ACUTE CHROMIUM EXPOSURE INDUCED ALTERATIONS IN BEHAVIORAL, ENZYMATIC AND GENO-TOXIC RESPONSES OF *CTENOPHARYNGODON IDELLA*

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Abstract. Heavy metals are the most significant pollutants because they are found throughout the environment. These metals are highly toxic and carcinogenic with ability to harm the organs and disrupt the biological systems of organisms even at very low levels. Therefore, in this study acute toxicity, behavior, catalase (CAT) activity and geno-toxic response of *Ctenopharyngodon idella* after exposure to chromium (Cr) was examined. To assess the acute toxicity (96-hr) fish was treated with various doses (10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 mg/L) of Cr. The CAT level was observed in liver, gills, brain and muscle of *C. Idella* and genotoxicity examined in blood cells by using micronucleus test. The LC_{50} and lethal conc. (96-hr) of Cr for *C. Idella* were computed as 45.11 and 75.58 mg/L, respectively. The behavioral alterations such as increased fin movement, mucus secretion, loss of equilibrium, hyperactive, swimming rate, jumping, and opercula movements of *C. idella* on exposure to Cr were observed. In present

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study significant (*P<0.05*) reduction in CAT activity in liver, gills, brain and muscle of Cr-treated *C. Idella* was noted as compared to control. The genotoxicity result revealed that nuclear abnormalities like number of micronuclei, notched and de-shaped nuclei was significantly (*P<0.05*) higher in Cd-treated fish as compared to control group.
Keywords: *catalase, micronucleus test, metals, toxicity, fish, organs*

Introduction

Due to their persistence in the environment and highly toxic effects on living organisms, heavy metal contamination is one of the most pressing global issues. As they are generally non-recyclable, heavy metals persist in the environment for extended periods. Heavy metals severely pollute water and pose significant health risks to both humans and animals. Chromium, a heavy metal that enters the environment through both natural and anthropogenic sources, is historically regarded as one of the most hazardous metals. Hexavalent chromium is reported to have genotoxic, carcinogenic, and mutagenic effects on living organisms (Laxmi and Kaushik, 2020).

Chromium (Cr) is found both in ground and surface water commonly. Principle sources of its pollution includes fossil fuel burning, grinding, welding, polishing of stainless steel and waste incineration (WHO, 1988). Its compounds are extensively used in chrome electroplating, dyes in textile industry, dipping, and anodizing. It also used in the product manufacture as catalysts and oxidants, purification of chemicals, fats and oils. Anthropogenic activities of Cr have become the most significant contributor to environmental contamination (Kumari et al., 2014).

The metal concentration becomes toxic to the organism when exceed the standard limit (Su et al., 2014) recommended by the United States Environment Protection Agency (USEPA) and WHO (Paul et al., 2014). In aquatic ecosystems, chromium toxicity is influenced by both abiotic and biotic variables. Biotic components include developmental stages of an individual, type as well as age of species. Abiotic components consist of oxidation state and concentration of chromium, water hardness, alkalinity, temperature and pH (Wang and Shi, 2001). Lake and river chromium concentrations can vary from 1 to 10 ug/L (De Mattia et al., 2004).

Alteration in fish behavior gives information about behavioural changes in aquatic animals that can be linked to physiological indicators (Hellou, 2011). The connection between the animal physiological and ecological processes is behavior, which is highly vulnerable to environmental signals and pollution exposure (Alonso and Valle, 2018). when a chemical concentration lower than the point at which it might cause death is applied to an animal, a change in behavior might be seen (Sharma, 2019). When a fish is first exposed to chromium, it exhibits a variety of behavioral changes, including a suspension of eating behavior, irregular swimming, and an accelerated operculum (Nagarajan et al., 2017).

