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Water-borne cadmium affects the genotoxicity, oxidative stress, and histopathology of the liver of the Freshwater Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

Heavy metal contamination of water poses a grave threat to aquatic organisms, including fish. One such heavy element that is harmful to fish is cadmium. The objective of the current investigation was to evaluate the liver of *Oreochromis niloticus* after it had been subjected for seven days to 1 mg and 5 mg l^{-1} of cadmium in water in terms of genotoxicity, oxidative stress, and histological reaction. The Comet test was used to evaluate the genotoxicity in the liver of fish. Seven-day cadmium exposure caused significant ($P < 0.05$) DNA damage in fish. Increased lipid peroxidation, superoxide dismutase, and catalase leads to oxidative stress in fish liver, which induces antioxidant defence mechanisms. Inhibitory effect of reduced glutathione and glutathione peroxidase were useful indicators of oxidative stress. The study examined the antioxidant response and oxidative stress biomarkers of the commercially significant fish species, *O.niloticus*. The decrease in protein content may be attributed to decreased protein synthesis during toxicity. The liver histopathology of affected fish was significantly altered, indicating the quality of the fish stocks. This has severe repercussions for humans, as these fish are consumed by the local population. The results of this study can be used to evaluate the stress that pollution causes in aquatic organisms. *O. niloticus* with stood the oxidative stress brought on by exposure to heavy metals by using antioxidant defence mechanisms. As a result, Glutathione levels and lipid peroxidation can serve as markers for the presence of heavy metal pollution.

***Key words**: Cadmium, Genotoxicity, Heavy metal, Histopathology, Oxidative stress*

Introduction

Freshwater fishes are exposed to a variety of pollutants created by humans. Fishes are remarkable geno-toxicological model organisms that serve as early warning systems for toxicant-induced environmental changes and degradations (Pawar, 2012). By studying the induction of oxidative stress, fish species can be used as environmental bio-indicators of heavy metals. Even at low doses, the well-known non-essential heavy metal cadmium (Cd) can harm aquatic life. Cadmium was found in high concentrations in the intestine, kidney, liver, gills, muscles, and reproductive organs of the Nile tilapia, *O. niloticus* (Otludl *et al*., 2017). As fish occupy the highest trophic level in aquatic systems, there is a greater

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likelihood that Cd will be transferred to higher organisms, such as humans. Antioxidant enzymes are necessary for the detoxification and elimination of toxicity-induced reactive oxygen species. (Guardiola *et al*., 2017; Abdel Tawwab *et al*., 2018). To our knowledge, there are no reports on how cadmium affects the oxidative stress on the hepatocytes of freshwater fish, *O. niloticus.* Consequently, the primary goals of the current investigation were

- To determine the degree of DNA damage caused by a 7-day exposure to $CdCl_2$ in the liver tissue of *Nile tilapia* by utilising the Comet assay technique.
- To evaluate the degree of lipid peroxidation and how the liver's SOD, CAT, GPx, and GSH antioxidant enzymes responded to exposure to waterborne Cd at 1 mg l^{-1} and 5 mg l^{-1} for 7 days in order to combat oxidative stress.
- To evaluate the protein content in the liver tissue as liver being the centre for various metabolisms is rich in proteins.
- To monitor the histopathological alterations in the liver of fish exposed to water borne cadmium for 7 days.

Materials and Methods

Oreochromis niloticus (Linnaeus, 1758), the freshwater Nile tilapia, approximately 50 ±5g were collected from Neendakara in the Kollam district of Kerala. Prior to the experiment, fishes underwent a three-week acclimatisation phase in 50 l glass tanks with tap water at 28°C (pH 7.2) and natural photoperiod (12l/12d). Every day, the water in the aquarium was changed, and the fish were given commercial fish food to eat. Purchased hydrated cadmium chloride from SD Fine Chem Limited in Mumbai, Maharashtra.

Experimental design : Acclimatized fish were separated into three groups of six fish each, and the experiment was carried out three times to ensure the reproducibility of the findings. Fish from Group One kept in tap water without chlorine served as the control. Group Two and Group Three animals were given 1 mg/l and 5 mg/l $CdCl_2$ for seven days, respectively. The experimental design was depicted in Table 1. Animals were starved for twenty-four hours prior to being sacrificed. After the experiment, the fish were given a 0.1% dose of 2-phenoxyethanol (SRL, Mumbai) to make them unconscious, and blood was taken from their caudal arteries using a heparin-coated syringe. For biochemical analysis, plasma was promptly separated (5,000xg for 5 minutes). The fish's spinal cord was then severed to cause its death and pieces of liver were removed and stored immediately at -20 °C for Comet assay, antioxidant, and histopathological examinations.

