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A Study of Cr (VI) Induced Toxicity on the Histology of Gill, Liver, Kidney of Freshwater Fish *Channa punctatus* (Bloch. 1793)

Anshika Singh and Vivek Kumar*

Department of Zoology, Isabella Thoburn College, Faizabad road, Lucknow, India
E.mail- anshika0833[at]gmail.com
Vivekkumarsb[at]gmail.com *Corresponding Author

Abstract: ***Environmental preservation is a major problem for the ecological balance on a worldwide scale. Chromium is a heavy metal that is regarded as one of the most pervasive pollutants in the aquatic environment. The most hazardous of all of its oxidation states is Cr (VI). Aquatic organisms absorb Cr through their gastrointestinal or respiratory tracts, so determining the influence there are histological parameters. The goal of the current experiment was to establish the subacute toxicity of Cr (VI) in light of its impact on histological parameters in the fish Channa punctatus, which had a length of 15-20 cm and a weight of 30-35 g, in a laboratory aquarium filled with 100 litres of water under natural 12D:12L conditions. Fish were exposed to three different Cr concentrations, including the control (LC_{50}/0, LC_{50}/5, LC_{50}/10, and LC_{50}/20). Live fish were slain for the preparation of gill, liver, and kidney slides for the weekly examination in order to determine histological parameters and effects such as vacuolization, necrosis, apoptosis, pyknotic nuclei development, etc. The impact only became apparent after chromium bioaccumulated and concentrated in the kidney, liver, and gills. A 4-week long experiment demonstrating changes in tissues compared to control tissues. The highest concentration (20 mg/l) has the greatest effect when compared to the lowest concentration (5 g/l). According to legend, Cr (VI) is a hazardous metal that can cause cancer and mutagenesis. Thus, we should provide disposable management of rubbish discharge and industrial effluents***

Keywords: *Channa punctatus*; chromium; toxicity; bioaccumulation; and histological criteria

1. Introduction

Water is a fundamental requirement for life and is regarded as a vital element for survival. The need to keep them pristine is considered a global concern because they are crucial to our ecology. Although water makes up 70% of the planet, only 3% of it is fresh water, and only 1.2% of it is used for drinking. Heavy metals are the primary component of water pollution, which is mostly caused by industrial discharge, population growth, the use of synthetic products, and other factors. Heavy metals are defined as metallic elements with a relative density that is five times greater than that of water (Garai et al., 2021). They are also thought of as the "trace elements," which are needed in very minute amounts to carry out various physiological tasks (Ding et al., 2018). Because of their capacity to interact with nuclear proteins and nucleic acids and cause oxidative degradation of biomolecules, heavy metals are capable of causing toxicity in living organisms (Paithankar et al., 2021). The problem has grown increasingly dangerous for the environment as a result of enterprises routinely releasing waste materials, including metallic pollutants, into the ground that surpass safe limits. Notwithstanding improvements in natural waste management systems, the entanglements caused by excessive metal release continue to have a negative, hostile impact on maritime life. Because of their long inventiveness, bioaccumulation, biomagnification, and non-biodegradability characteristics, lithophilic metals are recognised as being more harmful to the biological community and the centre collection of marine poisons because they can harm the systems of a wide range of species.Heavy metals have the potential to harm physiological processes, normal bodily function, and even cause death in extreme circumstances. When it reaches the threshold limits, Cr (VI), which is more toxic than Cr (III) in comparison, causes toxicity to the public's health. Although the formation of Cr(VI) from Cr(III) under oxidising conditions is extremely unlikely, it is possible (Das PK et al., 2021).Cr(VI) contamination and biosphere disruption are linked (Emamverdian A, et al., 2015).Understanding the amounts of heavy metals in aquatic environments and their inhabitants has been of great interest due to their hazardous consequences (Satapathy and Panda, 2018).In the past few decades, there has been an increase in interest in measuring the levels of heavy metal contamination in the aquatic environment and in the public food supply, particularly in fish (Velusamy et al., 2014).