An inescapable part of aerobic existence is oxidative stress. The oxidative stress is observed when the balance between reactive oxygen species (ROS) formation and antioxidant defenses in animals is disturbed (Nishida, 2011). Chemicals that result in ROS include petroleum pollutants, pesticides, and transitional metal ions (Slaninova et al., 2009; Lushchak, 2011). Before negative effects appear, in fish, catalase activity is considered to be a sensitive sign of oxidative stress (Gul et al., 2004; Sanchez et al., 2005). Catalase is a key antioxidant defense mechanism that eliminates H_2O_2, which flows through all cellular membranes and directly restricts a small number of enzymes (Atli et al., 2006).

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Fish are a key component in determining the impact of such pollutants on aquatic life since they are a great test model for determining the mutagenic and carcinogenic potential of aquatic creatures (Suresh et al., 2018). As signs of exposure to genotoxic chemicals, erythrocyte nuclear alterations (ENAs) have been studied (Grisolia et al., 2009). A test known as the micronucleus (MN) assay is used to determine genotoxicity and is one of the most sensitive indicators to identify DNA damage (Morita et al., 2011). The presence of micronucleus in polychromatic erythrocytes is employed as a genotoxic potential indicator (Chung et al., 2011).

Ctenopharyngodon idella commonly known as Grass carp is a freshwater fish species that are popular due to its fast growth rate and good commercial values (Qu et al., 2016). This fish is also popular in controlling aquatic vegetation (Cudmore et al., 2017). It is simple to upkeep *C. idella* in the laboratory settings and it holds significant commercial value in managing aquatic vegetation, and can serve as an effective indicator for appraising water quality (Vajargah et al., 2021).

The goal of present research was to evaluate the acute toxicity, behavioral, catalase activity and genotoxic response of *Ctenopharyngodon idella* against chromium toxicity.

Materials and methods

Fish sampling

Fisheries Research and Training Complex, Bahawalpur was visited to obtained the fish fingerlings of Grass carp (*Ctenopharyngodon idella*) and experiment was carried out at Fisheries Research under controlled conditions. Over the course of 15 days, fish were acclimated to the lab environment. Fish was fed twice a day during acclimatization. To ensure a healthy atmosphere, water was replaced after every 24 hr. Fish were shifted to a glass tank of 70 liters after an acclimatization period. Each aquarium contains 10 fish. During research trial, some water parameters were kept stable including temperature (28 °C), pH (7.25), and total hardness (220 mg/L). A capillary system air pump was used to provide continuous air to all of the test and control media.

Acute toxicity test

To estimate the LC_{50} and lethal concentration (96-h) of chromium (Cr) for *C. idella*, acute toxicity tests were carried out following the standard guidelines of APHA (2017) and OECD (2019). This experiment was performed with three replicates. In distilled water, pure salt of chromium chloride was dissolved for static bioassays. The test was start from 5 (mg/L) concentrations of Cr and gradually increases as 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 mg/L to find the LC_{50} and lethal concentration. The fish were not fed in acute toxicity test, and feeding had been stopped 24 hours before the experiment. The experiments were carried out with three replicates. During exposure, media were renewed after 96-h of exposure, and the Cr concentrations were adjusted to the initial values. In each aquarium, test organisms (n=10) were kept. Dead fish were removed to prevent contamination after recording mortality at 24-hour intervals. Additionally, a set of fish (n=10) was maintained in pure tap water.

Behavioral alterations

Behavioral alterations were observed after every 2-hr in both Cr-treated and control fish. Behavioral changes of fish including swimming rate, opercular movements,

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jumping, hyperactive, loss of equilibrium, fin movement and mucous secretion were observed daily.

Catalase activity

Fish was dissected after 4 days of exposure and tissues such as gills, muscles, liver and brain were homogenized in ice cold phosphate buffer. At 10,000 rpm and 4°C, the homogenates undergo for 10-minute centrifugation. Supernatants were extracted and processed for spectrophotometric analysis to determine the catalase (CAT) activity using Chance and Mehaly (1977) method.

