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Endocrine Disruption and Physiological Alteration ın Fish Exposed to Hexavalent Chromium

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ABSTRACT

Sensitivity detection of hexavalent chromium in Tilapia fish (*Oreochromis mossambica*) during toxicity test shows the physiological alteration and endocrine disruption. Biochemical impairments of hexavalent chromium at various sublethal concentrations, i.e., 10 ppm, 30 ppm, 60 ppm, and control for 24 hr, 48 hr, 72 hr, and 96 hr in common freshwater fish *Oreochromis mossambica* (Tilapia fish) was monitored during the diverse exposure periods. The blood sample was collected, and the ALP test was performed by spectrophotometric technique to observe cytotoxic or genotoxic effects. Behavioral and morphological alterations were elucidated in the course of the investigation. Muscle, skin, a gill of fish were collected to estimate total protein and carbohydrate to notice the growth and development pattern. Toxicity tests were determined with probit analysis in SPSS software. The maximum increase in the ALP content was 189.47% at 96 hr of exposure at 60 ppm of hexavalent chromium. The maximum decrease in carbohydrate content of muscle, gill, and skin was observed to be 43.33%, -50%, and -48.27%, respectively, and protein content as -64.93%, -44.44%, and -66.67%, respectively, at 96 hr LC50 of exposure at 60 ppm of hexavalent chromium. LC50 values indicated that Cr 6^+ was highly toxic in muscle, gill, and skin of *Oreochromis mossambica* as there was a drastic reduction of biochemical parameters (carbohydrate, protein). The increase in ALP content in male fish due to Chromium concentration revealed that Cr 6^+ acts as a potent endocrine disruptor. Hence the male fish was selected to find the precursor protein vitellogenin as a standard exposure indicator.

Key words : *ALP test, Endocrine disruptor, Spectrophotometer, Sublethal, Vitellogenin, Toxicity test*

Introduction

The arbitrary discharge of raw sewage wastes, industrial effluents, and other wastes pollute the majority of the environments and influence organisms' survival and physiological activities. Metals, in particular, tend to accumulate and undergo food chain magnification. Heavy metals affect all organisms and ecosystem processes, including microbial activities. The occurrence of heavy metals varies widely in fishes, depending on their development, age, and other physiological factors. Chromium is recognized to cause various health hazards in microorganisms, plants, animals, and human beings. Among the animal species, the fishes are inhabitants, which can be significantly affected by these toxic pollutants as toxicants can change the makeup of fish's surroundings and decrease oxygen level and food source. Heavy metals can have toxic effects on several organs.

Composite signaling networks within the neuroendocrine system control biological functions in higher organisms. The endocrine branch of this system is critically important in two broad classes of

biological functions – regulation of growth and development, and maintenance of internal homeostasis and normal physiological processes (temperature regulation, reproduction, metabolism, etc.)(Marty *et al.,* 2018).

Metals gather in aquatic organisms, including fish, and stick with water and sediments. Fishes are the consistent and straightforward biomarkers of pollution of the aqueous medium. When fish are exposed to superior metal levels in a polluted aquatic ecosystem, they take them up from their immediate environment. Chromium (Cr) and its compounds are chief industrial materials extensively used in leather processing, metallurgy, pulp production, mining, etc. Once in the environment, numerous Physico-chemical reactions decrease Cr (VI) to the moderately less toxic trivalent form, catalyzed by unusual reducing agents like sulfide compounds. The pH of water has a reflective power on reducing chromium in the environment (Velma *et al.,* 2009).