Fish and other aquatic species pick up contaminants either directly from contaminated water or indirectly by ingesting other aquatic organisms. In both fresh and salt water, DNA damage caused by direct mutagens and pro-mutagens can be detected in fish, a test organism (Fagr Kh. et al., 2015). To determine how chromium affects an organism, we employed the fish *Channa punctatus*. Fish can absorb heavy metals through their gills, mouth, or general body surface, although the gills are the most common entry point since polluted water enters the body through them most frequently. The body surface is the least common entry point.

2. Materials and Methods

Test Chemical

Analytical grade of CrO_3 Chromium (VI) Oxide Purified were used, manufactured by Merck Specialities Private Limited Shiv Sagar Estate'A' (Dr. Annie Besant Road, Worli Mumbai- 400018).

Test Animal

Channa punctatus was chosen as the test species for the current studies just because of its abundance. It is also regarded as the least expensive freshwater fish available. For the experimental setting more than 120 *Channa punctatus* specimens (Order: Ophiocephaliformes, Family: Ophiocephalide), each weighing 30±5g and measuring 15±2cm in length, were procured from a local fish market in Lucknow, India. Samples were treated with a 0.1% $KMnO_4$ solution to get rid of any possible cutaneous infection. The fish were acclimated for 10 days in laboratory before the experiment. They were kept in glass aquarium with a capacity of 100 liters of tap water that were monitored for water quality under natural lightning conditions and at room temperature. (pH 7.3, DO 7.5 mg/l, Total hardness 215.3 mg/l as $CaCO_3$, and alkalinity 133.2 mg/l as $CaCO_3$; APHA et al., 2017 was used to determine water quality at ambient temperature 25-27ºC). Fish were continuously fed commercial dry pellets (Tokyo fish food; Beijing, China). A minimum of 2% to 3% of their body weight was supplied in feed daily. Waste was taken from aquariums every third day, and an aerator was left on to maintain a constant level of oxygen in the water. The toxicant chromium trioxide (CrO_3) is employed at a variety of concentrations.

Experimental Design:
The whole experiment was divided into two sets. In first set of experiment the determination of LC_{50} 96h value of CrO_3 by Probit Analysis. In the second set of experiment three concentrations of Cr (VI) and control. Only the healthy fishes were chosen and exposure was continued for 28 days, the fish were divided into four groups having 15 fishes in each. Group I was retained as the control group by using just tap water whereas Group II, III and IV (LC_{50}/5, LC_{50}/10, LC_{50}/20 were treated with three sublethal concentrations of Chromium trioxide. Samples were collected at the intervals of 7th, 14th, 21th and 28th day. After the termination of desired duration 4 fishes were utilized for hisological slide preparation (n= 4 for each group).

Determination of LC_{50} 96h of test chemical
For the estimation of LC_{50} of 96 hours of test chemical chromium trioxide, several sets of experiments were designed to generate the raw data. For this purpose at least 10 concentrations were taken in logarithmic ratio and 10 fish were exposed to each concentration in glass aquaria. Mortality of fish was recorded after 24, 48, 72, and 96 h and dead fish were removed immediately from the aquarium. The raw data so generated for each concentration and chemical were loaded in a Core i3 computer having the required "Trimmed Spearman Karber" software and the LC_{50} values for 96 hours exposure period along with their 95% upper and lower confidence limits were obtained.

The data given in table 1 and 2 were analysed by the required software of "Trimmed Spearman-Karber Method" (Hamilton wt al., 1977) and the results obtained, are given below (Table-3).

