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# Adverse effect of chromium (VI) on genotoxicity, histology of brain and behavioral patterns of fish *Channa punctatus* (Bloch, 1793)

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**Abstract**
Nearly all of India's regions' water sources endure an intolerable burden of harmful pollution. Aquatic contamination is a result of waste product discharge and anthropogenic waste runoff. The environment has turned into a repository for chemical contaminants that seep into estuaries and other aquatic environments, immobilising the aquatic biota, including heavy metals. Heavy metals like chromium can be both helpful and destructive to living things. It is both highly carcinogenic and poisonous. Chemical contaminants are released into the water by numerous enterprises. Chromium is among the many contaminants present in these disposals. Because *Channa punctatus* is a readily available and popularly consumed freshwater fish, it was selected as the test species. The experiment was conducted over the course of 28 days in 4 distinct aquariums, 3 of which contained chromium at various concentrations (Group 2- $LC_{50}/5$; 20 mg/l, Group 3- $LC_{50}/10$; 10 mg/l, and Group 4- $LC_{50}/20$; 5 mg/l), and one aquarium (Group 1) served as the control. With an increase in the concentration of the heavy metal, the exposed fish exhibited more irregular swimming and became sluggish. Moreover, chromosome damage induced by chromium exposure increased the production of micronuclei in the interphase cells and increased the toxicant concentration. The findings indicated that acute chromium toxicity has a significant negative impact on normal histology of brain, genotoxicity and normal behaviour, which may be harmful to the fish population and ultimately to humans who eat fish.

**Keywords:** Chromium, heavy metal, genotoxicity, brain, behaviour, aquaculture

## 1. Introduction
One of the most prevalent trace elements in both seawater and the crust of the earth is chromium. The environment does not contain this element in its pure metal state. The main source of chromium is chromite, which is used to make ferrochrom alloys and chromium metal. It exists in the oxidation states of divalent (Cr2+), trivalent (Cr3+), or hexavalent (Cr6+). The two most stable forms among these are Cr3+ and Cr6+. Due to its non-corrosive character, poor membrane permeability, and low power of biomagnifications in the food chain, the Cr3+ oxidation state is less hazardous. Because of its intense oxidative potential and capacity to cross cell membranes, the Cr6+ state is more hazardous. Hexavalent chromium is thought to be more harmful than trivalent form on account of its simple permeability through the cell film. Hexavalent Chromium has two fundamental oxyanion frames CrO4 2- and CrO7 2- which are engaged with reversible change. After entering the cell, the hexavalent chromium promptly diminishes to its trivalent shape and complexes with intracellular macromolecules even with hereditary materials (Farag *et al*., 2006; Li *et al*., 2010) [13, 18]. The simple permeability and biotransformation property of hexavalent chromium is at last in charge of its harmfulness and mutagenic action (Velma *et al*., 2009) [28].
Chromium toxicity in aquatic ecosystems results from a variety of human activities, including leather tanneries, metal processing, petroleum refining, textile manufacture, alloy preparation, wood preservation, etc. Chromium [Cr (0)] does not normally occur in its elemental form. Organ size and chromium bioaccumulation are related. The amount of chromium in soft tissues and the shell decreases significantly as size and dimension increase. Many types of tissues accumulate chromium in diverse ways.

Its concentration has been shown to be highest in the fish's gills, kidneys, and liver, with little tendency for chromium to accumulate in the muscles. Due to the increased demand for fish as a relatively affordable source of animal protein that could make up for the current shortage of more expensive proteins, the impact of heavy metals on fish has received significant attention in Egypt. Hence, genotoxic pollutants have the potential to contaminate not just aquatic creatures but also the entire ecosystem and, in the end, people via the food chain.

Fish are particularly vulnerable to heavy metal contamination due to their continuous exposure to water, which serves as the primary medium for the transport of these contaminants. The toxic effects of chromium on fish have been studied extensively, and it has been reported to induce various physiological, biochemical and behavioural changes. Of particular concern is its ability to cause neurotoxicity in fish, which can alter their normal behaviour and survival rate. In fish, chromium toxicity can cause cerebral damage and based on previous studies it may cause significant changes in brain histology. Several studies have reported significant histopathological changes in the brain tissue of *Channa punctata* exposed to chromium trioxide. Mukherjee *et al*. (2014) [21] studied the histological changes in the brain tissue of *Channa punctata* following exposure to sublethal concentrations of chromium trioxide for 21 days. The study observed significant alterations in the brain tissue, including congestion, necrosis, and degeneration of neurons. These alterations suggest that chromium trioxide exposure can cause neurological damage in fish. Similarly, Sarker *et al*. (2019) [24] reported severe structural alterations in the brain tissue of *Channa punctata* exposed to different concentrations of chromium trioxide for 30 days. The study observed neuronal degeneration, necrosis, vacuolation, and gliosis in the brain tissue. The authors suggested that these alterations could be due to the oxidative stress induced by exposure to chromium trioxide.