Genotoxic response

Slides were immediately prepared with fish caudal vein blood and smear was then air dried. After that, slides were fixed and stained with methanol (10 minutes) and 10% Giemsa solution (8 minutes), respectively (Barsiene et al., 2004). Under an oil emersion lens, duplicate slides of Cr subjected fish were analysed to observe the nuclear alterations (NAs) and micronucleus (MN) frequencies (Fenech et al., 2003).

$$MN\% = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} x100 \qquad \text{(Eq.1)}$$

Statistical analyses

All data was reported as mean and standard deviation. The 96-hr lethal concentration and LC_{50} of chromium for fish were determined using Probit analysis. To highlight the statistically significant difference among various variables under study, One-way ANOVA was used. A non-parametric Mann-Whitney U-test was used to compare the frequency of MN and NAs between the treated and control groups. At $p < 0.05$ statistical significance was checked. By using Statistics 8.1 version statistical analyses were performed.

Results and discussion

Acute toxicity

Chromium caused toxicity in a concentration dependent manner. For *C. idella* the LC_{50} and lethal value (96 hr) of Cr was calculated 45.11 and 75. 58 mg L^{-1}, respectively (*Figure 1*). Handa and Jindal (2019) reported the LC_{50} (96 hr) of Cr for *C. idella* as 53.08 mg/L. Oliveira- Fitho et al. (2013) reported 96 hr LC_{50} for chromium as 123.1 mg/L for *Danio rerio*, 93.3 mg/L for *Hyphessobrycon eques* and 107.2 mg/L for *Oreochromis niloticus*. The LC_{50} and lethal conc. of Pb+Ni For *Ctenopharyngodon idella* (56.42 and 120.9 mg/L) and *Hypophthalmichthys molitrix* (55.85 and 128.44 mg/L) was reported by Naz and Javed (2013). According to Yılmaz et al. (2003) acute toxicity assay is a good choice to decide either a toxicant is harmful for water system. These assays are used to calculate the short-term impact of toxicants on aquatic animals during their life period (Hoffman et al., 2000; Ebrahimpour et al., 2010). The LC_{50} value of any heavy metal for fish is depends upon its susceptibility toward metal. The fish susceptibility toward

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different metals may vary even at the same level of that metal concentration (Das and Banerjee, 1980).

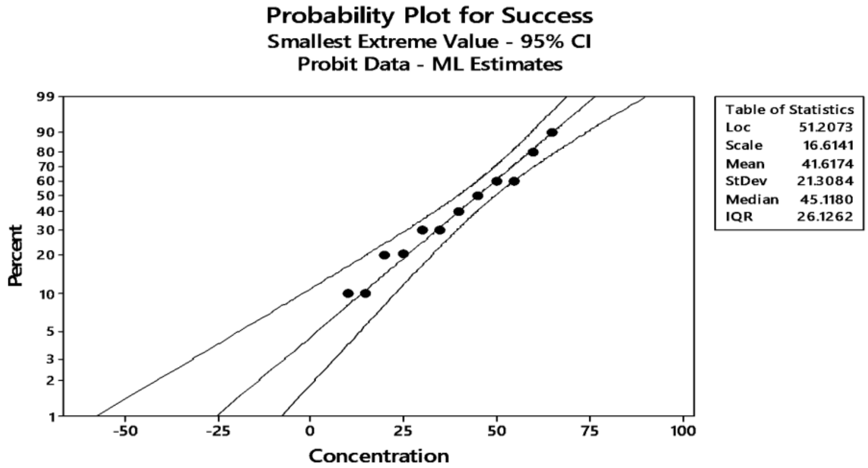

Figure 1. *96-hr acute toxicity of fish against chromium*

Behavioral changes

Results showed that the behavioral changes viz opercular and fin (dorsal and caudal) movements in the *control C. idella* remained essentially unaltered. Fish exposed to chromium exhibited abnormal behavior like opercular movement, jumping, hyperactivity, swimming rate and loss of equilibrium (*Figure 2*). They lost energy and generated an enormous amount of mucus throughout their entire body. The exposed fish's average swimming speed increased at a dose of 40 mg/L and then fall at a conc. of 60 mg/L. Lu et al. (2017) observed *Danio rerio* exhibited avoidance behaviour, increased speed in lower concentrations, and decreased speed at higher concentrations when exposed to zinc and chromium.