Table 1: Determination of LC_{50} chromium trioxide (CrO_3) by "Trimmed Spearman-Karbers Method"

S. No.	Concentration of Chromium trioxide (mg/l)	% Mortality in 96 h
1.	32	00
2.	42	10
3.	52	20
4.	62	30
5.	72	40
6.	82	50
7.	92	60
8.	102	70
9.	112	90
10.	122	100

Table 2: Analysis of LC_{50} and 95% confidence limits of Chromium trioxide

Exposure period (in hours)	Test Chemical	LC_{50} value (in mg)	95% confidence limits	% Spearman Karber Trim
96	Chromium trioxide	76.89	Lower 68.24 Upper 85.52	10

Histological Parameters: It is used to display the patterns of fish disease. The microscopic examination of altered morphology that indicates a disease process in an organism is known as histopathology. As histopathological events can quickly identify water contamination and communicate the health of exposed tissue, they can be considered effective, according to Liebel et al, (2013). Fish target organs are histopathologically analysed to detect cellular changes that occur.

Preparation of the slides: Test animals were slaughtered in accordance with 2013 OECD standards, and tissue from the gills, liver, and kidney was fixed individually in the fixatives Bouin's, Carnoy's, Methacarn, 10% neutral buffered formaldehyde (Sigma, UK), and Formal Saline. Paraffin wax is used for tissue embedding. Chilling was used to speed up solidification during embedding in order to encourage the development of a fine crystalline microstructure within the block. A Spencer microtome (Biocraft and Scientific Industries, Nagla Pandi, Agra-5) was used to section the tissues after they had been fixed by perfusion with osmic acid solution and embedded in camphor-naphthalene. After the embedding material was removed, the sections were stained with iron-hematoxylin and eosin according to Regaud's method.

Data Evaluation and Statistical Analysis

All statistical analyses were preformed with SPSS statistical program. All experimental data were expressed as mean ±Standard error (SE). Significant difference between experimental and control groups were compared by One-Way ANOVA (analysis of variance) followed by Least Significant Difference (LSD) ($P < 0.05$ and 0.001) using Statistics Package (SPSS) program version 27.

3. Results and Discussion

The percentage of damage varies when the fish *Channa punctatus* is exposed to three different concentrations of sublethal chromium trioxide, including the control. As shown in tables 1, 2, and 3, *C. punctatus* exposure to sublethal concentrations of chromium (VI) for 7, 14, 21, and 28 days resulted in several notable and substantial changes in the histology of the gill, liver, and kidney. The damage percentage as CDSL (Complete Disintegration of Secondary Lamella), as compared with control, gradually increased from 2.49±0.56 to 16.59±2.67 after 7 days of exposure at $LC_{50}/20$ concentration, as indicated in Table 1 of gill tissues. The maximum percentage damage of CSDL was shown at concentration $LC_{50}/5$ after 28 days of exposure, which is 42.62±2.35, which was approximately 16 times greater than the control. The gradual alteration in percentage damage was shown in the graph at all concentrations of chromium trioxide after the exposure of days 7, 14, 21, and 28. The median concentration of $LC_{50}/10$ showed moderate changes, with the highest change occurring after 28 days of exposure and being 34.54±2.51, which was 9 times higher than the lowest concentration of $LC_{50}/20$. Moreover, another parameter of histology ESBV (extremely swollen blood vessels) was seen in the gills as a result of sub chronic exposure to CrO_3, which is also shown in Table 1. The initial damage percentage of the ESBV at 7 days of exposure at concentration $LC_{50}/20$ was 22.58±2.48 as opposed to 7 days of control fish 2.63±0.45, showing about 7 times more modification after exposure. Yet when fish were exposed for 28 days at $LC_{50}/5$ as opposed to controls, there was a progressive rise in ESBV changes. In comparison to control, it exhibits a 15 times larger percentage damage alteration in ESBV. When fish were exposed to $LC_{50}/10$ concentrations, the gills, a main organ in the pathway of toxicant exposure, showed mild changes.