As fishes are more sensitive to heavy metal pollution, they accumulate in their tissues and pose poisoning impacts on fish health (Authman, 2015) [7]. Heavy metals contamination is considered a poisoning agent for the health of fish. These metals can effectively influence a fish's vital operations and reproduction, weaken the immune system, and induce pathological changes. As such, fish are used as bio-indictors, playing an essential role in monitoring heavy metals pollution. Harmful devastating metals contaminate freshwater bodies, attacking aquatic species and constituting an ecological issue. The trophic level exchanging conceivably harmful and devastating metals in the natural human ways of life, particularly in fish, has significant ramifications for human wellbeing (Ali & Khan, 2018a, 2018b) [1-2]. Heavy metals from industrial, agricultural, and mining activities discharged into rivers are trapped by sediments that pass and bio-magnify along the food chain. Heavy metals are persistent, non-biodegradable, non-soluble, and food chain energetic; they increase their concentration (bio-amplification) and the change in tropic level. Their toxic effect in aquatic systems is a primary focus of scientists due to their health risks to living organisms and human beings via the food chain. So these heavy metals cause severe diseases in fish and human beings (Nyairo *et al*., 2015) [22]. Heavy metals are generally viewed as unsafe because of their risk, persistency, and bio-accumulative nature.

Consequently, the primary goals of the current research are to analyze hexavalent chromium's potential for genotoxicity in *Channa punctatus* as well as the histopathology of brain and behavioural alterations.

## 2. Materials and Methods

### 2.1 Test Chemical

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

### 2.2 Test animal and Acclimatization

A snakehead, freshwater fish, *Channa punctatus*, was acquired from the neighborhood fish market at Taadikhana, Lucknow to laboratory. To get rid of external diseases, the treatment lasted for two minutes and used a 0.05% potassium permanganate ($KMnO_4$) solution. Prior to the trial, fish were exposed to lab conditions for 14 days. To keep the aquarium water oxygen-saturated and the temperature at 25±2 °C, aeration was used.

### 2.3 Estimation of $LC_{50}$ 96 h concentration of test chemical

The median lethal concentration ($LC_{50}$ 96 h) is typically defined as the concentration of chemical in water that, under a specific set of experimental conditions, kills 50% of the animals in a population. Studies on acute lethality were crucial for describing the harmful effects of substances. The Trimmed Spearman Karber's method was used in the current experiment to estimate the $LC_{50}$ 96 h. The raw data needed to calculate the $LC_{50}$ after 96 h of exposure was generated by several sets of tests. The logarithmic ratio was used to choose the top 10 concentrations (i.e., 75.8, 76, 76.2, 76.4, 76.6, 76.8, 77, 77.2, 77.4, and 77.6). In each experiment, 10 fish were given the test chemical while having their behavioral activities closely monitored. Fish deaths were noted on a regular basis. To assess the validity of each experiment, it was carried out three times. The raw data generated in this manner was loaded into a Pentium IV computer outfitted with the Trimmed Spearman Karber method software in order to estimate the median fatal concentration of the test chemical chromium VI. The values for the various exposure times were collected, along with their 95% upper and lower confidence levels. For the batch of fish exposed, the median lethal dose ($LC_{50}$) of chromium was discovered to be 76.7 mg/L. The method is widely accepted because it is simple to use, has good statistical properties, can be programmed so that all calculations can be done on a computer, has the ability to analyse multiple sets of data using a single statistical method, and can calculate $LC_{50}$ values with internal endpoints that have a 95% confidence level.

### 2.4 Experimental setup

The experiment the fish were divided into 4 groups having 40 fish in each. Fish from group 2, 3, and 4 were treated to a sub-lethal concentration of chromium trioxide of $LC_{50}/5$; 15.34 mg/L, $LC_{50}/10$; 7.67 mg/L and $LC_{50}/20$; 3.84 mg/L respectively for 28 days while fish from group 1 were kept as control group without any treatment. During the experiment, adequate aeration was given.