After long-term Cr(VI) exposure, spawning decreases and shows signicantly lower than before exposure (Ni and Shen, 2021). The health hazards associated with exposure to chromium are reliant on unit oxidation states. The hexavalent form is more toxic than the trivalent form (Praveena *et al.,* 2013). Protein content in the muscle of *Mystus canvass* exposed to 1/3 of sublethal concentration of electroplating industrial effluent chromium (0.25%) for 30 days has been studied (Palanisamy *et al.,* 2011). After 24,48,72 hrs and 15 days exposure the muscle showed significant depletion from - 9.77%, -20.45%, -26.14% and-31.82% over the control. The toxicity of Cr is affected by species, body sizes and life stages of the organisms, the pH of the water, and to a lesser extent, by hardness, salinity, and temperature (Holdway, 1988). Zn, Ni, Cr, Cu, Cd, and Pb in the skin, gills, intestine, liver, and muscle of two freshwater fishes, *Wallago attu* and *Labeo dyocheilus* occupying different feeding habits in the same natural ecosystem were examined (Yousafzai *et al.,* 2010). The heavy metal concentration in fish tissues reveals past exposure via water and food, and it can display the current situation of the animals before toxicity influences the ecological balance of populations in the aquatic environment.

The vitellogenins (Vtgs) are glycolipophospho proteins that play a crucial role in constituting nutritional reserves for embryo development in non mammalian vertebrates (Carducci *et al.,* 2021). The presence of both VTGs in untreated male *Tilapia* was detected with the ELISAs using relatively high plasma volumes. Their presence in males was confirmed by VTG-like immunoreactive materials eluting from the ion-exchange column at the same positions as tVTG-200 and tVTG-130. The concentrations of the VTGs in males were several orders of magnitude lower than in vitellogenic females (Kishida and Specker, 1993).

During recent years, substantial consideration has relied on metals' fates and their derivatives in the aquatic environment. Human activities and increased use of metal-containing fertilizers in agriculture could lead to a continued increase in the concentration of metal pollutants in freshwater reservoirs as an outcome of water runoff (Adeyemo *et al.,* 2010). The test substance used in this present study, chromium (VI) or hexavalent chromium (Cr^{6+}), is not common in nature, unlike trivalent chromium. It is generally used as a chemical model in ecotoxicity studies for two main reasons: its significance as a pollutant that threatens aquatic ecosystems and its importance as a metallic element about toxicants used in the standard toxicity tests (Domingues *et al.,* 2010). Omnivores bioaccumulate more heavy metals than carnivorous fish in natural habitats (Bawuro *et al.,* 2018). The field and the laboratory experiments reveal the accumulation of heavy metals in fish. This is chiefly dependent upon metals concentration in ambient water and exposure period. However, other factors such as water salinity, pH, hardness and temperature, ecological needs, size and age, life cycle, capture season, and feeding habits of fish also play a significant role in metal contamination (Canli and Atli, 2003).

Materials and Methods

Test species

A male specimen of *Oreochromis mossambica (Order: Perciformes, Family: Cichlidae*) weighing 60 ± 5 g was used to experiment. These tilapia fish were netted with the help of the local fisherman from the fishing pond of Sambalpur town. All samples were acclimated for 1 week in 15 aerated fiberglass tanks. Fishes were shifted to fishery bags and were transported to the laboratory. Forty eight male fish species of 10-15cm (*Tilapia* Fish) were identified. Acclimatized fish were fed daily with a formulated feed and commercially available food such as pellets, rice

bran, flakes, plankton, brine shrimp, krill, etc. Dead fish were immediately removed with special plastic forceps to avoid possible deterioration of the water quality (Gooley *et al.,* 2000).

The physico-chemical parameters of the tap water were measured by standard methods (APHA, 2012). Fish were maintained in glass aquaria containing commonly used potable tap water (pH 7.28 ± 0.05, DO 5.83 ± 1.0 mg/L >95% saturation under natural photoperiod (13L: 11D) and ambient temperature (26.02 °C).