Fish liver are detoxifying organ that exhibit some abnormalities after being exposed to chromium trioxide for 28 days. It exhibits flaws such as necrosis, vacuolization, apoptosis, and cytoplasmic degenerations, among others. Nonetheless, as necrosis and vacuolization are better suited for the liver, authors have put them here. According to Table 2, the percentage of liver tissue that experienced necrosis after exposure to the $LC_{50}/20$ concentration for 7 days grew steadily from 1.37±0.59 to 10.94±2.39, roughly 5 times the control level. The gradual changes were depicted on a graph for all chromium trioxide concentrations after exposure for days 7, 14, 21, and 28; the largest percentage of necrotic damage was seen at conc. $LC_{50}/5$ after 28 days of exposure, or 35.78±2.68 as compared to control. The damage percentage of the control group did not change 28 days further, while all changes were seen in the three groups of liver tissues exposed to Cr (VI). Similar to this, table 2 also shows liver necrosis and vacuolization. When fish were exposed to CrO_3 for 7 days at a concentration of $LC_{50}/20$ versus the control 2.43±0.88 to 22.25±1.09, they showed eight times the variation in damage percentage as the control, as well as changes in vacuolization damage percentage. Maximum vacuolization was recorded at $LC_{50}/5$ concentration for a total exposure length of 28 days, compared to the control, which was 2.53±0.82–48.63±1.17, indicating an 18-fold increase in the vacuolization damage percentage.

Table 3 displays the abnormalities in the *C. punctatus*, kidney that were noticed after exposure to CrO3, such as CRRT (Cavity Reduction in Renal Tubules) and hypertrophy. When chromium exposed to concentrations $LC_{50}/5$, $LC_{50}/10$, and $LC_{50}/20$ were compared to control, it was discovered that some changes in CRRT were significantly different. Particularly at concentration $LC_{50}/5$ is 44.95±2.65 after 28 days of exposure, the CRRT (Cavity Reduction in Renal Tubule) value was significantly 16 times higher in the treated fish groups. After a 28-day exposure, the concentration $LC_{50}/20$ is 25.76±2.45 had the lowest harm percentage of all the concentrations when compared to the control 2.53±0.59; a similar finding was previously demonstrated by Samudra Gupta et al. in 2017. Comparable changes can be seen in the hypertrophy damage percentage when compared to the control. With exposure to chromium trioxide for 48 days, the maximum hypertrophy was seen at the concentration $LC_{50}/5$. After 28 days of exposure, the initial damage percentage change in the control group was 3.41±0.58, and the $LC_{50}/5$ value was 57.73±2.74, which was 15 times higher than that of the control group. The least significant changes were seen at conc.$LC_{50}/20$, which was observed at 30.17±2.45 after 7 days of exposure to Cr (VI), which was approximately 8 times larger than the control damage percentage after 7 days of exposure 3.33±0.46.

Table 1: Mean value and SE observed in the histology of gill after the exposure of 28 days of CrO_3 in *Channa punctatus.*

Groups	Conc. mg/l	Exposure period (in days)	CDSL	ESBW
Group 1	$LC_{50}/0$ (Control)	7 days	2.49±0.56	2.63± 0.45
	$LC_{50}/0$ (Control)	14 days	2.52±0.57	2.45±0.53
	$LC_{50}/0$ (Control)	21 days	2.54±0.51	2.81±0.66
	$LC_{50}/0$ (Control)	28 days	2.51±0.62	2.55±0.47
Group 2	$LC_{50}/5$ (CrO_3)	7 days	38.56±2.19	35.38±2.25
	$LC_{50}/5$ (CrO_3)	14 days	37.34±2.28	37.42±2.38
	$LC_{50}/5$ (CrO_3)	21 days	39.57±2.25	39.34±2.37
	$LC_{50}/5$ (CrO_3)	28 days	42.62±2.35	41.69±2.44
Group 3	$LC_{50}/10$ (CrO_3)	7 days	25.37±2.33	30.23±2.28
	$LC_{50}/10$ (CrO_3)	14 days	27.63±2.48	32.92±2.75

	$LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3)	21 days 28 days	31.63±2.58 34.54±2.51	36.23±2.63 37.47±2.87
Group 4	$LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3)	7 days 14 days 21 days 28 days	16.59±2.67 18.6±2.55 20.64±2.59 23.38±2.66	22.58±2.48 25.37±2.45 28.39±2.58 31.05±2.69s