When fish exposed to chromium, it exhibits a variety of behavioral changes, including a suspension of eating behaviour, irregular swimming, and an accelerated operculum (Svecevicius, 2007). The same results have also been reported by Begum et al. (2006) who observed the *Gambusia affinis* subjected to greater concentrations of chromium showed irregular floating and loss of equilibrium. They were drowsy and released huge amounts of mucous throughout their bodies. When *E. danricus* was subjected to Cu (Vutukuru et al., 2006) behavioural signs of acute poisoning, such as loss of balance, darting movements, grouping and surfacing was noted. When *Danio rerio* was subjected to Cr and Zn avoidance behaviour, speed rise at lower concentrations, and enhanced when concentration was high (Lu et al., 2017).

Batool et al. (2014) examined *Channa Marulius* and *Wallago Attu's* behavioral reactions to acute exposure of cadmium and copper. The fish displayed increased surface behaviour, hyperactivity and abnormal swimming. Fish (*Chanos chanos*) exposed to high concentrations of cadmium chloride had altered behaviour, including abnormal swimming and movements of fin (Biuki et al., 2010). Hesni et al. (2011) investigated that when exposed of lead nitrate to milkfish (*Chanos chanos*) displayed altered behaviour, including swimming patterns that were downwards and vertical, greater mucus production, balance loss and hyperactivity.

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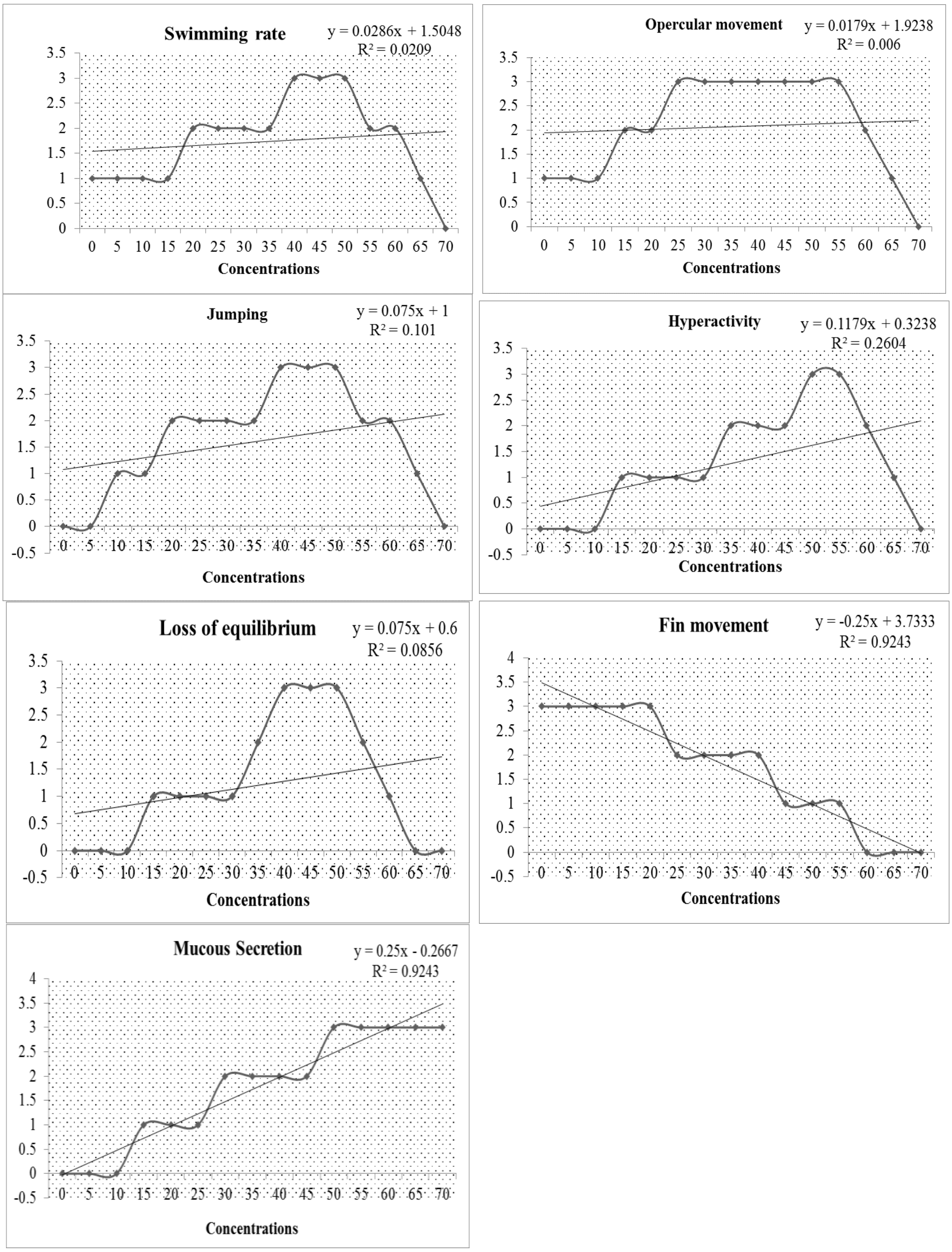

