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Evaluation of Catalase Activity, Gill Histology and Genotoxic Effects of Cadmium in Tilapia (*Oreochromis niloticus*) Fingerlings

(Penilaian Aktiviti Katalase, Histologi Insang dan Kesan Geno Toksik Kadmium pada Anak Ikan Tilapia (*Oreochromis niloticus*))

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ABSTRACT

This research was designed to assess the toxicity of cadmium (Cd), and its effects on catalase (CAT) activity, histology of gills and geno-toxicity of *Oreochromis niloticus*. The acute toxicity of Cd (96-h) for fish was computed as 56.021 mg/L (LC_{50}) and 80.7336 mg/L (LC_{100}) using Probit method. The inferences showed that catalase level was significantly ($P<0.05$) lower in Cd treated *O. niloticus* as compared to control that was metal-stress free. It followed the order as: intestine<gills<muscles<brain. Results of gills histology showed that Cd-exposure caused prominent damage to both primary and secondary lamella. The histological alterations included fusion and curling of secondary lamella, hyperplasia in secondary lamella, epithelial lifting, and aneurysm observed in gills. Geno-toxic results showed that a significant ($p<0.05$) increase in micronuclei, de-shape and notched nuclei in erythrocytes of Cd-exposed *O. niloticus* was found as compared to control. The inferences of this study confirmed the genotoxic properties of Cd. This study will be helpful in understanding the histological and geno-toxic changes in fish body kept under Cd stress. This study will also help in the development of a specific approach to minimize the negative and genotoxic impacts of cadmium. Furthermore, tilapia can be used as a good bio-indicator for detecting toxic impacts associated with water pollution.

Keywords: Catalase; heavy metals; histology; tilapia; toxicity

ABSTRAK

Penyelidikan ini direka untuk menilai ketoksikan kadmium (Cd) dan kesannya terhadap aktiviti katalase (CAT), histologi insang dan geno toksik *Oreochromis niloticus*. Ketoksikan akut Cd (96-jam) untuk ikan dihitung sebagai 56.021 mg/L (LC_{50}) dan 80.7336 mg/L (LC_{100}) menggunakan kaedah Probit. Inferens menunjukkan bahawa tahap katalase secara signifikan ($P<0.05$) lebih rendah dalam Cd dirawat *O. niloticus* berbanding kawalan yang bebas

tekanan logam. Ia mengikut tertib sebagai: usus<insang<otot<otak. Keputusan histologi insang menunjukkan bahawa pendedahan Cd menyebabkan kerosakan yang ketara kepada kedua-dua lamela primer dan sekunder. Perubahan histologi termasuk gabungan dan lencongan lamela sekunder, hiperplasia dalam lamela sekunder, pengangkatan epitelium dan aneurisme yang diperhatikan dalam insang. Keputusan geno toksik menunjukkan bahawa peningkatan ketara ($p<0.05$) dalam mikronukleus, nyah-bentuk dan nukleus bertakuk dalam eritrosit *O. niloticus* terdedah Cd didapati berbanding kawalan. Inferens kajian ini mengesahkan sifat geno toksik Cd. Kajian ini akan membantu dalam memahami perubahan histologi dan geno toksik dalam badan ikan yang disimpan di bawah tekanan Cd. Kajian ini juga akan membantu dalam pembangunan pendekatan khusus untuk meminimumkan kesan negatif dan ketoksikan geno kadmium. Tambahan pula, tilapia boleh digunakan sebagai penunjuk biologi yang baik untuk mengesan kesan toksik yang berkaitan dengan pencemaran air.

Kata kunci: Histologi; katalase; ketoksikan; logam berat; tilapia

Introduction

In the last few decades, pollution caused by heavy metals has become a center concern of environmentalists (Fazal et al. 2018). Residuals of heavy metals get continuously increased in air and water causing pollution to our environment (Javed & Usmani 2019). Despite advancements in environmental waste management systems, issues of negative influence of metallic waste pollution to aquatic bio-life are not being solved until today (Adhikari, Ghosh & Ayyappan 2006). Lakes, ponds, rivers, and other water bodies are being polluted due to the increase of industries (Fazal et al. 2021). Urbanization has also played an important role in creating aquatic pollution (Praveena et al. 2013). Large amounts of these heavy metals accumulate in the aquatic ecosystems as a result of land-based activities which includes untreated waste discharge into water bodies, agricultural runoff, domestic storm water runoff, and sewage discharge. All these activities are considered as the source of contamination in aquatic ecosystems (Shah et al. 2020).

Short term but severe exposure of toxicant with living body is defined as acute toxicity. And the particular concentration that kills half of the population is termed lethal concentration (LC_{50}). Fish has sensitive interaction with its habitat and any change in its surroundings did not go undetected. Thus, fish in aquatic system act as bioindicators (Woody et al. 2010). Fish are good bio-indicators of metal contamination because they occupy the top place in aquatic food web (Thangam, Jayaprakash & Perumayee 2014). Due to its widespread cultivation and distribution in aquatic habitats in subtropical and tropical climates, the Nile tilapia is a species of commercial and ecological importance (Mattioli et al. 2020).