Preparation of the test solution

Cr (VI) standard (Sigma – Aldrich, 99.9%), 1000µg/ml was prepared by dissolving 2.889g potassium dichromate in deionized water to make one liter. Four aquaria were taken for the experiment. Chromium tested concentrations were 10, 30, 60 ppm. In the 2nd, 3rd, and 4th aquarium 10ppm, 30ppm, and 60ppm concentrations of hexavalent chromium were prepared in 20 liters of tap water, and the first aquarium was taken as control. Twelve male *Tilapia* fishes were transferred to each aquarium for the experiment.

Methods

Acute toxicity tests were carried out to calculate the LC50 for hexavalent chromium. Toxicity was recorded after 24, 48, 72, and 96 h, and LC50 values and confidence limits (95% CLs) were calculated. Four individuals were taken out from each container at 24hr, 48hr, 72hr, and 96hr intervals. The lethal concentration (LC50) is the concentration of a substance in water causing the death of 50 % of the tested aquatic population. The World Health Organization recommends a maximum allowable concentration of 0.05 mg/l of Cr (VI)in drinking water. Also, LC50 values were calculated from the data obtained in acute toxicity bioassays, using Finney's (1971) method of 'Probit analysis' and SPSS computer statistical software. In Finney's method, the LC50 value is derived by fitting a regression equation arithmetically and graphical interpolation by taking logarithms of the test chemical concentration on the x-axis and the probit value of percentage mortality on the y-axis (Finney, 1971). The 95% CLs of the LC50 values obtained by Finney's method were calculated with Mohapatra and Rengarajan (1995). Probit transformation adjusts mortality data to assumed normal population distribution, resulting in a straight line. The LC, 10, 30, 60 values were derived using simple substitution probit of 10,30 and 60, respectively. In Finney's method, the LC50 value is derived by fitting a regression equation arithmetically and graphical interpolation by taking logarithms of the test chemical concentration on the x-axis and the probit value of percentage mortality on the y-axis (Finney, 1971).

Blood samples were collected from each euthanized fish by inserting a needle into the caudal region's musculature perpendicular to the fish's ventral surface until blood entered the syringe. Increasing blood levels of Alkali-labile protein-bound phosphoprotein (ALP) in male and immature fish as an indirect measurement of vitellogenin (Hallgren *et al.,* 2012) has been established as a biomarker for endocrine disruption. At the end of the acute test, the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) was determined for each endpoint measured.

After the ALP test, the skin, gill, and muscles were collected from the euthanized fish and stored in a deep freezer to estimate biochemical constituents. The protein content in skin, gill, white muscle was analyzed by following the method of Lowry *et al.,* 1951, and the carbohydrate content in skin, gill, the white muscle was analyzed by the Anthrone method (Samseifter *et al.,* 1949). In the meantime, the fishes were observed regularly to study behavioral and morphological changes.

Results and Discussion

Behavioral and morphological change

Treatment followed with sublethal concentrations of hexavalent chromium in the fishes showed exciting aggressive behavior and symptoms of restlessness. An erratic swimming pattern was also observed. Erratic opercula movement was also noticed due to difficulties in normal respiration. Black and white spots were found on the skin (Plate 3.1.2). The eye became cloudy, almost to the point of whiteness, and the fish lost vision (Plate 3.1.3). Gill appeared pale persisting discoloration was common on the body (Plate 3.1.1). The tail and fins do not seem to function normally. There was a loss of balance, and the fish could not maintain its position in the column of water. Acute poisoning by the Chromium compound causes excess mucous secretion, damage in the respiratory epithelium, injury in the mouth (Plate 3.1.4), and fish death occurs with suffocation

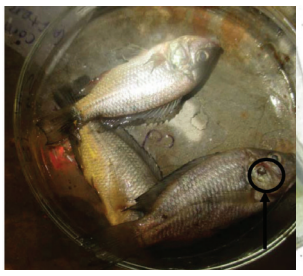

Plate 4.1.1 Gill affected due to hexavalent chromium

Plate 4.1.2 Black spot on outer surface of fish affected by hexavalent chromium

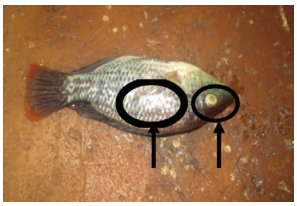

Plate 4.1.3 Whiteness of the eye and scales became disappeared in fish affected by hexavalent chromium