***Figure 2.** Alteration in swimming rate, Opercular movement, jumping movement, Hyperactivity, Loss of equilibrium, Fin movement and Mucous secretion of C. idella subjected to chromium*

Hesni et al. (2011) observed that lead exposure caused numerous behavioral abnormalities in freshwater fish. Some of these issues were loss of balance, breathing issues, slower movement, upturning in water, sinking to the bottom of the tank, and high secretion of mucus. All treatments resulted in aberrant behaviour, though the severity of

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the symptoms increased due to increased lead acetate concentrations. Lack of coordination between the nervous system and muscles can cause erratic movements and irregular swimming, which can be brought on by acetylcholine excess in synaptic and neuromuscular connections (Rao et al., 2005). Loss of balance is accompanied by irregular and jerky swimming movements, this might be associated with a decrease in brain cytochrome C oxidase activity, which results in cytotoxic hypoxia and damages the part of the brain responsible for maintaining equilibrium.

Catalase activity

In this research, C. *Idella* indicated a considerable decline in CAT activity after 96-hr exposure to Cr in liver, brain, muscle and gills as compared to control. Among four tissues' CAT activity followed order: gills ≥ liver ≥ brain ≥ muscle of fish due to Cr exposure (*Figure 3*). Suppression of CAT activity as a result of the production of catalase inhibitors. Lower CAT activity in the kidney of *Cyprinus carpio* after subjected to different concentrations of chromium was noted by Kumar et al. (2013). Due to heavy metals and H_2O_2 denaturation, fish from contaminated areas have lower levels of CAT activity (Kubrak et al., 2013; Karadag and Firat, 2014). By converting H_2O_2 into water, the essential antioxidant enzyme catalase defends cells from the harmful effects of H_2O_2. When it is absent, H_2O_2 accumulates as a result, an increase in the generation of hydroxyl radicals (Abele and Puntarulo, 2004). It has been found because the organ with the greatest level of activity is the liver of antioxidant enzymes and is more vulnerable to oxidative stress than other tissues. This may be because the liver undergoes a number of oxidative processes and has the highest degree of free radical production; hence, liver tissues were considered to be the best approach to demonstrate CAT activity sensitivity to metal. In previous investigations, same results have also been assessed by Lushchak et al. (2009).