Cadmium (Cd) lies in category of non-essential elements. Small amounts of cadmium could be dangerous to fish body due to slow rate of removal (Capillo et al. 2018). Cadmium is considered to be the most dangerous pollutant due to its high tendency to accumulate in living tissues (Abbas et al. 2019). It can bioaccumulate and bio-magnify quickly in numerous fish tissues such as in the liver, kidney, gills, and muscles due to a long half-life (Barwick & Maher 2003; Cao et al. 2012). Metabolic systems get affected by Cd includes disrupted enzyme functioning, decrease in enzyme activity and lowered mitochondria working especially in lipid peroxidation. Changes in DNA like breakdown of fragments and other nuclear abnormalities are also included (Almeida et al. 2009).

As a result of Cd build up in tissues, the generation of reactive oxygen species (ROS) increases. These species can target the biological components like lipids and proteins, resulting in peroxidation of lipid, protein carbonylation, and cause changes in various systems such as antioxidants defense and biotransformation process (Cao et al. 2012). The catalase enzyme protects animals against oxidative damage. Metallic ions and hydrogen free radicals are activated by hydrogen peroxide (Barynin et al. 2001). Catalase protects fish from the dangers of hydrogen peroxide by converting it into hydrogen and water (Zhang & Jin 2008).

In laboratory and field research, biomarkers like histological alterations have been frequently studied to assess the impact of pollutant on fish (Van der Oost, Beyer & Vermeulen 2003). While studying the environment, utilizing histological biomarkers is helpful because it allows examining specific organs (Padrilah et al. 2018). Both in marine and freshwater habitats, examining gills

give accurate information about quality and quantity of contaminants (Fernandes et al. 2007). Gills are the most sensitive organ in a fish's body for detecting harmful substances because their epithelia are extremely reactive to hazardous exposure (Evans, Piermarini & Choe 2005).

The first organs that are directly exposed to environmental pollutants are the gills. Large surface area of the gills allows for better interaction with metals and also absorption (Fernandes et al. 2007). Gills play important role in fish body which includes not only respiration but maintenance of ion equilibrium and excretion also (Padrilah et al. 2018). Changes in gill histology are easier to understand (Fanta et al. 2003) and give clear clues of damages in fish body. Sensitivity of gills towards metal exposure gives researchers a chance to investigate metallic pollutants in different habitats (Jimenez-Tenorio et al. 2007). Metals while interacting to gills brought changes in shape and structure of gills, reducing the gill capacity to carry out respiration, ion and water balance around the fish body (Fernandes et al. 2007). Due to Cd exposure, chances of histological changes in gill layers increases (Cao et al. 2012).

Fish have been recognized as useful and sensitive bio-indicator for assessing the genotoxic and cytotoxic effects of contaminants in aquatic bodies (Ozkan et al. 2011). According to Banerjee and Flores-Rozas (2005) cadmium is well recognized as a carcinogen metal as well as genotoxic, apoptotic (Kim et al. 2005; Watjen et al. 2002) and teratogenic (Hovland et al. 2000). Micronucleus test is a good technique for identifying environmental stress; it is one of the most helpful biomarkers for assessing the mutagenic effects in fish (De-Jesus et al. 2016). Normal nuclear morphology changes, such as anomalies in the nuclei of erythrocytes caused by cell division failure or cell death, are also thought to be signs of genotoxic damage (Furnus et al. 2014).

One of the most rapidly used techniques for genomic alterations includes micronuclei (MN) assay. It was originally developed in mammalian species but now widely applied in studying fish, mussels, sea urchin, crabs, and oysters (Bolognesi & Hayashi 2011). Micronuclei assaying is quite easier in comparison with microscopic analysis of chromosomal aberrations during metapahase. As fish and other aquatic fauna have small chromosomes, micronuclei assay becomes a more suitable technique. Due to these reasons, MN assay proved to be a more applicable biomarker in the analytical study of environmental bio-monitoring (Bolognesi & Hayashi 2011). Thus, this study has been planned to evaluate the effect of Cd on *O. niloticus.*

Materials and Methods

Sample collection and acclimatization

A total (n=150) fish, *Oreochromis niloticus* (age= 5 months) were collected from the Fisheries Research and Training Complex, Bahawalpur, Pakistan. Polythene bags were used to transport it to the Fisheries Research laboratory, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan. Dechlorinated tap water was used to acclimatize fishes for the next 10 days. During this period fish was fed twice daily.

Toxicity test

Oreochromis niloticus was used as an experimental organism to determine the 96 h LC_{50} and lethal toxic level of cadmium (Cd). The chloride salt of cadmium as $CdCl_2$ was dissolved in distilled water to make a stock solution for toxicity tests. Toxicity tests were conducted with 10 fish in each aquarium (100 L). The concentration of Cd was started at 5.0 mg L^{-1} and increased gradually to 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 65, 70 and 75 mg L^{-1}. No feed was given in this period to avoid debris and fecal matter. Water was replenished at 24 h interval. The fish mortality was observed after every 12 h period. During this trial, water pH, temperature, dissolved oxygen level, total hardness and total ammonia, calcium, magnesium and carbon dioxide level were measured (APHA 1998).

Catalase activity

After getting LC_{50} (96 h), fish was dissected and organs including gills, brain, heart and muscles were isolated. Homogenization of organs was completed in the presence of cold sodium phosphate (60 mM, pH 7.0) buffer by ratio of 1:4 (w/v) using a blender. Next, homogenization and centrifugation were done at temperature 4 °C, with speed 10,000 rpm and time limit of 15 min. The supernatant was obtained and stored to use later. The catalase (CAT) activity was measured by adopting the Chance and Mehaly (1977) protocol.