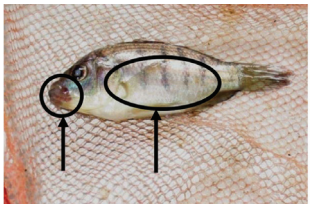

Plate 4.1.4 Mouth was affected coloured and was disappeared due to hexavalent chromium.

symptoms.

Biochemical Analysis

In order to find out the effect of protein and carbohydrate content, the test organisms were treated with concentrations of 10 ppm, 30 ppm, and 60 ppm of hexavalent chromium for different exposure periods, i.e., 24 hours, 48 hours, 72 hours, and 96 hours. As treatments with 10 ppm increase transaminase enzyme activity, the lethal concentration at 100 ppm did not measure enzyme activity. 60 ppm is considered the sublethal concentration, and 30 ppm is taken as an intermediate concentration. Seasonal variations in carbohydrate metabolism and sucrose-related enzyme under Cr stress were observed, and Cr(VI) stress (at ≥ 50) caused a reduction in sugar and starch content (Rai and Mehrotra, 2008). Treatment with 30, 60, and 90 ppm of Cr (VI) enhanced lipid peroxidation as indicated by an elevation in MDA level and also H_2O_2 level. The activity of GR increased at ≤60 ppm Cr (VI) and then declined. (Rai *et al*., 2006). The ability to tolerate and reduce Cr(VI) concentration of 10, 20, and 30 ppm by the presence of growth in the form of turbidity and decolorization of 10, 20, 30 ppm was shown by the presence of growth in the form of turbidity and decolorization of the medium from yellow to pale yellow or colorless. A single organism was isolated from the sewage, which individually showed resistance to Cr(VI) up to concentrations greater than 60 ppm. It can reduce concentration as 10 ppm and as high as 60 ppm with 100 and 66.66 percent reduction, respectively, at 55 °C in aerobic conditions (Gore *et al.*, 2016).

The ALP content (μg.ml^{-1} in the blood plasma of Tilapia during 24 hr, 48 hr, 72 hr, and 96 hr exposure at 10 ppm concentration of hexavalent chromium was found to be 0.03, 0.04, 0.041, and 0.05 respectively, at 30 ppm concentration of hexavalent chromium it was found to be 0.05, 0.06, 0.65 and 0.075 respectively. At 60 ppm concentration of hexavalent chromium, it was found to be 0.07, 0.09, 0.10, and 0.11, respectively. The ALP content (μg.ml^{-1} gradually increases at 10 ppm, 30 ppm and 60 ppm concentrations, respectively, compared with control, which was found to be 0.02, 0.03, 0.03, and 0.038.

The carbohydrate content (g/100 g) in muscle tissue, gill, the skin of Tilapia during 24 hr, 48 hr, 72 hr, and 92 hr exposure to different concentrations of hexavalent chromium i.e., 10 ppm, 30 ppm, and 60 ppm were analyzed, and the values are depicted in Table 1. The carbohydrate contents for the above conditions gradually decreased compared with the control.

The protein content (g/100g) in muscle tissue, gill, the skin of Tilapia during 24 hr, 48 hr, 72 hr, and 92hr exposure to different concentrations of hexavalent chromium i.e., 10 ppm, 30 ppm, and 60 ppm were analyzed, and the values are depicted in Table 1. The protein contents for the above conditions gradually decreased when compared with control.