According to Batool et al. (2014) catalase activity was decreased after acute exposure to metals (Cr and Cd) in fish. According to Mohanty and Samanta (2016), *Notopterus notopterus* muscle tissues exposed to iron, copper, nickel, copper, lead, cadmium, and zinc from the Mahanadi River have considerably lower catalase activity. Acute exposure to metals including Cu, Cd, Zn, Cr, and Fe, the kidney of tilapia and liver's catalase levels were reduced (Atli and Canli, 2010). *Heteropneustes fossilis* and *Channa striatus* showed changes in antioxidant enzymes in liver, kidney, brain, and gills when captured from metal polluted (Ni, Cr, Cd and Pb) Kali River of northern India (Fatima and Usmani, 2015).

According to Atli and Canli (2010) responses of different enzymes to metal exposures depend on the toxicant's composition, the exposed organ, and the exposure method. A peroxisomal enzyme named CAT primarily breaks down H_2O_2 into O_2 and H_2O (Fatima and Usmani, 2015). Metal ion binding to catalase's SH groups, which causes an increase in O-2 and H_2O_2 radicals, may be the cause of the catalase inhibition (Atli and Canli, 2010). According to Orun et al. (2008) when in contact with metals (Cd^{+2}, Cr^{+3}, and Se), *Oncorhynchus mykiss's* liver's catalase level significantly decreased. Exposure of heavy metals showed significant decreased in brain, liver and gills catalase of *Oreochromis niloticus* (Rehman et al., 2021). Metals mixture significantly decreased the CAT level in brain, gills, muscles, liver, heart and kidney of *Channa striata* (Arshad et al., 2018). The fish, *C. mrigala* had lower CAT level in brain, kidney, muscle and liver due to metal mixture treatment (Naz et al., 2018).

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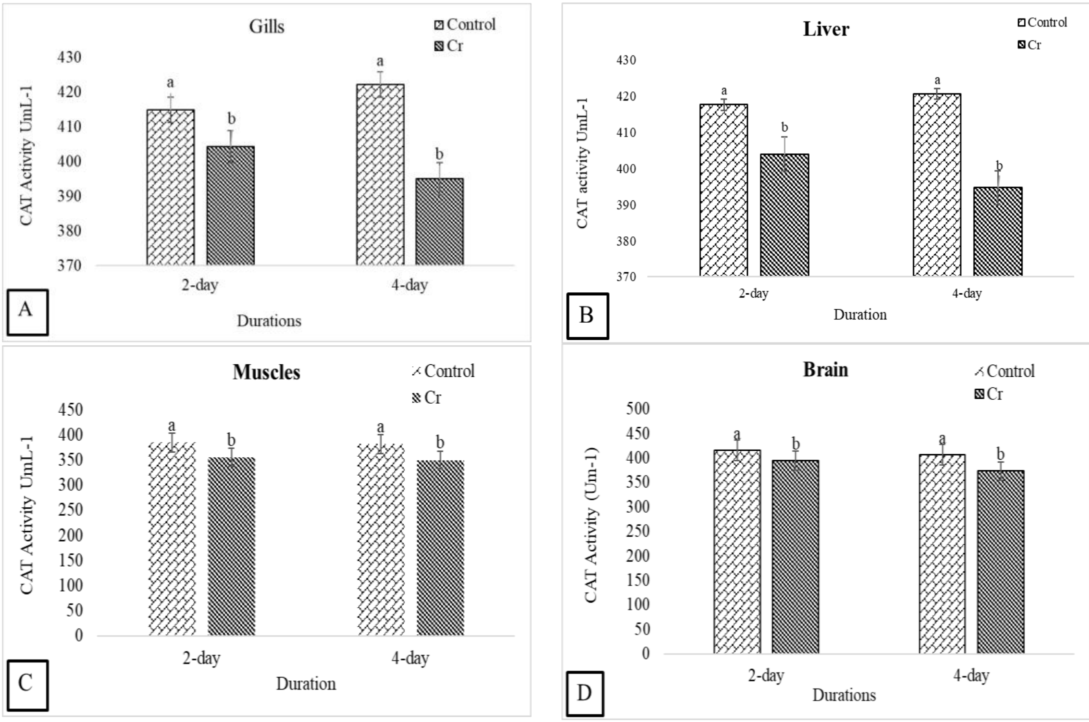