Gill histology

For light microscopic examination of gill samples, paraffin-embedding technique was used (Suvarna, Floyd & Bancroft 2012). The steps included in this technique

were washing, dehydration, clearing, infiltration, sectioning/tissue mounting. The prepared slides were studied under OPTIKA® Italy light microscope (40X magnification) provided with a camera and linked with a computer (Adam et al. 2019).

MICRONUCLEUS TEST FOR GENO-TOXIC STUDY

To observe the MN and NAs, slides were prepared from blood of both control and Cd-treated fish by following the protocol Barsiene et al. (2006). The Fenech et al. (2003) criterion was adopted for scoring abnormalities. MN frequency was calculated as follows

$$MN\% = \frac{Number\ of\ cell\ containing\ micronucleus}{Total\ number\ of\ cells\ counted} \times\ 100$$

STATISTICAL ANALYSES

The Probit analyses was applied to determine LC_{50} and lethal value of Cd for *O. niloticus* (Hamilton, Rusoo & Thurstan 1977). The software Minitab and Statistix 8.1 version were used for analyses. One-way ANOVA was applied on results gained from oxidative stress study ($P< 0.05$). Mann Whitney U test was performed to check the difference between frequencies of micronuclei and other nuclear abnormalities (Steel, Torrie & Dickey 1996).

RESULTS AND DISCUSSIONS

TOXICITY TEST

Figure 1 shows the relationship between increasing concentration of Cd and mortality rate of *O. niloticus*. In the present study, 96 h toxicity of cadmium LC_{50} and lethal value for *O. niloticus* was computed as 56.021 and 80.7336 mg/L, respectively. Similarly, Samuel (2021) reported the value of LC_{50} (96 h) of Cd for *C. gariepinus* as 79.72 mg/L. Ali, Abed and Ellah (2018) also reported the toxicity of Cd to *G. holbrooki* as 50.178 mg/L. Valiallahi and Pourabbasali (2019) studied the LC_{50} of Cd for *Huso huso* as 28 mg/L. The LC_{50} (96 h) of Cd to *T. Mossibica* was computed as 36.848 mg/L (Goswami et al. 2016). Many researchers reported the lethality of Cd for various fishes as *O. niloticus* (Bakar et al. 2014), *O. javanicus* (Puspitasari, Purbonegoro & Putri 2018), *A. japonica* (Ahn et al. 2020), *Cyprinus carpio* (Delahaut et al. 2020; Yallappa & Asiya Nuzhat 2018; Ta et al. 2018), *C. idella* and *H. nobilis* (Vajargah & Hedayati 2017), *D. rerio* (Al-Sawafi, Wang & Yan 2017), *C. carpio* and *C.*

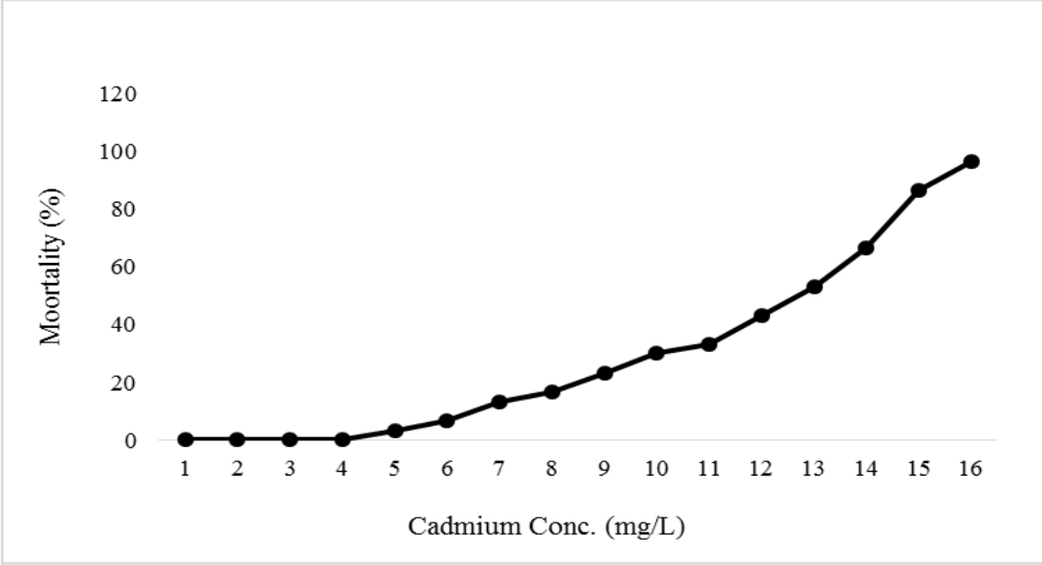

FIGURE 1. Relation between cadmium concentration and mortality rate of *O. niloticus*