It was observed (Table 1) that the percent increase in ALP content (μg.ml^{-1} over zero concentration of hexavalent chromium at 10, 30, and 60 ppm was 50%, 150%, and 250% respectively for 24hr of the exposure period. After 48hr of exposure, the ALP content increased over zero concentration of hexavalent chromium at 10, 30, and 60ppm was 33.33%, 100%, and 200%, respectively. After 72hr of exposure, the ALP content increased over zero concentration of Hexavalent chromium at 10, 30, and 60ppm was 36.67%, 116.67%, and 233.33%, respectively. After 96hr of exposure, the ALP content increased over zero concentration of Hexavalent chromium at 10, 30, and 60ppm was 31.58%, 97.37%, and 189.47%, respectively.

The percent decrease in carbohydrate content (g/100g) in muscle over zero concentration of

hexavalent chromium at 10, 30, and 60 ppm was 17.65%, 26.47%, and 35.29%, respectively for 24hr of the exposure period, for 48hr it was found to be 18.18%, 27.27% and -34.85% respectively, for 72hr of exposure period it was found to be 16.13%, 29.30%, and 35.48% respectively, and for 96hr of the exposure period, the carbohydrate content was 16.67%, 28.33% and -43.33% respectively.

In gill, over zero concentration of hexavalent chromium at 10, 30, and 60 ppm, the carbohydrate content was 20%, 26.67%, and 33.33% for 24hr of the exposure period, 23.23%, 30%, and 43.33% respectively for 48hr exposure period, 24.14%, -31.03% and 44.83% for 72hr of the exposure period and at 96hr of exposure period it was found to be 32.14%, 42.86%, and 50% respectively. The percent decrease in carbohydrate content (g/100g) in the skin over zero concentration of hexavalent chromium at 10, 30, and 60 ppm was -16.13%, 25.81%, and 32.26%, respectively 24hr of the exposure period. At 48hr of exposure period it was -16.67%, 28.33%, and 33.33%, for 72hr of exposure period it was found to be 17.24%, 27.59%, and -37.93% respectively and for 96hr of exposure period the carbohydrate content was -20.69%, 37.93%, and 48.27% respectively.

The percent decrease in protein content (g/100g) in muscle over zero concentration of hexavalent chromium at 10, 30, and 60 ppm was -30.95%, 47.62%, and 58.33%, respectively, for 24hr of the exposure period, for 48hr of exposure period it was 40.24%, 52.44%, and 58.54% respectively, for 72hr of exposure period the protein content was 39.24%, 56.96%, and 62.02% respectively, for 96hr of exposure period it was found to be 45.45%, 61.04%, and 64.93% respectively. In gill over zero concentration of hexavalent chromium at 10, 30 and 60 ppm was -11.43%, 30%, and 40% respectively for 24hr of the exposure period, for 48hr of exposure period the

Table 1. Protein, carbohydrate content and ALP values measurement per exposure in muscles, gill, skin