Table 2: Mean value and SE observed in the histology of Liver after the exposure of 28 days of CrO_3 in *Channa punctatus.*

Groups	Conc. mg/l	Exposure Period (in days)	Necrosis	Vacuolization
Group 1	$LC_{50}/0$ (Control) $LC_{50}/0$ (Control) $LC_{50}/0$ (Control) $LC_{50}/0$ (Control)	7 days 14 days 21 days 28 days	1.37±0.59 1.38±0.48 1.65±0.63 1.57±0.59	2.43±0.88 2.42±0.87 2.52±0.81 2.53±0.82
Group 2	$LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3)	7 days 14 days 21 days 28 days	10.94±2.39 19.02±2.46 22.87±2.62 26.44±2.39	22.25±1.09 23.83±1.08 26.31±1.05 27.87±1.07
Group 3	$LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3)	7 days 14 days 21 days 28 days	13.63±2.57 21.06±2.72 24.07±2.69 28.69±2.45	29.16±1.12 32.2±1.12 34.77±1.19 38.22±1.15
Group 4	$LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3)	7 days 14 days 21 days 28 days	16.99±2.77 24.46±2.59 31.48±2.82 35.78±2.68	40.77±1.17 44.1±1.15 45.68±1.18 48.63±1.17

Table 3: Mean value and SE observed in the histology of kidney after the exposure of 28 days of CrO_3 in *Channa punctatus.*

Groups	Conc. mg/l	Exposure period (in days)	Hypertrophy	CRRT
Group 1	$LC_{50}/0$ (Control) $LC_{50}/0$ (Control) $LC_{50}/0$ (Control) $LC_{50}/0$ (Control)	7 Days 14 Days 21 Days 28 Days	3.3±0.46 3.56±0.56 3.58±0.48 3.41±0.58	2.31±0.57 2.52±0.46 2.54±0.63 2.53±0.59
Group 2	$LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3) $LC_{50}/20$ (CrO_3)	7 Days 14 Days 21 Days 28 Days	30.17±2.45 31.93±2.68 35.14±2.61 37.14±2.65	20.15±2.67 20.89±2.35 22.87±2.55 25.76±2.45
Group 3	$LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3) $LC_{50}/10$ (CrO_3)	7 Days 14 Days 21 Days 28 Days	41.71±2.38 43.48±2.33 45.74±2.59 49.18±2.62	30.07±2.68 31.66±2.49 33.69±2.74 36.67±2.56
Group 4	$LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3) $LC_{50}/5$ (CrO_3)	7 Days 14 Days 21 Days 28 Days	51.67±2.58 53.76±2.69 56.1±2.58 57.73±2.74	38.84±2.44 40.97±2.75 42.76±2.63 44.95±2.65

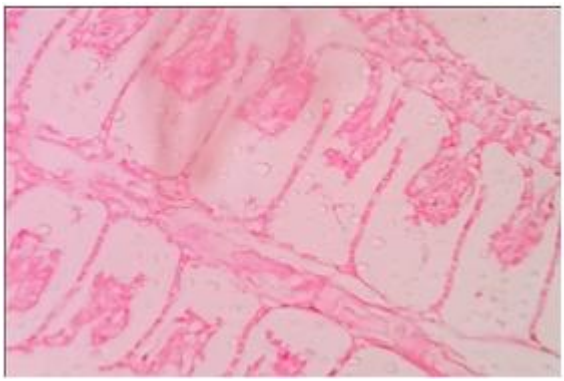


Figure 1: Control fish gill showing normal PGL and SGL.