According to our research, fish can be exposed to sub-lethal concentrations of chromium trioxide during $7^{th}$, $14^{th}$, $21^{st}$, and $28^{th}$ day exposure periods of 15.34 mg/L ($1/5^{th}$), 7.67 mg/L ($1/10^{th}$), and 3.84 mg/L ($1/20^{th}$). To find out the consequences of prolonged exposure to low quantities of harmful chemicals,

chronic toxicity studies were carried out. This kind of test is crucial for assessing and comprehending the possible damage that chemicals provide to the aquatic ecosystem. The test solution (or toxicant) concentrations that have sub-lethal effects are lower than those that have acute effects. Chronic toxicity tests generally are longer-term tests that measure the effects of exposure to relatively lower, less toxic concentrations.

**2.5 Physicochemical evaluation of test medium**

Several physico-chemical characteristics were investigated. By using the evaporation method, the total dissolved solids (TDS) of the water and fixed residue were determined. By using the sodium thiosulfate titration method, the dissolved oxygen (DO) and biochemical oxygen demand (BOD) of water were assessed. Using the titration of potassium dichromate and sodium thiosulfate, chemical oxygen demand (COD) was determined. All these parameters of test medium were found within the limits suggested by APHA *et al*., 2017 [5] and were suitable for the survival of fish.

**2.6 Histopathological analysis**

Histopathological analysis of the brain tissue is a useful tool to evaluate the structural and functional alterations induced by heavy metal toxicity. After every 7 days of exposure to chromium during the 28 days experiment, all the fish were euthanized, and the brain tissue was removed and undergo the standard histological process, the brain tissues were fixed in 10% formalin, followed by dehydration in ethanol into paraffin wax. The thin sections of the tissues were acquired from the blocks prepared, and the sections are then be stained with hematoxylin and eosin. Histological changes in the brain tissue of *Channa punctata* exposed to varying concentrations of chromium are quantified. Histomorphometric analysis is conducted to quantify structural changes with specialized software. The obtained data undergoes statistical analysis using one-way analysis of variance (ANOVA), and significance will be set at $P<0.05$.

**2.7 Genotoxicity test**

**Micronuclei Test**

Micronuclei, which are little extranuclear entities, are formed during mitosis when a-centric chromosomal fragments or chromosomes are absent from either daughter nucleus. MN thus consists of either entire or incomplete chromosomes, or it consists of microscopic particles made up of incomplete or fragmented chromosomes that lag behind during the anaphase stage of cell division. During telophase, these fragments might not be incorporated into the nucleus of the offspring cells but rather form one or more micronuclei in the cytoplasm (Schmid, W. 1975) [26].

**Preparation of MN slides**

Take a 1 ml heparinized syringe and used a cardiac puncture to get 0.05–0.1 ml of blood. Using a different glass slide, I made a thin smear of blood on a slide that had already been cleaned. The slides were air-dried for an entire night at ambient temperature in a dust- and moisture-free environment. The slides were repaired by submerging them in 100% methanol for 5–10 minutes. Slides should be air-dried for at least an hour. Slides should be stained for two to three minutes in May-Grunnwald. Solution 1. DD water was used to wash and dry the slides. Slides should be stained for three to six minutes in Grunnwald's 2. Slides were cleaned with D.D. water and then dried. 30 minutes were spent staining the slides with 6–10% Giemsa stain in phosphate buffer (working solution). Wash the slides with DDW to thoroughly remove all Giemsa particles. The slides were air-dried overnight. DPX-mountant was used to make the slides permanent, and they were then dried overnight on a hot plate at 60 °C. Using 40/10 X objective lenses, I examined the slides under a microscope and graded the micro-nucleated cells.

**Scoring of MN slides**

Only MNs that could be distinguished from the main nucleus, had boundaries that could be readily defined, and had the same colour as the nucleus were scored. To ascertain the frequency of MN, it was estimated that there were MN in at least 1000 cells or fish. The effect of the test chemicals on micronucleus frequencies was investigated across four treatment periods using the one-way ANOVA and Duncan's multiple range test. The significant level of micronucleus frequencies at each assessment period, fluctuations in micronucleus frequencies between succeeding evaluation periods, and controls were all evaluated using the same test.

**2.8 Analysis of Behaviour**

Fish behaviour is an important endpoint to evaluate the toxic effects of heavy metals on the nervous system. Alterations in the normal behaviour patterns of fish can indicate an adverse neurological impact of heavy metals. The behaviour of the fish changed after exposure in each aquarium, and this was periodically monitored as well as photographed and recorded with a camera.