Exposure duration (hr)	Conc (ppm)	ALP (ml^{-1})	Carbohydrate (gm/100 g)			Protein (gm/100 g)		
			Muscle	Gill	Skin	Muscle	Gill	Skin
24hr	0	0.02	0.34	0.30	0.31	2.100	1.75	1.675
	10	0.03	0.28	0.24	0.26	1.450	1.550	1.150
		(50%)	(-17.65%)	(-20%)	(-16.13%)	(-30.95%)	(-11.43%)	(-31.34%)
	30	0.05	0.25	0.22	0.23	1.100	1.225	0.875
		(150%)	(-26.47%)	(-26.67%)	(-25.81%)	(-47.62%)	(-30%)	(-47.76%)
	60	0.07	0.22	0.20	0.21	0.875	1.050	0.750
		(250%)	(-35.29%)	(-33.33%)	(-32.26%)	(-58.33%)	(-40%)	(-55.22%)
48hr	0	0.03	0.33	0.30	0.30	2.050	1.675	1.625
	10	0.04	0.27	0.23	0.25	1.225	1.500	1.100
		(33.33%)	(-18.18%)	(-23.33%)	(-16.67%)	(-40.24%)	(-10.45%)	(-32.31%)
	30	0.06	0.24	0.21	0.215	0.975	1.200	0.850
		(100%)	(-27.27%)	(-30%)	(-28.33%)	(-52.44%)	(-28.36%)	(-47.69%)
	60	0.09	0.215	0.17	0.20	0.850	0.975	0.700
		(200%)	(-34.85%)	(-43.33%)	(-33.33%)	(-58.54%)	(-41.79%)	(-59.92%)
72hr	0	0.03	0.31	0.29	0.29	1.975	1.625	1.625
	10	0.041	0.26	0.22	0.24	1.200	1.400	1.000
		(36.67%)	(-16.13%)	(-24.14%)	(-17.24%)	(-39.24%)	(-13.85%)	(-38.46%)
	30	0.065	0.22	0.20	0.21	0.850	1.100	0.800
		(116.67%)	(-29.03%)	(-31.03%)	(-27.59%)	(-56.96%)	(-32.31%)	(-50.77%)
	60	0.10	0.20	0.16	0.18	0.750	0.925	0.575
		(233.33%)	(-35.48%)	(-44.83%)	(-37.93%)	(-62.02%)	(-43.08%)	(-64.61%)
96hr	0	0.038	0.30	0.28	0.29	1.925	1.575	1.575
	10	0.05	0.25	0.19	0.23	1.050	1.325	0.925
		(31.58%)	(-16.67%)	(-32.14%)	(-20.69%)	(-45.45%)	(-15.87%)	(-41.27%)
	30	0.075	0.215	0.16	0.18	0.750	1.050	0.675
		(97.37%)	(-28.33%)	(-42.86%)	(-37.93%)	(-61.04%)	(-33.33%)	(-57.14%)
	60	0.11	0.17	0.14	0.15	0.675	0.875	0.525
		(189.47%)	(-43.33%)	(-50%)	(-48.27%)	(-64.93%)	(-44.44%)	(-66.67%)

protein content was -10.45%, 28.36%, and 41.79% respectively, for 72hr of exposure period it was found to be -13.85%, 32.31% and 43.08% respectively and for 96hr of exposure period the protein content was -15.87%, -33.33% and 44.44% respectively. In skin, over zero concentration of hexavalent chromium at 10, 30, and 60 ppm was -31.34%, 47.76%, and 55.22%, respectively, for 24hr of the exposure period. For 48hr of the exposure period, it was -32.31%, 47.69%, and 59.92%, respectively, it was found to be -38.46%, 50.77%, and 64.61%, respectively, for 72hr and 96hr of the exposure period. It was -41.27 %, 57.14% and 66.67%s respectively.

Two-way ANOVA was performed to observe the significant difference between the concentration and exposure duration. Two-way analysis of variance for ALP test revealed that the difference was statistically significant for concentration and exposure duration, i.e., F_1 (504.2727) and F_2 (263.5455) at 10 ppm, F_1 (343.8947) and F_2 (24.68421) at 30 ppm and F_1 (154.6364) and F_2 (5.74026) at 60 ppm at $p \leq 0.05$ level of significance respectively. Two-way analysis of variance for carbohydrate content in muscle tissue revealed that the difference was statistically significant for concentration and exposure duration, i.e., F_1 (331.8) and F_2 (37.93333) at $p \leq 0.05$ level of significance. Two-way analysis of variance for carbohydrate content in gill revealed that the difference is statistically significant for concentration and exposure duration, i.e., F_1 (135.1488) and F_2 (19.31405) at $p \leq 0.05$ level of significance. Two-way analysis of variance for carbohydrate content in skin revealed the difference for concentration and exposure duration, i.e., F_1 (123.0134) and F_2 (14.93318) at ≤ 0.05 level of significance. Two-way analysis of variance for protein content in muscle tissue revealed that the difference is statistically significant for concentration and exposure duration, i.e., F_1 (469.5) and F_2 (22.25676) at $p \leq 0.05$ level of significance. Two-way analysis of variance for protein content in gill revealed that the difference is statistically significant for concentration and exposure duration, i.e., F_1 (1238) and F_2 (87) at $p \leq 0.05$ level of significance. Two-way analysis of variance for protein content (g/100 g) in skin revealed that the difference is statistically significant for concentration and exposure duration, i.e., F_1 (580.3939) and F_2 (20.87879) at $p \leq 0.05$ level of significance.