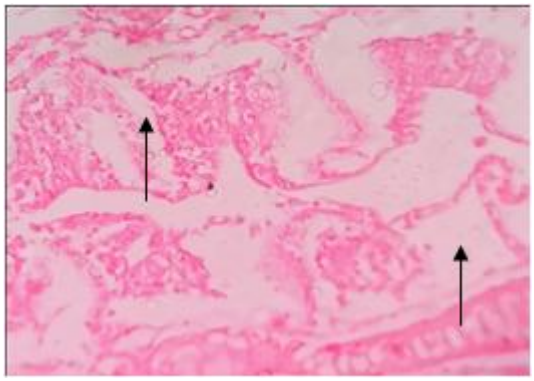


Figure 2: Exposed gills showing ESBV and CDSL

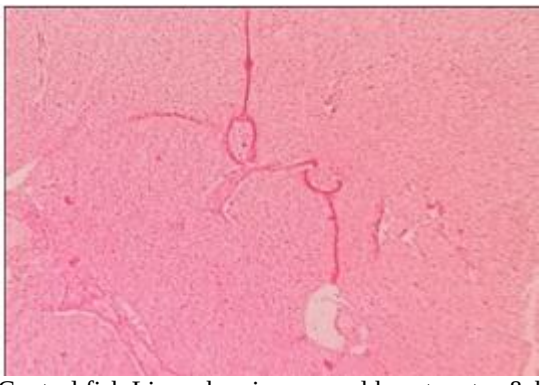


Figure 3: Control fish Liver showing normal hepatocytes & blood sinus

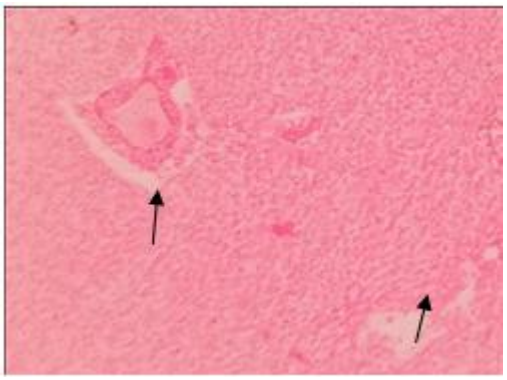


Figure 4: Exposed liver showing Vacuolization and Necrosis

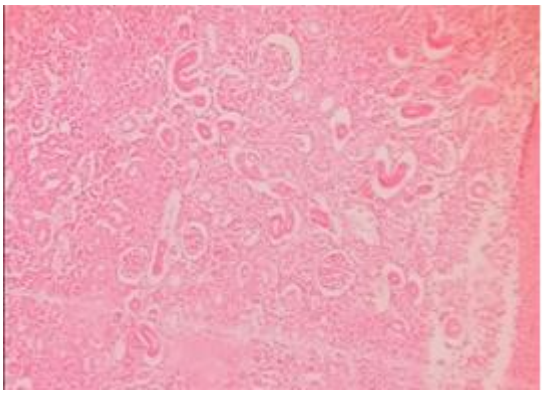


Figure 5: Control fish Kidney showing normal renal tubules.