Comet test procedure

The three-layer method for the comet test (Singh *et al*., 1988) was slightly modified (Tice *et al*., 2000). Pre-coating fully frosted slides with 1 ml of 0.75% agarose with a normal melting point was done while they were held at 4 °C. In a 1:1 ratio, 10µ l of low melting point agarose and blood samples were combined, and the mixture was pipetted over the top of the agarose layer in the first place.As a final protective layer, NMA (80µl) was applied. The slides were incubated after every stage for 10 minutes at 4 °C to let the agarose crystallise. Prior to use, DMSO (10%) and Triton X- 100 (1%) were added to a cold lysing solution. After lysis, the DNA was unwound in electrophoresis buffer for 20 minutes (300 mM NaOH and Na_2EDTA, PH13). The same buffer was used for electrophoresis, which was carried out using an electrical supply of 300mA for 20 minutes. Final steps included drying the slides and staining them with ethidium bromide after three to five-minute washes with neutralisation buffer (0.4 L Tris, pH 7.5). The slides were captured using an Opitka Pro5 CCD camera attached to an Olympus CKX41 inverted epifluorescent microscope. Tritek's comet-scoring software was used to record comet scores, which were then statistically connected.

Determination of Antioxidant enzymes

- The method of pyrogallol auto oxidation by superoxide radicals was used to test the SOD activity in the liver tissue extract, and the results were expressed in units per milligram of protein (Marklund and Marklund, 1974).
- The decrease in H_2O_2 absorbance at 240 nm, which was used to calculate the liver catalase activity, was given as mol/mg protein/min (Aebi, 1984).
- The Ellman technique (1959) was used to assess the GSH levels in liver tissue. 1 ml of tissue that had been homogenised in PBS was taken out and put in a test tube. Along with 1.3 ml of distilled water and 0.2ml of DTNB, 0.5 ml of phosphate buffer (0.2M, pH 8) was added (0.6mM). The contents were thoroughly combined, and

the spectrophotometer was read at 420 nm (Moron *et al*., 1979).

- Flohe and Gunzler's method (1984) was used to measure GPx activity in the liver. A reaction mixture containing one millilitre and 0.3 millilitres of tissue supernatant and phosphate buffer was created. The reaction was stopped by adding 0.5 ml of 5% TCA after 15 minutes of incubation at 37 °C. The tubes were spun at a speed of 3000 g for 5 minutes before the supernatant was removed. Following mixing, the absorbance was assessed at 420 nm. nM GSH was utilised per minute per mg of protein to measure the GPx activity.
- The assay relied on the formation of an MDA-TBA adduct from the reaction of MDA and TBA (Ohkawa *et al*., 1979). 2 ml of TBA-TCA-HCl reagent were well combined with 1 ml of liver tissue homogenate. For 15 minutes, the solution was boiled in a bath of boiling water. Centrifuging was used to separate the precipitate for 10 minutes at 1,000 rpm after the flocculant had cooled. The sample's absorbance at 535 nm was evaluated in comparison to a blank without any tissue homogenate. LPO was determined by measuring the amount of MDA that produced per milligram of protein, or n moles of MDA.
- The amount of protein in the liver was determined using a method developed by Lowry *et al*. (1951) and using bovine serum albumin as a reference.

Histopathological analyses: The histopathology of the liver of fish subjected to various doses of cadmium chloride, as well as the control, was investigated using a microtome. Depending on the staining technique, liver tissues were preserved in 10% formalin, cleaned in running water, and dehydrated by passing through a succession of ethanol concentrations (60-100%). Clark's technique for haematoxylin-eosin staining was utilised (1981). Mounting the stained sections with DPX mountant. The nuclei were blue while the cytoplasm was pink. The photomicrographs were captured using Qwin software and a Leica microscope (Leica, Jena, Germany).

Statistical analysis: Data were statistically analysed using the SPSS programme to compare means at a significance level of $P< 0.05$ using one -way analysis of variance.