Figure 3. *Effect of chromium on CAT activity of [A] gills, [B] liver [C] brain and [D] muscles, of L. rohita*

Geno-toxicity

In the current research, exposure of Cr to fish *C. Idella* resulted in significantly increase in nuclear abnormalities like micronuclei, notched and De shaped nuclei as compare to control group (*Figures 4-5*). Kumar and Garg (2021) reported a significant rise in the frequency of MN in *Carassius auratus* gibelio erythrocytes exposed to chromium. Singh et al. (2019) examined the impact of arsenic on *Channa punctatus* using MN test. Result revealed higher number of notched nuclei in arsenic treated group. Ghaffar et al. (2016) found that *Labeo Rohita* RBCs exposed to heavy metals had a higher frequency of micronuclei. Matsumoto et al. (2006) reported the impacts of Cr in *O. niloticus* RBCs. Results showed a significant rise in notched nuclei.

Micronucleus (MN) test is a helpful experimental method for determining the genotoxic characteristics of substances exit in the aquatic environment (Al-Sabti and Metcalfe, 1995). At least one cell cycle in a cell is required to identify MN, which manifest when a whole chromosome or chromosomal fragment fails to migrate with one of the two daughter nuclei formed during mitosis (Udroiu, 2006).

Kumar and Garg (2021) also noted the dramatically increased in MN and NAs in RBCs of four fish species exposed to copper. The results of this experiment confirmed previous evidence of copper's genotoxic effects on fish. Even at extremely low concentrations, mutagens found in aquatic environment can cause responses in fish. These mutagens may lead to the development of micronuclei in cells. However, compared to other species, the healing process in fish is extremely slow. In order to examine for the presence of harmful substances in aquatic environments, fish might be put to use as a model species (Mahboob et al., 2014). Additionally, it was shown that arsenic greatly increased the frequency of micronuclei in *Channa punctatus*, but it dramatically decreased in control fish (Patowary

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et al., 2012). Minhas et al. (2022) also reported the micronucleated and binucleated RBCs of *C. mrigala* because of cobalt metal. Abdullah et al. (2021) noted the considerable MN and DN production due to Pb and Cd exposure in fish RBCs. Razzaq et al. (2021) reported that the cobalt and chromium mixture induced significantly higher mean MN and DN in *Labeo rohita* by using micronuclei assay.