**2.9 Statistical analysis**

Values were expressed as mean ± standard deviation (SD). The LC50 value of chromium was determined through probit analysis. The data were analyzed by using one-way ANOVA, followed by post hoc test to find out the statistically significant differences among treated values with control. Statistical analysis was executed by using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA) at level of significance $p< 0.05$.

**3. Results**

**3.1 Histopathology of Brain**

On exposure for a period of 28 days to Chromium trioxide, compared to the control, degenerative changes were observed in the neural cells of the brain of the fish *Channa punctatus*. Necrosis of neurons, intracellular oedema and congestion of neural cells were noticed. Degeneration of neural cells with the cytoplasmic vacuolization was also observed (Fig 1a and 1b). Histopathological alterations were registered in the tissue from brain are summarized in Table 1, shown in Figures 1a and 1b.

The percentage of necrosis in the brain tissue of fish increased, in group 1 which served as control, the values ranged from 1.38±0.31 to 1.58±0.38 after the duration of 7, 14, 21 and 28 days respectively. Group 2 recorded the highest increment of necrosis in brain tissue in all exposure periods as follows 17.0±1.12, to 35.79±1.54 after the period of 7, 14, 21 and 28 days respectively, out of which percentage of necrosis in the brain tissue in 28$^{th}$ day recorded highest during the experiment. In group 3 the values ranged from 13.63±1.13, to 28.69±1.32 in their respective period. Group 4 recorded the minimum percentage of necrosis as compared to the other exposed groups which ranged 10.94±1.12 to 26.45±1.28 after

the duration of 7, 14, 21 and 28 days respectively. As compared to control group all three exposed groups showed the increment in percentage of necrosis in the brain tissue of fish, hence from the results it is clear that the damage percentage of necrosis increased significantly ($P < 0.05$) after the exposure of all three concentration of Chromium trioxide of fish *Channa punctatus.*

Vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. In group 1 which served as control, the values ranged from 2.43±0.54 to 2.54±0.54 after the duration of 7, 14, 21 and 28 days respectively. Group 2 recorded the highest increment of vacuolization in brain tissue in all exposure periods as follows 40.77±1.31, to 48.63±1.36 after the period of 7, 14, 21 and 28 days respectively. In group 3 the values ranged from 29.17±1.27, to 38.22±1.28 in their respective period. Group 4 recorded the minimum percentage of vacuolization as compared to the other exposed groups which ranged 22.26±1.23 to 27.88±1.23 after the duration of 7, 14, 21 and 28 days respectively. As compared to control group all three exposed groups showed the increment in percentage of vacuolization in the brain tissue of fish, *Channa punctatus.*