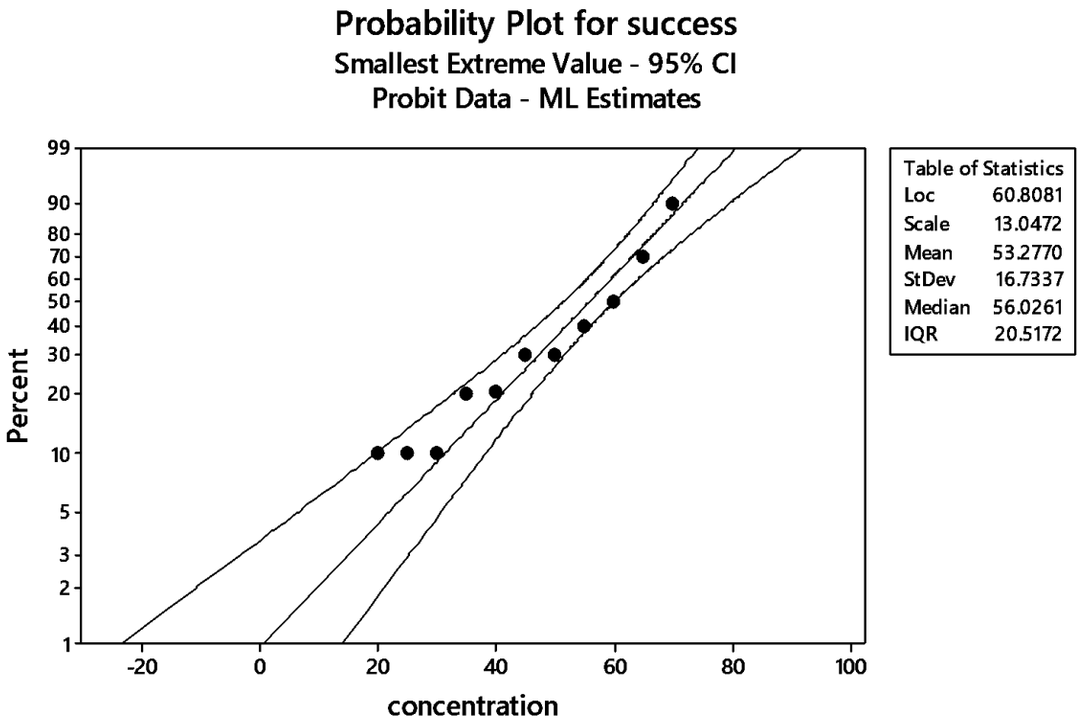

FIGURE 2. 96-h acute value of Cd for fish *O. niloticus*

auratus (Yalsuyi et al. 2017). Heavy metals add a toxic effect by interfering with metabolism and by causing mutagenesis. Heavy metals have the potential to disrupt normal body functions and physiology, affecting fertility, and even cause death in severe cases (Fatima et al. 2020).

CATALASE ENZYME

In the present study, significantly ($P>0.05$) lower CAT level in brain, muscles, gills, and intestine of Cd-stressed fish was recorded as compared to control. The level of CAT in organs of fish was noted as: intestine<gills<muscles<brain. The inferences also showed that CAT level continuously dropped off during the whole experimental period in organs of Cd-treated fish (Figure 3). Similarly, Naik, Shyama and D-Costa (2020) also documented the decline in CAT level of *O. mossambicus* due to $CdCl_2$. Exposure of metal mixture (Pb+Ni) significantly decreased the CAT activity in gills, liver, kidney, brain, muscle, and heart of *Channa striata* (Arshad et al. 2018). Rehman et al. (2020) observed decreased catalase in gills, brain, and liver tissues of metals exposed *O. niloticus*. The acute exposure of Fe+Ni+Pb+Zn mixture to *C. mrigala* caused significant decline of CAT in brain, liver, muscle, kidney, and gills (Naz et al. 2018).

The Cd induced reduction in kidney CAT activity was noted in fish, *C. batrachus* and *O. niloticus* (Atli et al. 2006; Kumar et al. 2009), catfish, *R. quelen* (Pereira et al. 2016), *G. maculatus* (Mcrae, Gaw & Glover 2018) and due to Cd+Pb in *O. niloticus* (Ahmed et al. 2016). Cadmium causing decrease in gill CAT activity was reported by Pandey et al. (2008) in *C. punctate,* Kumari, Khare and Dange (2014) in *Labeo rohita*, and Dabas et al. (2014) in *L. rohita*. Mani et al. (2014) studied the lower CAT activity in brain tissues of *A. arius* after Cd-exposure.

A potential pathway for reduction in CAT activity in kidney of *Dicentrarchus labrax* due to Cd is that Cd

