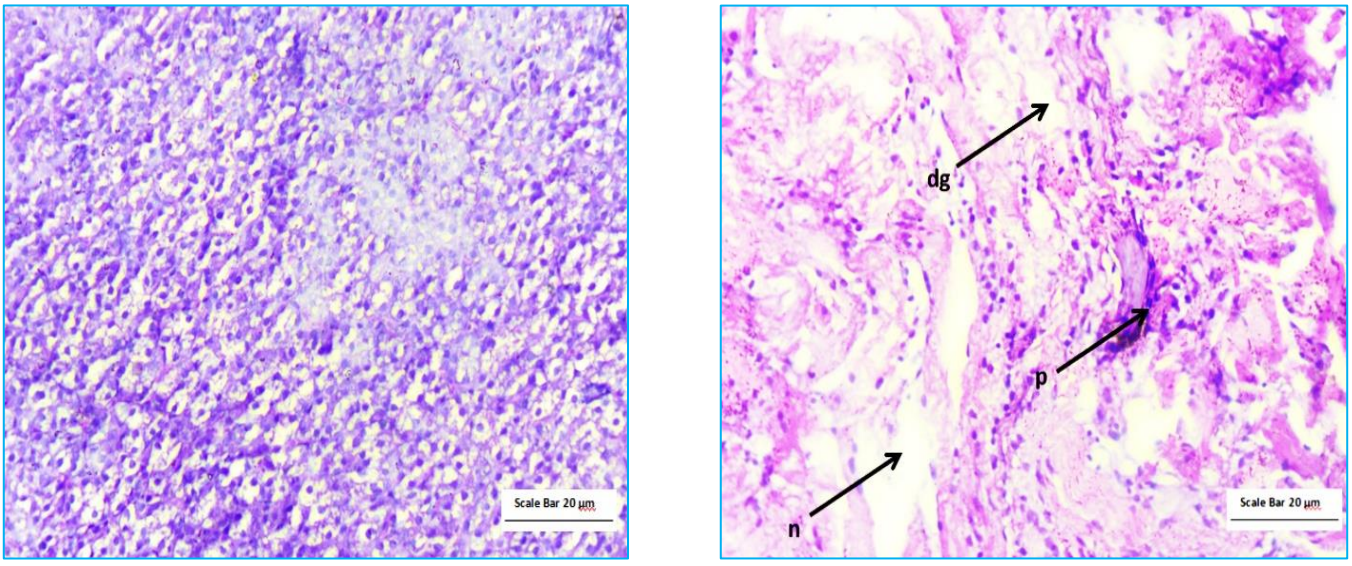
Fig 1 Graph showing the median lethal concentration (LC_{50}) of chromium at 96 hours exposure period

Alteration in behavioral expression of test fish during their exposure to chromium

No behavioral abnormalities were observed in the control fish. Fish treated with heavy metal at the concentration level from 30 to 50 mg/L exhibited mild (around 30 to 35% of individuals showing abnormalities) to moderate (around 45 to 50% of individuals showing abnormalities) behavioral responses for the initial 48 h but afterwards fish started to show severe (around 60 to 70% of individuals showing abnormalities) behavioral reactions in terms of hyperactivity, jerky movement, abnormal swimming, disability of equilibrium, abnormalities in ventilatory function, mucus secretion as well as abnormalities in skin pigmentation. In most cases, fish treated to higher concentrations of chromium exhibited severe behavioral irregularities such as very fast swimming, skin discolouration, loss of scales, jumping and instability with severe jerky movements, speedy opercular movement and surfacing with gulping of air. The results were showing in the (Table 2).

Table 2 Behavioral expressions of *Labeo rohita* on exposure to lethal dose of chromium within the 96 hours exposure period

Conc. (mg/l)	Hyper activity	Jerky movement	Abnormal swimming behavior	Loss of equilibrium	Abnormal ventilator function	Mucous secretion	Abnormal skin pigmentation
			24 Hours				
0	-	-	-	-	-	-	-
30	-	-	+	-	-	-	-
35	+	-	+	-	+	-	-
40	+	-	+	-	+	-	-
45	+	+	++	+	+	-	-
50	+	+	++	+	+	-	-
			48 Hours				
0	-	-	-	-	-	-	-
30	+	-	+	-	-	-	-
35	+	+	+	-	+	-	-
40	+	+	+	+	+	-	-
45	++	+	+	+	+	+	-
50	++	++	++	++	++	++	+
			72 Hours				
0	-	-	-	-	-	-	-
30	+	+	+	+	+	+	-
35	+	+	+	+	+	+	-
40	++	++	+	++	++	+	+
45	++	++	+	++	++	++	++
50	+++	+++	++	+++	+++	+++	++
			96 Hours				
0	-	-	-	-	-	-	-
30	+	+	+	+	+	+	+
35	++	++	++	++	++	++	+
40	++	++	++	++	++	++	++
45	+++	+++	+++	+++	+++	+++	++
50	+++	+++	+++	+++	+++	+++	+++



a) b)