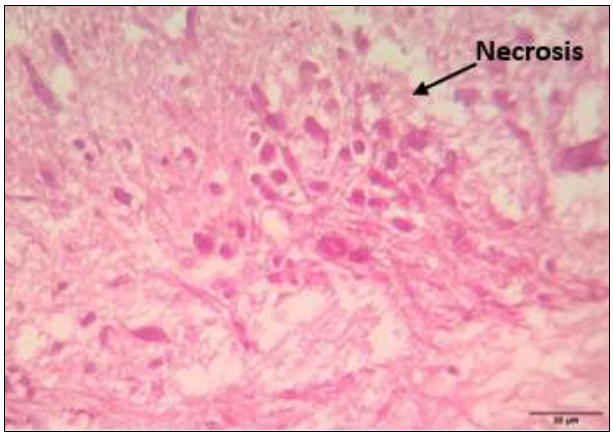



**Fig 1a:** Histology of Brain tissue of fish, Channa punctatus showing necrosis after exposure to Chromium trioxide

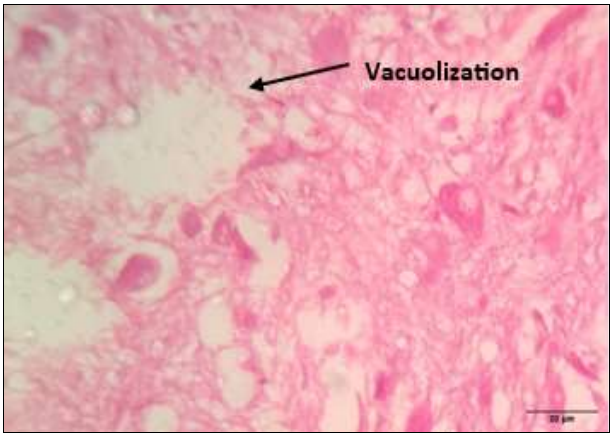
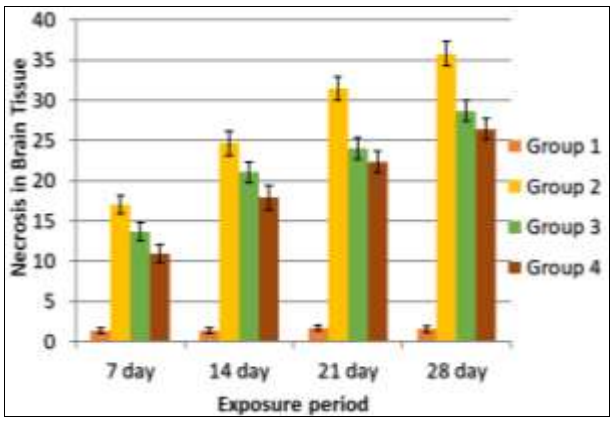


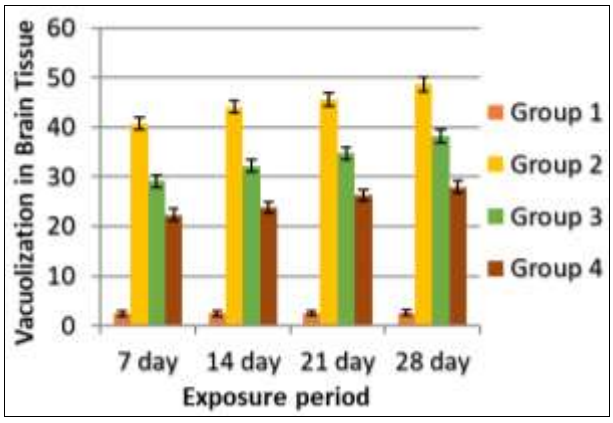

**Fig 1b:** Histology of Brain tissue of fish, Channa punctatus showing vacuolization after exposure to Chromium trioxide

**Table 1:** Mean Percentage and SE of Brain Tissue Damage in fish *Channa punctatus* when treated with Chromium trioxide

| Groups | Conc. (mg/l) | Exposure period(days) | Necrosis Mean (%) ±SE | Vacuolization mean (%) ±SE |
|---|---|---|---|---|
| Group 1 | Control | 7 | 1.38±0.31 | 2.43±0.54 |
| | | 14 | 1.38±0.38 | 2.43±0.53 |
| | | 21 | 1.66±0.34 | 2.53±0.53 |
| | | 28 | 1.58±0.38 | 2.54±0.54 |
| Group 2 | $LC_{50}/5$ | 7 | 17.0±1.12 | 40.77±1.31 |
| | | 14 | 24.66±1.51 | 44.10±1.28 |
| | | 21 | 31.49±1.48 | 45.68±1.35 |
| | | 28 | 35.79±1.54 | 48.63±1.36 |
| Group 3 | $LC_{50}/10$ | 7 | 13.63±1.13 | 29.17±1.27 |
| | | 14 | 21.06±1.29 | 32.21±1.29 |
| | | 21 | 24.07±1.34 | 34.78±1.25 |
| | | 28 | 28.69±1.32 | 38.22±1.28 |
| Group 4 | $LC_{50}/20$ | 7 | 10.94±1.12 | 22.26±1.23 |
| | | 14 | 17.91±1.54 | 23.83±1.22 |
| | | 21 | 22.33±1.38 | 26.31±1.19 |
| | | 28 | 26.45±1.28 | 27.88±1.23 |




**Graph 1:** Mean Percentage and SE of necrosis in Brain Tissue damage in fish *Channa punctatus* when treated with Chromium trioxide




**Graph 2:** Mean Percentage and SE of vacuolization in Brain Tissue damage in fish *Channa punctatus* when treated with Chromium trioxide

### 3.2 Genotoxicity Test

The generation of micronuclei (MN) was used to assess genomic instability. As chromium content rises, micronuclei occur more frequently. To ascertain the frequency of MN, the number of MN in at least 1000 blood cells or fish was estimated.