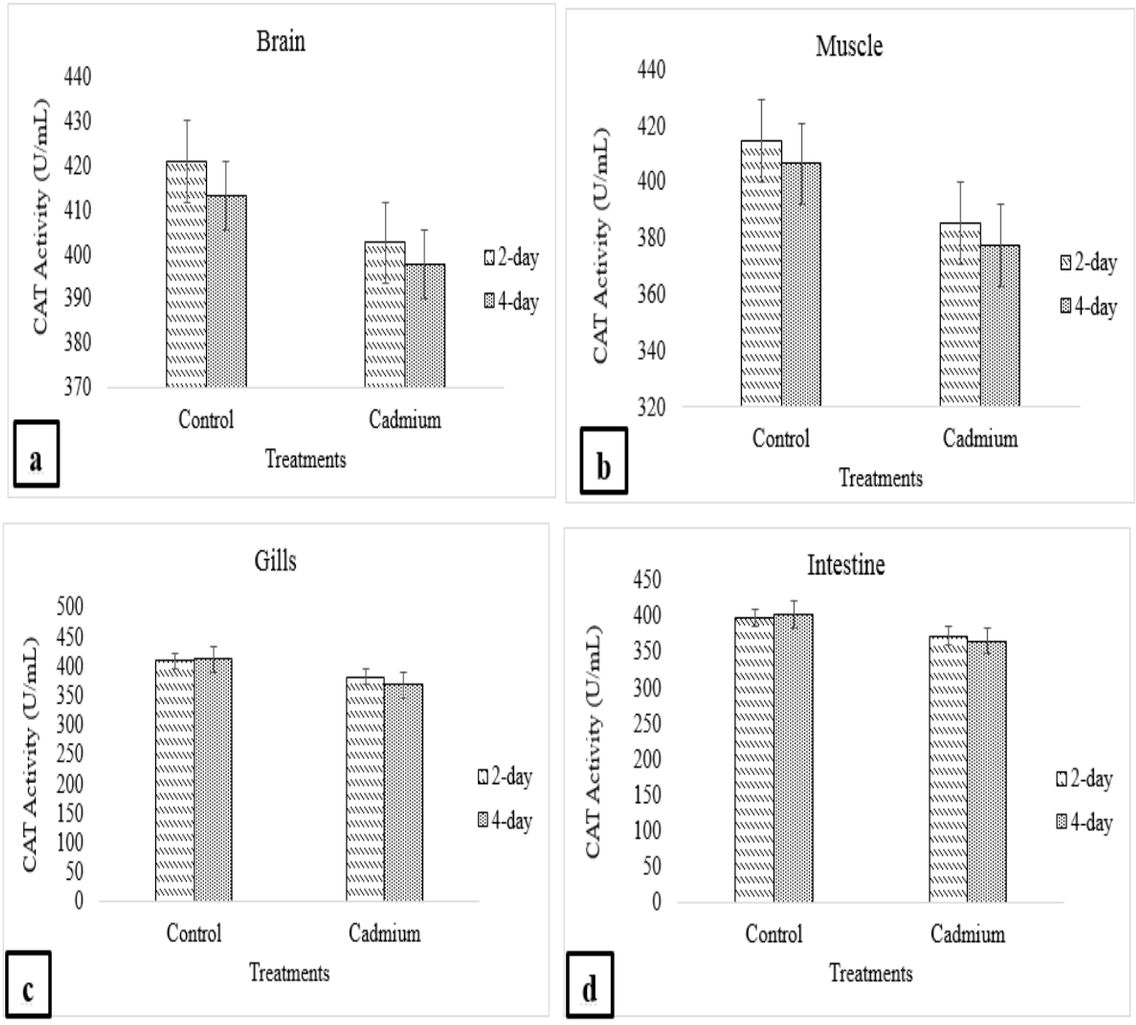

FIGURE 3. Activity of CAT in organ of *O. niloticus* a) brain b) muscles c) gills d) intestine

can cause the structural changes in enzyme by directly binding, in addition to this Cd can a reduced the synthesis of CAT (Romeo et al. 2000). Cadmium has the ability to bind directly with active sites of antioxidant enzymes which inhibits its activity or may disturb the three dimensional shape of enzyme (Wang et al. 2015).

GILL HISTOLOGY

Table 1 representing account of changes induced by Cd treatment in *O. niloticus*. According to Poleksic and Mitrovic-Tutundzic (1994) the histological changes in gills were classified into four stages viz. where +(0) = Normal, + (1) = lower damage, ++(2)= moderate damage and +++(3) = severe damage, on the basis of severity of the lesions. Lower damage (+) involves changes, which do not alter the normal function of the gill and healing of gill can occur with improvement of the environmental conditions; Moderate damage (++) are more severe and harm the normal gill function. These elisions are reparable, but they can lead to severe alterations in the case of chronic pollution; and severe damage (+++) cause irreversible injury, which recovery is not possible, even

with improvement of the water quality.

Figure 4(a) represents the normal gills of tilapia kept under control water. In the present study, changes due to Cd exposure in *O. niloticus* were reported, which includes epithelial lifting in secondary lamellae (Figure 4(b)), aneurysm in secondary lamella (Figure 4(c)), hyperplasia in secondary lamellae (Figure 4(d)), curling filaments of secondary (Figure 4(e)) and fusion of secondary lamella. Similarly, many changes viz. fusion and curling of secondary lamellae, aneurysm and hyperplasia in gills of Cd-stressed *O. niloticus* was studied by Otludil, Akin and Erhan (2017). According to Macedo et al. (2020) interaction of metallic ion (Cd) with gills caused upward lifting of epithelial and fusion of secondary lamella in *Astyanax lacustris.* Histological alteration in gills including lifting of epithelia, lamellar fusion and aneurysms was noted in metals (cadmium, zinc and nickel) exposed *C. carpio* (Todorova et al. 2019). Lead and cadmium induced alterations in gills such as epithelial lifting and hyperplasia were also observed by Ahmed et al. (2014) in *Anabastestudineus* and Patnaik et al. (2011) in *C. carpio*. The fusion of secondary lamella (Pandey et al. 2008; Prabhahar et al. 2012; Selvanathan, Vincent & Nirmala 2013), curling of secondary lamellae (Selvanathan, Vincent & Nirmala 2013) and aneurisms (Liu et al. 2011; Selvanathan, Vincent & Nirmala 2013) in gills of metal-exposed fish were observed.

In laboratory experiments, histopathological examinations have proven to be a sensitive method for detecting direct effects of chemical substances on fish target organs. The dose and duration of exposure determine the degree of pathological alterations. Histopathological studies have been done to help figure out how exposure to contaminants affects different biological responses (Bose et al. 2013).