Fig 2 (a) Transverse section of fish liver without exposure to chromium (Control); (b) Transverse section of fish liver after 96 hours of exposure to chromium (Exposed to LC_{50} Concentration), arrows are showing degeneration of hepatocytes (dg), necrosis (n) and pigmentation (p)

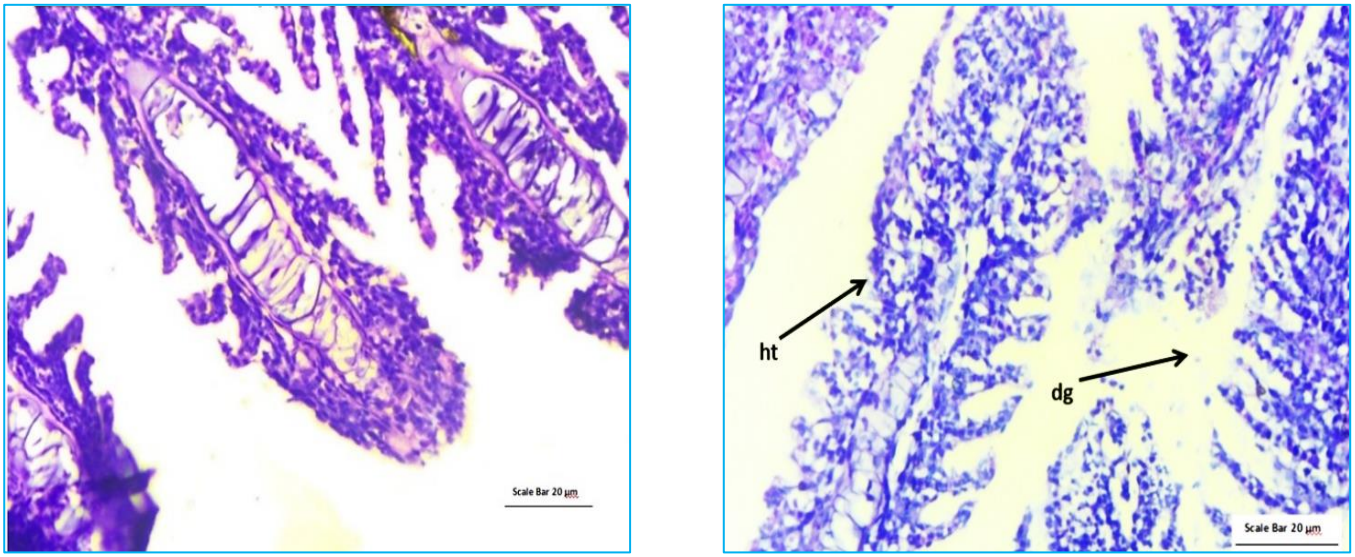
Histopathological responses of liver and gills of Labeo rohita to the 96 hours exposure to chromium

Histopathological transformations in liver

Fish in the control group showed typical histology of liver including hepatocytes (polygonal in shape with a prominent nucleus), sinusoids (fenestrated), regular hepatic portal vein and red blood cells. Vacuolization in hepatocytes, hepatocytes fusion, melano-macrophages center, pyknotic nuclei along with congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes are reported for the exposure to chromium after 96 h [10] (Fig 2).

Histopathological responses in gill

The histopathological alterations in the gills of Labeorohita are illustrated after 96-h exposure to chromium. Fish in the unexposed group (control) observed normal structure in the epithelial cell, primary lamellae and secondary lamellae. Severe deformation and loss of secondary lamellae happened during the exposure to chromium (33.4 mg/L) after 96-h [11] (Fig 3).



a) b)

Fig 3 (a) Transverse section of fish gill without exposure to chromium (Control); (b) Transverse section of fish gill after 96 hours of exposure to chromium (Exposed to LC50 Concentration), arrows are showing degeneration of primary lamellae (dg) and hypertrophy of secondary lamellae (ht)