The frequencies of MN in blood erythrocytes of fish *Channa punctatus* in group 1 which served as control was recorded as 0.20±0.04, 0.23±0.02, 0.26±0.03 and 0.30±0.04 after the duration of 7, 14, 21 and 28 days respectively. Group 2 recorded the highest increment in MN frequencies in all exposure periods as follows 2.35±0.06, 2.85±0.05, 3.23±0.07 and 3.43±0.06 after the period of 7, 14, 21 and 28 days respectively, out of which MN frequency in 28th day recorded highest 3.43±0.06 during the experiment. In group 3 the recorded frequencies of MN were 1.17±0.05, 1.66±0.06, 2.16±0.05 and 2.54±0.06 in their respective period. Group 4 recorded the minimum MN frequencies as compared to the other exposed groups which were 0.88±0.05, 1.26±0.05, 1.48±0.07 and 1.66±0.07 after the duration of 7, 14, 21 and 28 days respectively. As compared to control group all three exposed groups showed higher frequencies of MN induction, hence from the results it is clear that the frequency of MN increased significantly ($p< 0.05$) after the exposure of all three concentrations of Chromium trioxide in erythrocytes of fish *Channa punctatus* showed in Table 2 and Graph 3.

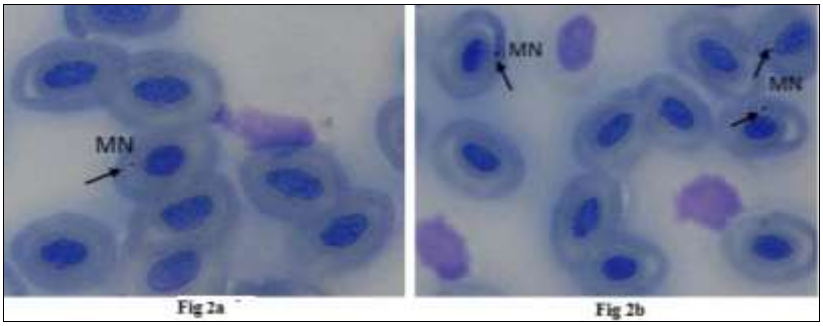
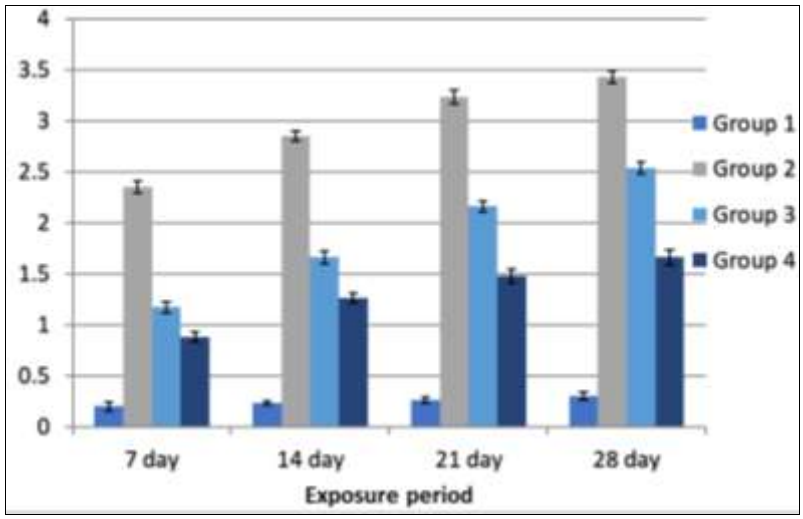



**Fig 2a, b:** Microphotographs showing MN induced by Chromium trioxide




**Graph 3:** MN frequency induced by Chromium trioxide in all treated groups up to 28th day

**Table 2:** Micronucleus frequencies in peripheral erythrocytes of fish *C. punctatus* exposed with Chromium trioxide

| Groups | Conc. (mg/l) | Exposure period(days) | Total cells scored | Total no. of MN | Frequency of MN mean (%) ±SE |
|---|---|---|---|---|---|
| Group 1 | Control | 7 | 3054 | 6 | 0.20±0.04 |
| | | 14 | 3073 | 7 | 0.23±0.02 |
| | | 21 | 3047 | 8 | 0.26±0.03 |
| | | 28 | 3047 | 9 | 0.30±0.04 |
| Group 2 | $LC_{50}/5$ | 7 | 3052 | 71 | 2.35±0.06 |
| | | 14 | 3044 | 88 | 2.85±0.05 |
| | | 21 | 3056 | 98 | 3.23±0.07 |
| | | 28 | 3064 | 104 | 3.43±0.06 |
| Group 3 | $LC_{50}/10$ | 7 | 3045 | 37 | 1.17±0.05 |
| | | 14 | 3057 | 52 | 1.66±0.06 |
| | | 21 | 3044 | 67 | 2.16±0.05 |
| | | 28 | 3067 | 77 | 2.54±0.06 |
| Group 4 | $LC_{50}/20$ | 7 | 3055 | 28 | 0.88±0.05 |
| | | 14 | 3078 | 38 | 1.26±0.05 |
| | | 21 | 3054 | 46 | 1.48±0.07 |
| | | 28 | 3056 | 52 | 1.66±0.07 |