Gills are the most sensitive sites in fish body to identify presence of toxic substance because its epithelia are quite reactive towards toxic exposure (Evans, Piermarini & Choe 2005). Moreover, gills are organs, which are directly exposed to environmental toxicants. They are paradoxically and highly susceptible to toxic chemicals because, first, their large surface area makes it easier for toxicants to interact with and be absorbed, and second, their detoxification system is not as strong as the liver. Additionally, the rapid absorption of toxicants through the gills results in the rapid toxic response in the gills (Fernandes et al. 2007).

The edema is an accumulation of fluid that causes swelling in tissue that results in inflammation. The cell organelles were distracted by fluid collection, which reduced cell permeability, resulting in their disappearance and swelling (Rennika & Nurlita 2013). In severe conditions, edema may result in hyperplasia, which could block the inter-lamellae and cause the space between them to be filled with fresh cells, thickening the epithelium at the base of the lamellae (Robert 2001). The fusion of gill lamellae was a result of epithelial hyperplasia. The epithelial cells that suffered with hyperplasia and lamellae can result in significant reduction in surface area of gills required for respiration, interrupting the gills' blood flow, interfering with metabolic functions, and ultimately causing fish mortality (Modu et al. 2012).

TABLE 1. Histological alterations in treated and control group of *O. niloticus*

Histological alterations	Control	Treated
Fusion of secondary lamella	-	+++
Curling filaments of secondary	-	+
Aneurysm	-	++
Hyperplasia in secondary lamella	-	+++
Epithelial lifting	-	++

Normal (-), low damage (+), moderate damage (++), Severe damage (+++)

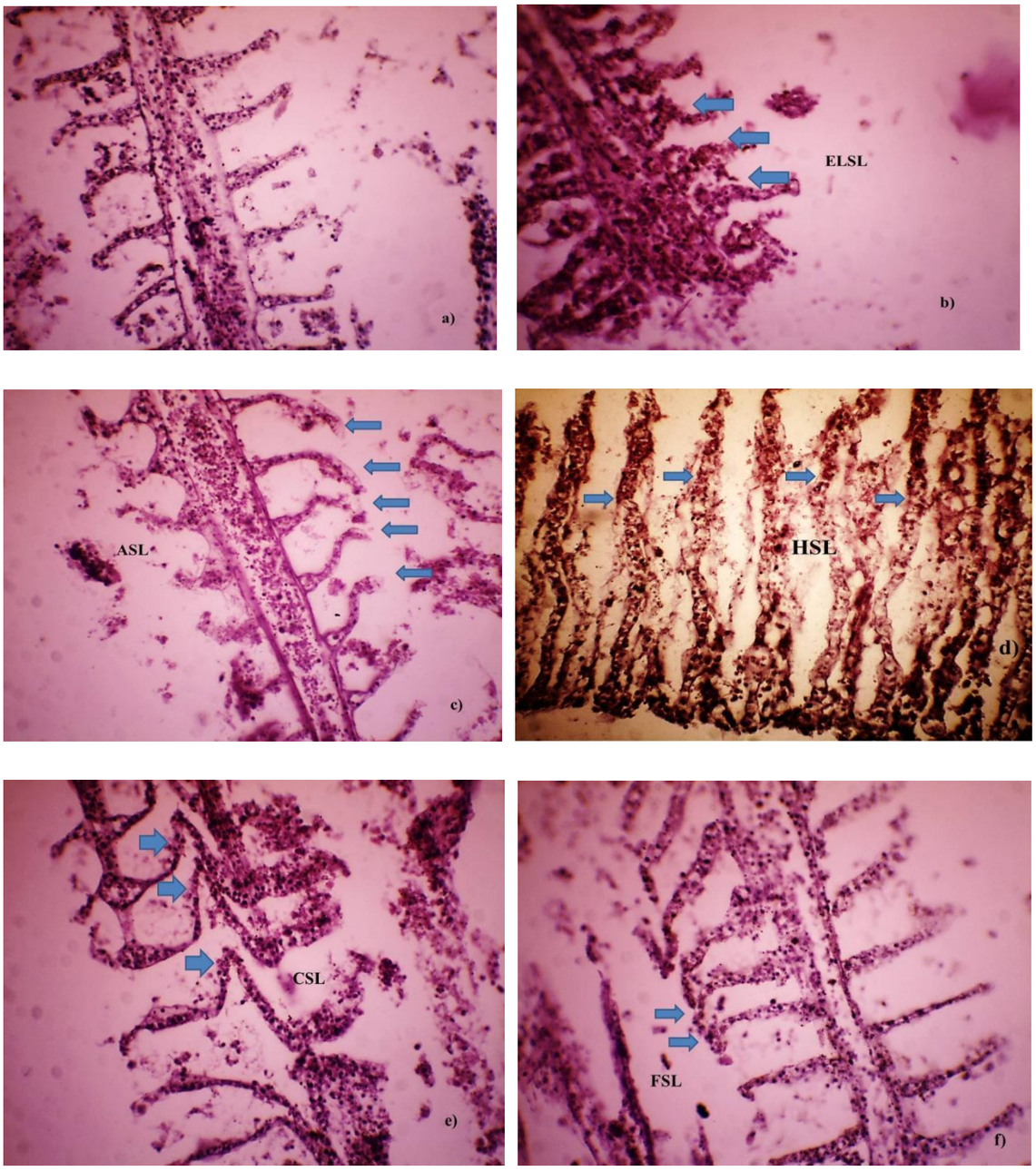

FIGURE 4. a) Gill histology of tilapia; Control group, b) epithelial lifting in secondary lamella (ELSL), c) aneurysm in secondary lamella (ASL), d) hyperplasia in secondary lamella (HSL), e) curling of secondary lamella (CSL), and f) fusion of secondary lamella (FSL)