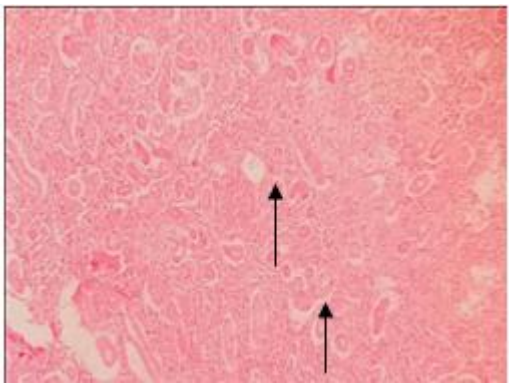
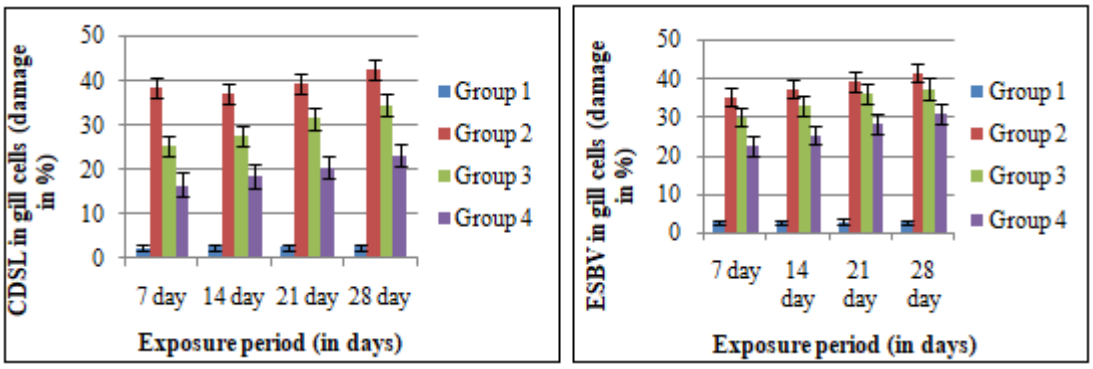
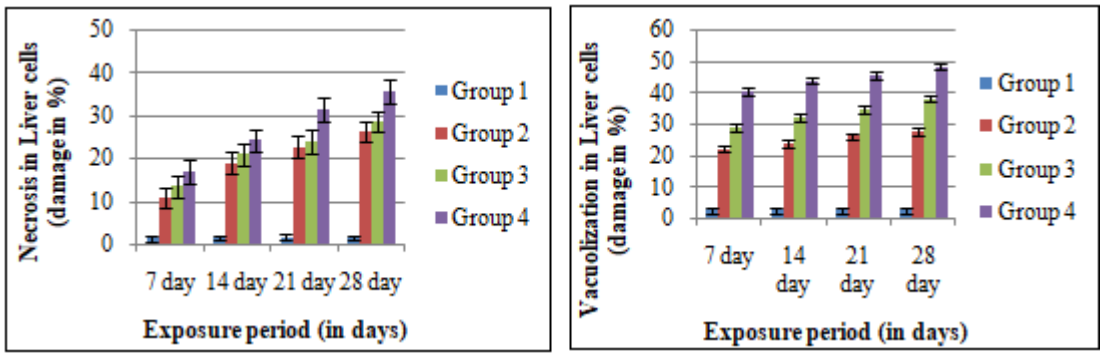


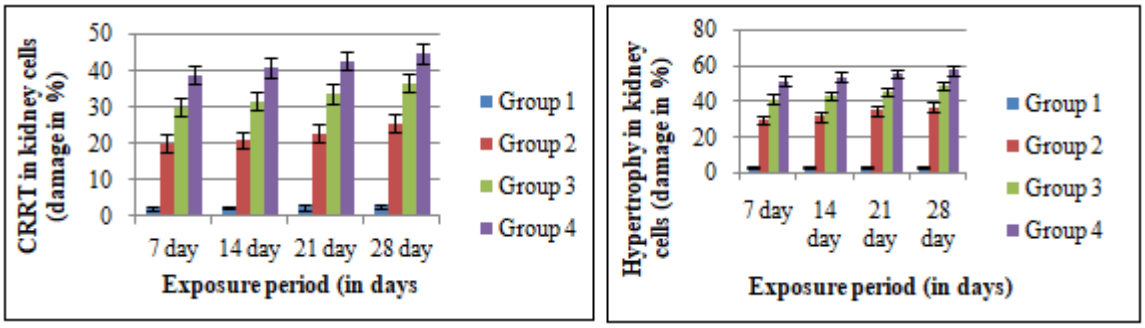
Figure 6: Exposed kidney cells showing CRRT and Hypertrophy



Graph 1 & 2 Effect of Hexavalent chromium on Fish gills showing CDSL & ESBV damage in percentage.



Graph 3 & 4 Effect of Hexavalent chromium on fish liver cells showing necrosis and vacuolization damage in percentage



Graph 5 & 6: Effect of Cr (VI) on fish kidney cells showing CRRT and Hypertrophy damage in percentage.