### 3.3 Behavioral Changes

The exposed fish occasionally displayed jerky movements and inconsistent swimming, which could be signs of neurological harm to the fish because it means the fish's nerves and muscles are no longer under proper control. The fish exhibit air gulping to meet their oxygen needs since they are unable to absorb enough oxygen from the water due to toxicant buildup in their gills. As opposed to normal fish, the exposed fish also grew lethargic and moved much less.

**Fig 3a:** Lethargic behavior

**Fig 3b:** Erratic swimming

**Fig 3c:** Air gulping

**Fig 3d:** Jerky movement

## 4. Discussion

As histopathology is the microscopic study of diseased or damaged tissue, it is an important tool of anatomical pathology since accurate diagnosis of diseases usually requires histopathological examination of samples. These studies would help in assessing the extent of pollution in the ecosystem by the pollutants such as heavy metals and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ systems of an organism. In the present study, it is clearly indicated that Chromium trioxide has induced pronounced pathological changes in the brain of the fish *Channa punctatus* (Fig 1a and 1b). The histopathological responses of the fish reveal the percentage of damage caused by this heavy metal to the brain of the fish.

Several authors have reported different histopathological alterations in the brain of fish after exposing to different

chemical substances. The major histological changes observed in the Chromium trioxide-treated brain of the fish showed degeneration of the nerve cells, vacuolization and necrosis of the brain cells. Anita Kumari *et al*., (1998) [4] have made similar observations which are in conformity with the present study on the brain of Catlacatla. The same effects appeared in the Fish *Catla catla* (Bose *et al*., 2013) [1] and fish *Carassius gibelio* (Berillis *et al*., 2014) [9] when exposure to Heavy Metals and Toxic Cyanobacteria, respectively. In the study of Savari *et al*. (2020) [25] that treated the brain of spotted grouper *Epinephelus coioides* with methylmercury, the results showed hyperemia, some extent of hemorrhage, karyolysis, necrosis, nuclear dust, hyper chromatin, vacuolation, endothelium hypertrophy, cloudy swelling, hydropic degeneration, and ectopic granular accumulation. This study's results are very similar.

The quantity of micronuclei grows along with the concentration of the toxicant. Genotoxicity is the disruption of the genome through either DNA damage or disturbance of the DNA's normal division and distribution during mitosis. MNT is one of the most commonly used genotoxicity tests used to evaluate the potential genotoxicity of environmental pollutants. In particular, it enables a practical and simple application in genotoxicological research using aquatic creatures. Due to their fast bio-concentration and bio-accumulation of waterborne toxins, fish can be used as excellent experimental animals to evaluate the toxic and genotoxic potential of aquatic toxicants for environmental risk assessment (Icbelpaeme *et al*., 1996) [14]. One of the best instruments for both *in vivo* and *in vitro* genotoxicity research is the MN test. For the purpose of evaluating the genotoxicology of chromium compounds on micronuclei, numerous fish species, target tissues, and chromium compound varieties have been used. The most frequently used cells or target tissue to evaluate the genetic harm caused by aquatic pollutants in fishes using the MN test are peripheral blood erythrocytes because they may be used without having to go through the laborious cell preparation processes. Significantly increased levels of micronuclei in peripheral blood erythrocytes were also observed by Yadav & Trivedi, (2009) [30] in fish *Channa punctata* exposed to 1/10$^{th}$ of 96 h LC50 of CrO3 after all exposure periods. They observed maximum frequency of micronuclei after 96 h of exposure period. In comparison to a negative control, Jha *et al*., n.d. found a statistically significant increase in the frequency of MN in the peripheral blood erythrocytes of fish Channa punctatus using trivalent chromium compound. Kumar *et al*. (2012) [32] investigated the genotoxicity of a hexavalent chromium compound ($K_2Cr_2O_7$) in a freshwater mussel fish, *Channa punctatus*, in an aquatic, static bio-system and discovered that Cr (VI) is a potentially genotoxic compound and that the MNT is a sensitive and quick method of detecting genetic effects.