MICRONUCLEUS TEST FOR GENO-TOXIC STUDY

In the present study, Cd treated *O. niloticus* erythrocytes showed an increase in nuclear geno-toxicity. The significantly increased number of micronuclei, notched and de-shape nuclei was found in RBCs of Cd-treated fish. Negligible or nearly zero percent nuclear abnormalities were found in control *O. niloticus* (Figure 5). In Cd-treated *O. niloticus,* formation of micronuclei, notched and de-shape nuclei was found to be greater with increase in exposure duration (Figure 6).

Micronucleus (MN) and nuclear abnormalities (NAs) assays can be efficiently used to assess the geno-toxic effects and chromosomal damage (Qualhato et al. 2017) that occur due to environmental toxicants. Many authors used all these techniques in their research studies to assess the cytotoxicity, mutagenicity and geno-toxicity in aquatic animals (Almeida et al. 2019; Witeska 2013).

The induction of micronuclei in RBCs of different fishes due to metals $CdCl_2$ exposure was observed by many researchers like Ergene et al. (2007) in *Alburnus orontis, Clarias gariepinus* and *Mugil cephalus*, Ossana, Eissa and Salibian (2009) in *C. carpio*, Guner, Dilek and Muranli (2011) in *G. affinis*, Parveen and Shadab (2012) in *C. punctatus,* Bakar et al. (2014) in *O. niloticus*, Arkhipchuk and Garanko (2005) in common carp, crucian carp and Mozambique tilapia, Naik, Shyama and D-Costa (2020) in *O. mossambicus* and Mahrous et al. (2015) in *O. niloticus.* The Cd-exposed *O. niloticus* showed increased formation of micronuclei and NAs in RBCs (Bakar et al. 2014; Ozkan et al. 2011). Our results are similar to Jiraungkoorskul et al. (2007) who noted the formation of notched nuclei in RBCs of Cd-stressed *O. niloticus, Peprilus triacanthus* and *P. altus*. Witeska, Kondera and Szczygielska (2011) also reported Cd caused significant increase in de-shaped nuclei in erythrocytes of *C. carpio*. Jindal and Verma (2015) observed the notched nuclei formation in RBCs of $CdCl_2$ treated *L. rohita*. Fatima et al. (2015) reported the increased number of micronuclei in red blood cells of *C. striatus* and *H. fossilis* captured from metals polluted Kali River. Minhas et al. (2022) also reported the cobalt induced MN and binucleated in *Cirrhinus mrigala.* Drąg-Kozak et al. (2022) also confirmed the higher number of MN and NAs in erythrocytes of Cd and Zn exposed Prussian carp.

Cadmium has the ability to stimulate the production of free radicals, which cause the oxidation of biomolecules like DNA, proteins and lipids resulting in different pathological disorders in organisms (Mahrous et al. 2015). The harmful effects of Cd impair normal Fe and Cd metabolism, diminish the reduced glutathione, alkaline phosphatase, Calcium-ATPase, and alter cell membrane structure and function through peroxidation of lipids. Moreover, it modifies the DNA repair system (Abdelazim et al. 2018). This has caused genetic material damage in exposed animals, including fish, and is consequently having additional genotoxic effects (Naik, Shyama & D-Costa 2020). Metals are clastogenic and aneugenic chemicals that can break chromosomes into acentric pieces and cause chromosome lagging, respectively. These compounds also have an impact on the spindle apparatus (Saleh & Alshehri 2011).