CONCLUSION

Acute toxicity of chromium to different fish species varies with the fish species concerned, age of the fish, health status of the fish, method of toxicity test employed and also oxidation status of the metal. 96 hours LC_{50} value of chromium to *Labeo rohita* therefore deviates slightly from the previous reported value of 39.4 mg/l. The study revealed several behavioral abnormalities of *Labeo rohita* in response to acute exposure to chromium. The reported behavioral changes such as hyperactivity, increased erratic swimming, excessive mucus secretion, discoloration, loss of scales, loss of equilibrium, increase jerky movement, gasping and decreased fin movement are similar to behavioral expressions observed in *Labeo rohita* following exposure to chromium. Behavioral abnormalities reduce the ability of fish to successfully respond to different environmental stimuli, leading to decreased for aging success and enhanced susceptibility to predation. Histology of liver of *Labeo rohita* exposed to chromium (33.4 mg/L) revealed pyknotic nuclei with congestion of RBCs (red blood cells) in HPV (hepatic portal vein) of hepatocytes, hepatocytes fusion, vacuolization in hepatocytes, melano-macrophages canter and disintegration of hepatocytes cell membrane with oozing of cytoplasmic content. Our findings are comparable with the histological alterations of liver in *Labeo rohita* exposed to chromium. The present study showed that there is a strong link between liver damage and toxicants. Histopathology of gill of *Labeo rohita* exposed to chromium (33.4 mg/L) presented thickening of primary lamellae epithelium, shorting of secondary lamellae, epithelial hyperplasia and lamellar fusion (fusion of secondary lamellae) with thickening of primary lamellar epithelium along with collapsed secondary lamellae. To validate our findings, similar histological alterations are also found in the gill of *Labeo rohita*. Exposure of fish to pollutants such as pesticides collapses gills which may disrupt gas exchange efficiency resulting in respiratory disorders, osmoregulatory dysfunction and ion-regulation imbalance to force the excretion of nitrogenous waste products.

Acknowledgement

Authors are grateful to Department of Zoology, University of Calcutta and Department of Zoology, Bidhannagar College for providing the infrastructure and constant support during this study.

Conflict of interest

Both the authors don't have any conflict of interest from this research work.

LITERATURE CITED

1. Mushtaq F, Chen X, Veciana A, Hoop M, Nelson BJ, Pane S. 2022. Magnetoelectric reduction of chromium (VI) to chromium (III). *Appl. Mater. Today* 26. Doi: https://doi.org/10.1016/j.apmt.2021.101339

2. Mohammed K, Sahu O. 2019. Recovery of chromium from tannery industry waste water by membrane separation technology: health and engineering aspects. *Sci. Afr.* 4. Doi: http://dx.doi.org/10.1016/j.sciaf.2019.e00096
3. Bashir MS, Ramzan N, Najam T, Abbas G, Gu X, Arif M, Qasim M, Bashir H, Shah SSA, Sillanp M. 2022. Metallic nanoparticles for catalytic reduction of toxic hexavalent chromium from aqueous medium: a state-of-the-art review. *Sci. Total Environ*. 154475. Doi: https://doi.org/10.1016/ j.scitotenv.2022.154475
4. Kumar N, Chandan NC, Bhushan S, Singh DK, Kumar S. 2023. Health risk assessment and metal contamination in fish, water and soil sediments in the East Kolkata Wetlands, India, Ramsar site. *Science Reporter* 13: 1546. Doi: https://doi.org/10.1038/s41598-023-28801-y
5. Sanyal T, Kaviraj A, Saha S. 2017. Toxicity and bioaccumulation of chromium in some freshwater fish. *Human Ecol. Risk Ass: An Int. Journal* 23(7): 1655-1667. Doi: https://doi.org/10.1080/10807039.2017.1336425
6. Senthamilselvan D, Chezhian A, Suresh E. 2015. Acute toxicity of chromium and mercury to Lates calcarifer under laboratory conditions. *International Journal of Fisheries and Aquatic Studies* 2(4): 54-57. Doi: http://dx.doi.org/10.13140/RG.2.1.4014.8640
7. Finney DJ. 1971. *Probit Analysis*. Cambridge University Press, Cambridge. pp 333.
8. Mhadhbi L, Beiras R. 2012. Acute toxicity of seven selected pesticides (Alachlor, Atrazine, Dieldrin, Diuron, Pirimiphos-Methyl, Chlorpyrifos, Diazinon) to the marine fish (Turbot, *Psetta maxima*). *Water Air Soil Pollution* 223: 5917-5930. Doi: http://dx.doi.org/10.1007/s11270-012-1328-9
9. Bancroft JD, Gamble M. 2007. *Theory and Practice of Histological Techniques*. 6th Edition, Edinburgh: Churchill Livingstone. ISBN: 978-0-443-10279-0
10. Vutukuru SS. 2003. Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton). *Bulletin of Environmental Contamination and Toxicology* 70(1): 118-123. doi: 10.1007/s00128-002-0164-9. PMID: 12478433
11. Vutukuru SS. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health* 2(3/4): 456-462. doi: 10.3390/ijerph2005030010. PMID: 16819101