Behaviour offers a special perspective that connects an organism's physiology, ecology, and environment (B. Kumari *et al*., 2017) [16]. It also enables the organism to respond appropriately to internal and external inputs so that it can best handle the challenge of surviving in a changing environment. Through a mechanism involving anion (phosphate) transport, chromium (VI) actively enters cells. Since this method is not usable by chromium (III) (Valko *et al*., 2005) [27]. Fish mucus protects fish from chromium pollution because it reduces the oxidative state of Cr(VI) and thus its penetration (Arillo, 1990) [6]. One of the behaviors of the fish that have been treated with chromium to prevent the entry of toxicants into their bodies is to secrete vast amounts of mucus, as was seen in the current study. In fish treated with chromium, the development of locomotory responses, the frequency of swimming motions, and the length of activity were all dramatically changed. Several studies that demonstrate that toxicants can impair fish swimming ability and activity as well as startle responses (Carlson *et al*., 1998) [11] confirm the findings of our investigation (Little & Finger, 1990; Weis & Weis, n.d.; Zhou & Weis, 1998) [19, 29]. The sluggish movement and lack of balance in *Channa punctatus* treated with Cr (VI) support the findings (Mishra & Mohanty, 2008) [20]. The fish treated with chromium were found to have abnormal breathing. Respiration is a rhythmic neuromuscular process that is controlled by both ambient cues and an endogenous biofeedback loop. Acute contaminant exposure can cause a reflexive cough and a gill purge to rid the opercular chamber of the irritant. It can also cause a rise in the rate and amplitude of the respiratory cycle when the fish changes the amount of water in the respiratory stream. As exposure increases, the respiratory cycle may become erratic, primarily as a result of diminished input and changes to the endogenous pacemaker. Diamond *et al*. (1990) [12] also discovered that exposure to various pollutants changed the frequency and amplitude of bluegill opercular cycles and cough responses. The accumulation of acetylcholine at cholinergic synapses as a result of suppression of acetylcholinesterase (AchE) activity may potentially be the cause of all the symptoms listed above, resulting in excessive stimulation (K. Kumari *et al*., n.d.).

## 5. Conclusion

Hexavalent chromium is thought to be a hazardous metal, having mutagenic, carcinogenic, and other negative effects on biota, despite the fact that chromium is a common metal in the environment and trivalent chromium is also necessary for bio life. According to research, chromium has an impact on the experimental organism's physiology, Behavior, histology, biochemistry, genetics, and immune system. Trivalent chromium is a necessary component of some enzymes, whereas hexavalent chromium, which can pass through bio membranes, has been discovered to be hazardous to freshwater fish. Fish have been seen to lose their bodily equilibrium after acute exposure to a 50% fatal dosage, becoming agitated and breathing more slowly while secreting more mucus. Chronic chromium exposure in fish has been linked to genotoxic effects, including DNA breakage and the presence of micro nucleated (MN) and nucleated (BN) RBC. As a result, even though all of the hazardous notations are dose-dependent, it can be argued that chromium-contaminated industrial effluent discharges are causing significant changes in aquatic life. Several studies have reported the induction of significant histopathological changes, alterations in behaviour and biochemical changes in the brain tissue of *Channa punctata* following exposure to chromium trioxide. These findings suggest that exposure to chromium trioxide can cause significant damage to the brain tissue of fish, which can alter their normal behaviour and survival rate. The implications of these findings for the ecological health of aquatic ecosystems and human health are significant and highlight the need for strict regulations and precautionary measures to prevent the harmful effects of chromium trioxide on aquatic life and human health. This review will help future researchers gain a better understanding of chromium ecotoxicology and risk

assessment, as well as the findings of this review highlight the need for strict regulations and precautionary measures to prevent the harmful effects of chromium trioxide on aquatic life and human health. The use of alternative, less toxic chemicals in industrial processes and better waste management practices can significantly reduce the release of toxic heavy metals into the environment.

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**7. Author's contributions**
All writers contributed to the completion of this work. The experimental planning, statistical analysis of the data, graph production and text writing were all done by author Priyanshi Yadav. The selection of test animals and the experiment's execution were handled by author Priyanshi Yadav. The work was edited in its entirety by author Priyanshi Yadav. Vivek Kumar, the author, oversaw the experiment and provided advice on article development. The final text was reviewed and approved by both writers.

**8. Compliance with ethical standards**
According to the guidelines set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the University of Lucknow in Lucknow established an Institutional Animals Ethics Committee (IAEC) with registration number 1861/GO/Re/S/16/CPCSEA. The authors carried out the experiment in accordance with the CPCSEA's specified procedures.

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