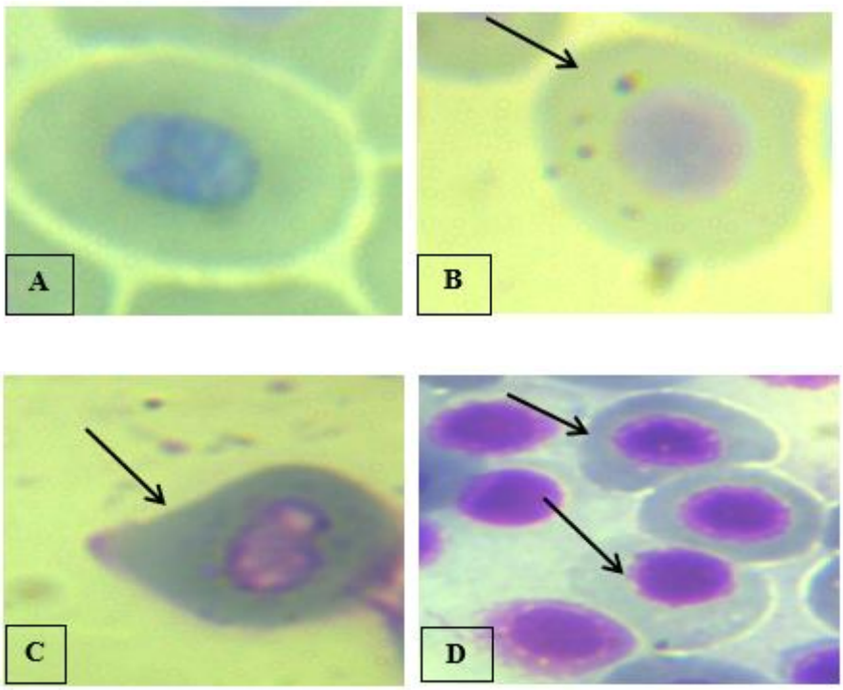

FIGURE 5. A) Normal Nuclei B) Micronuclei C) Notched nuclei D) De-shaped nuclei

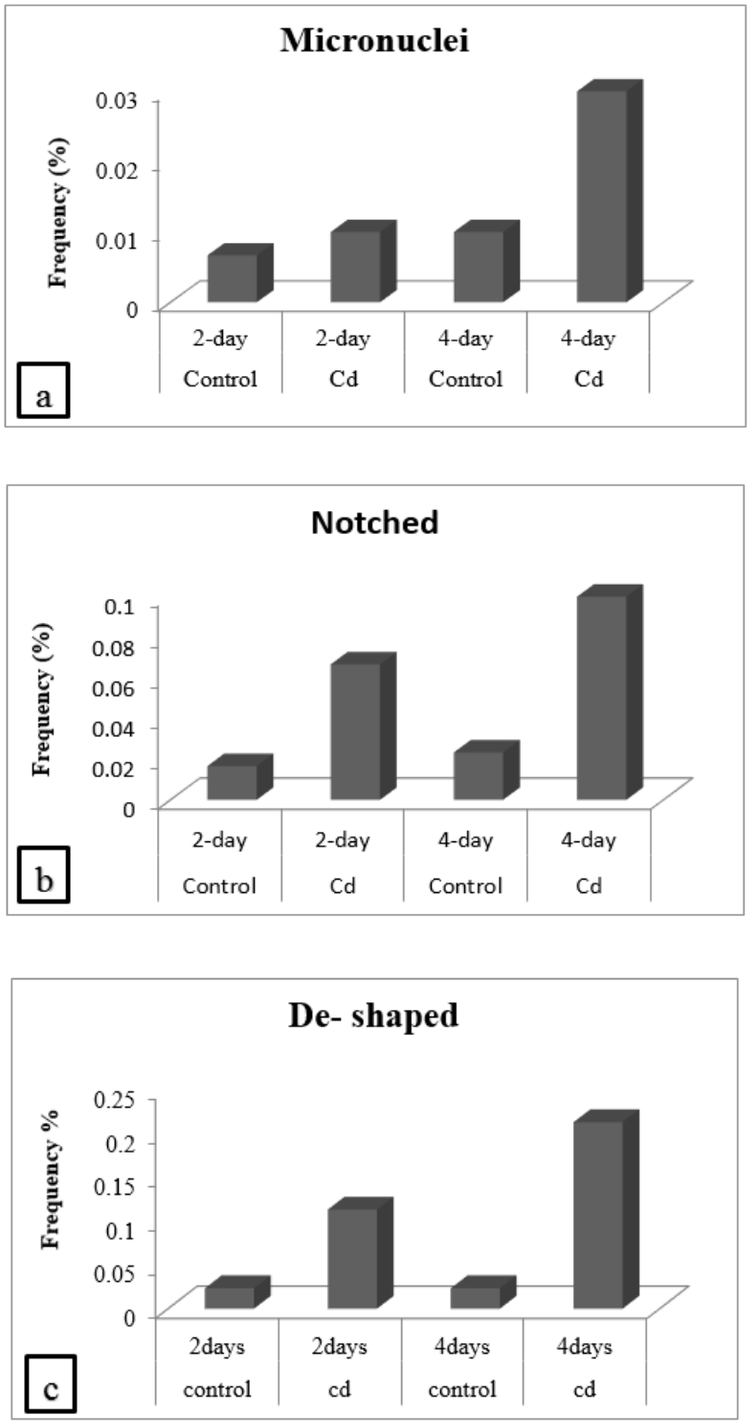

FIGURE 6. Frequency (%) of a) micronuclei, b) notched nuclei and c) De- shaped nuclei in RBCs of *O. niloticus*

In a variety of cell types, micronuclei and nuclear abnormalities develop during the proliferative stage of cell cycle. During cell division, a micronucleus is created at telophase when an entire chromosome or its fragment become encapsulated in a nuclear envelope and adopt the characteristics of an interphase nucleus, which is affectedly smaller in size (Al-Sabt & Metcalfe 1995). The precise mechanisms that cause the formation of nuclear abnormalities are unknown, but all these are applied as markers to identify the genotoxic and mutagenic responses of contaminated exposed fish species (Barsiene et al. 2006; Da-Rocha et al. 2011). Lately, it has been suggested that significant nuclear abnormalities may also result from physical pressures, such as the compression of the nucleus during migration through small areas, which may cause the rupture of the nuclear envelope (Shah, Wolf & Lammerding 2017).

Conclusion

The present study shows that acute toxicity of Cd induced a significant change in enzyme activity, histological parameters and genotoxicity of fish, which may lead to the death of fish. The integrated use of all parameters can serve as a good biomarker to detect metal pollution not only in laboratory conditions but can also be used in field studies like in the natural aquatic environment. Furthermore, there is a need to understand the mechanism of genotoxicity induced by metals and other toxicants in fish as well as other aquatic individuals.

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