The fish *Channa punctatus'* gills, liver, and kidney were shown to have undergone histological changes as a result of sublethal concentrations of chromium (VI) after subchronic exposure, according to the overall observed outcomes of tissues and data in the current investigation. Gills showed completely disintegrated secondary lamellae and much enlarged blood vessels. Fish exposed for 28 days had gills with higher levels of ESBV and CDSL. Similar types of findings were found in zinc-treated *Heteropneustes fossilis* (Hemlata and Banerjee in 1977) and treated *Cirrhinus mrigala* (Gupta and Kumar in 2006) respectively. The principal organ of fish that is exposed to toxins first is thought to be the gills. It is required for fish to breathe. The secondary gill lamellae (SGL) in control fish appeared as finger-like structures. The primary gill lamellae were attached to each side of the SGL, which was narrow and

slender (PGL). As seen in Fig. 2, the secondary gill lamellae are strongly vascularized and encircled by a thin layer of epithelial cells (K. Muthukumaravel et al., 2013).

4. Discussion

Glycogen is digested, filtered, and stored by the liver. Many enzymes produced by the liver were also retained in the gallbladder. Energy from food is stored by the liver. In a healthy liver, there are many hepatocytes and a lot of sinus blood (fig. 3). Chromium exposure resulted in vacuolation, a loose assembly of liver cells, histolysis, and degeneration borders in the fish (Figure 4). After 28 days of exposure, the damage becomes more serious and progressive. On the T.S. of the control liver, a continuous mass of hepatic parencymal cells organised in cords surrounding blood vessels can be seen. Hepatic cells are polygonal in shape, with a rounded nucleus in the centre and homogeneous cytoplasm (A Kumar Singh et al., 2019).Fish liver histological changes have received a lot of attention (Maftuch Maftuch et al., 2018).The findings of the current study in chromium-treated *Channa punctatus* were consistent with those of the earlier researchers, particularly in the vacuolization, necrosis, and shrinking of nuclei, which were also noticeable in the present investigation. Large-scale buildup of these metals in the liver was the main cause of the development of necrosis, congestion of hepatic blood vessels, and vacuolization in *Channa punctatus* treated with chromium. The liver is a crucial organ for the detoxification of harmful and undesirable chemicals. *Channa punctatus* exposed to chromium (VI) underwent histological analysis, which demonstrated bulging of the gill lamellae's tips, changes in the pillar cells' organization, shrinking of the epithelial cells, collapsed blood capillaries in the primary gill lamellae, and cell necrosis (Jayakumar N et al., 2017). The kidney is a crucial organ for excretion, osmoregulation, and homeostasis maintenance. It is also in charge of selective reabsorption, which aids in the preservation of bodily fluid volume and pH, including blood and erythropoieses (Iqbal F et al., 2004).High concentrations of dietary Cr (VI) induced noticeable changes in kidney histology, such as renal tubular separation, vacuolation, aggregation of red blood cells in the sinusoid, and degeneration of renal tubules. Histological analysis of the kidneys of fish fed on Cr (VI) treatments revealed significant induction of Cr toxicity (Ahmed et al., 2022).

5. Conclusion

Channa punctatus, a common freshwater fish, had severe histological lesions and many cellular changes in its liver, gills, and kidney. These changes may have been caused by a buildup of heavy metals in the tissues that was above the WHO/FAO recommended limits. The discharge of industrial effluents into freshwater bodies must therefore be strictly controlled to prevent the influx and buildup of heavy metal toxicants in aquatic life (Saravpreet Kaur et al., 2018).

Contribution of Author

This experimental work was carried out in collaboration among all authors. Author VK did the experimental designing, statistical analysis of the data and preparation of graphs, drafting of the manuscript as well as final editing of manuscript. Author AS did the collection of test animal and execution of the experiment. Author SPT did the supervision of the experiment and guidance in the formation of manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

As the requirements of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India, an institutional Animal Ethics Committee (IAEC) vide registration no. 1861/GO/Re/S/16/CPCSEA, University of Lucknow. The author has followed the protocols as stated in the CPCSEA for execution of experiment.

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