Behavioral and morphological changes

In the present investigation, toxicity tests were conducted to evaluate the adverse effects of the heavy metal hexavalent chromium on the freshwater fish *Oreochromis mossambica*. Previous studies clearly showed that the acute exposure resulted in the instantaneous death of fish because of an induced increase in mucous production, causing suffocation or direct detrimental effect on the gill epithelium. The eye became cloudy, almost to whiteness, and the fish lost vision. Greyish white marks or patches on the body of the fish were seen.

Acute poisoning by Chromium compounds causes excess mucous secretion damage in the gill respiratory epithelium, and the fish may die with symptoms of suffocation. Vera *et al.,* (2011) Chromium compounds also cause renal failure, leading to osmoregulatory ability and respiration in fish (Mishra and Mohanty, 2008).

Chronic hexavalent chromium exposure induced alterations in the gill, kidney, and liver of a freshwater teleost, *C. punctatus*; the severity of the lesions was dose and duration dependent. The toxicity of the metal also affected the growth and behavior of sh (Mishra and Mohanty, 2009). On acute exposure, the cortisol level was raised compared to control (Mishra and Mohanty, 2009). Behavioral manifestations of acute toxicity like copious secretion of mucus, loss of scales, discoloration, surfacing, and darting movements were observed in *Labeo rohita* exposed to higher concentrations of potassium dichromate. (Vutukuru, 2005)

Biochemical changes

The hexavalent chromium-induced depletion in liver glycogen, total protein, and total lipid profiles has been reported (Saxena and Tripathi, 2007). Nguyen and Janssen (2002) studied the effect of chromium on the African catfish (*Clarias gariepinus*). Virk and Sharma (2003) assessed the effects of acute toxicity of chromium on fingerlings of the *C.mrigala.* After 45 days of exposure significant decline in the protein and carbohydrate content of gills was observed.

Examination of the effects of potential Endocrine-disrupting chemicals (EDCs) begins only at levels at which there are no apparent stress or discomfort to the animal so that it is potentially equally able to reproduce (Ackermann *et al*., 2002). Reproductive dysfunction at such low concentrations can be caused either by direct action on the gamete or indirectly by modulation of the endocrine system so that the game development takes place during an imbal-

ance hormonal environment (Okai *et al.,* 2004).

Conclusion

Cr (VI) induced toxicological pathology in fish is influenced by various factors such as species, age, environmental condition, exposure time, and exposure concentration. The exact causes of fish death are multiple and depend mainly on time-concentration combinations. Hence bioindicators and biomarkers can be used as early-warning indicators of the presence of stressors. Biomarkers can offer additional biologically and ecologically relevant information, which is a valuable tool for establishing guidelines for effective environmental management. So, it can be stated that fish biomarkers are necessary for monitoring environmentally induced alterations to assess the impact of xenobiotic compounds (i.e., heavy metals) on fish. Also, it is recommended that treatment of all kinds of wastewaters, sewage, and agricultural wastes be conducted before discharge into the aquatic system. The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring. The increasing emphasis on assessing and monitoring the estuarine ecosystem is needed to deploy appropriate biological indices.

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Conflict of Interest

The authors have no conflict of interest to declare. All co-authors have seen and agreed with the manuscript's contents, and there is no financial interest to report. We clarify that the submission is original work and is not under review at any other publication. Competent authorities did ethical approval for designing and conducting field research on fish.

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