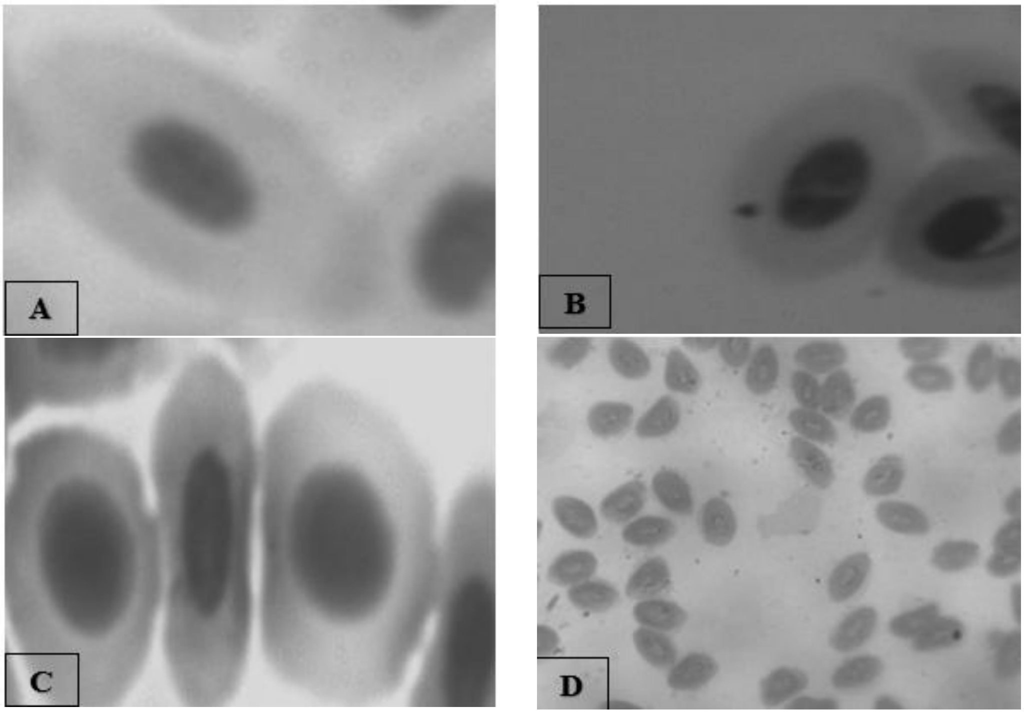

Figure 4. *[A] Normal Nuclei [B] Micronuclei [C] D shaped Nuclei [D] Notched Nuclei*

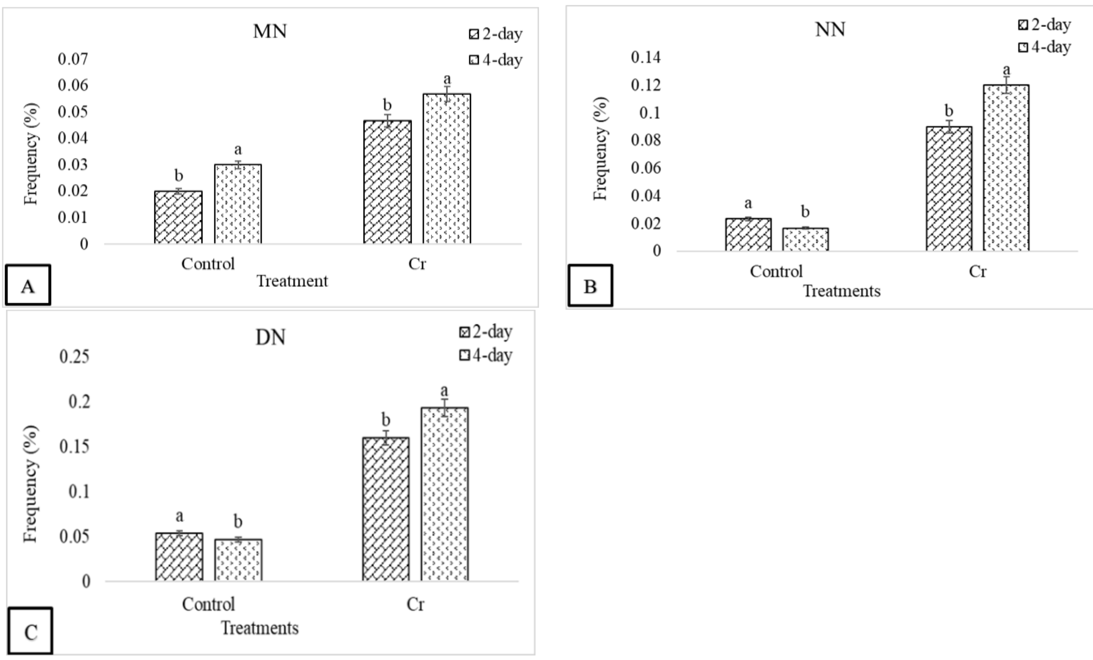

Figure 5. *Frequency of Nuclear Anomalies in fish [A] Micronuclei [B] Notched nucei [C] De-shaped nuclei*

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Conclusion

In conclusion, exposure to chromium affected the behaviour, catalase activity, and genotoxic reactions of *Ctenopharyngodon idella* (grass carp). These results highlight the requirement for efficient monitoring and management techniques to reduce the effects of chromium contamination on the environment and protect the wellbeing of aquatic ecosystems. Moreover, to understand the underlying processes and long-term effects of chromium exposure on fish populations and their ecosystems, more study is necessary.

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