Results and Discussion

Figure 1. depicts the comet assay results of *O. niloticus* liver tissue exposed to cadmium in comparison to the control. There was considerable damage in DNA, as determined by % tail DNA, in the liver tissues of fish subjected to high doses of cadmium chloride at 5 mg/l for 7 days as compared to the corresponding control. The liver tissues of fish subjected to 1 mg and 5 mg/l $CdCl_2$ showed signifi-

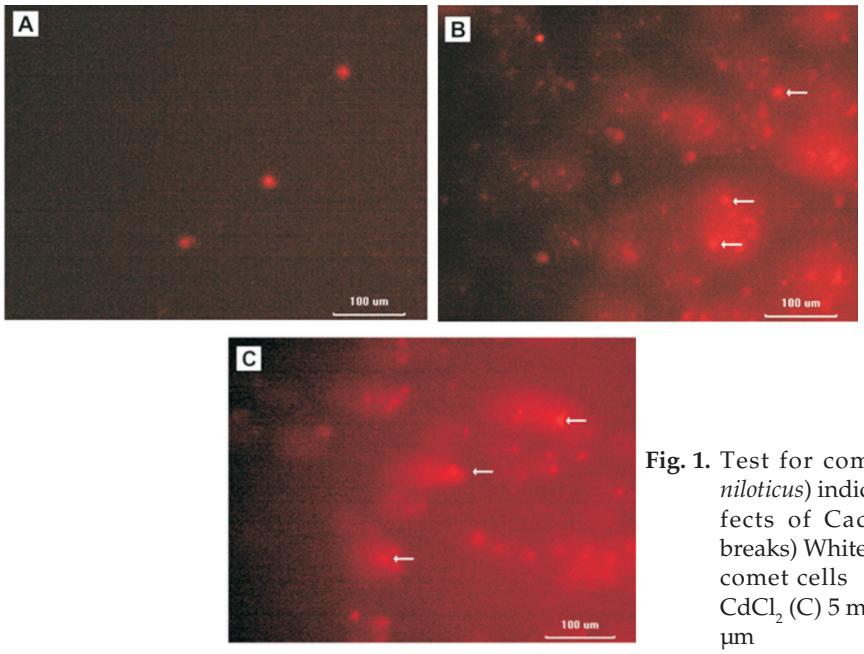

Fig. 1. Test for comets in fish liver (*O. niloticus*) indicating the genotoxic effects of Cadmium (many DNA breaks) White arrows indicate to the comet cells (A) Control (B) 1 mg $CdCl_2$ (C) 5 mg $CdCl_2$. Scale bar: 100 µm

cantly higher damage in DNA (P< 0.01) than the control sample. Significantly (P< 0.01) more DNA damage was detected in liver tissues exposed to 5 mg/l $CdCl_2$ compared to 1 mg/l $CdCl_2$. The higher the chemical concentration, the longer the comet tail and the shorter the comet head; consequently, the DNA fragmentation will be greater. The greatest damage to DNA was observed in tilapia exposed for seven days to the highest $CdCl_2$ concentration. Table 2. displays the average comet length and tail length of *O. niloticus* liver samples contaminated with cadmium.

The level of SOD, CAT, and LPO in the liver tissue of the cadmium-treated groups was considerably higher (P< 0.05) than that of the control group. (Fig. 2 A, B, C). When compared to the control

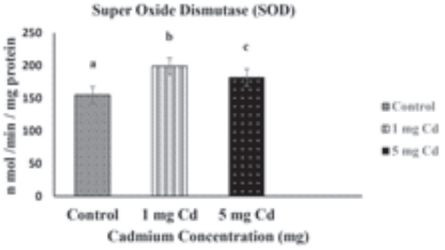

Fig. 2A. Level of SOD in the liver of *O.niloticus* exposed to water carried cadmium for 7 days. Data are shown as means ± SEM (n = 6).

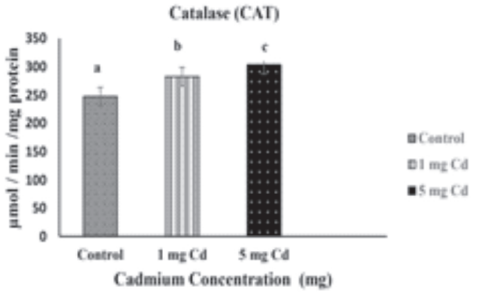

Fig. 2B. Level of Catalase in the hepatocytes of *O.niloticus* on seven days exposure to water-borne cadmium. Data are represented as means ± SEM (n = 6).

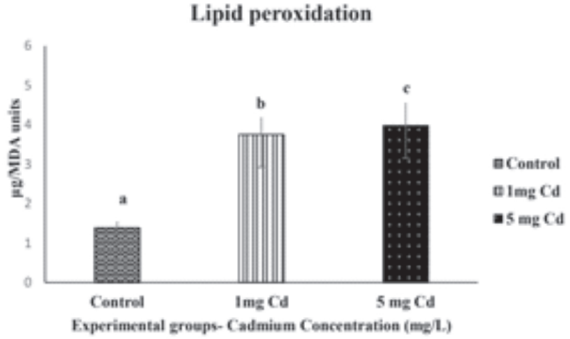

Fig. 2C. *O. niloticus* liver LPO was induced after exposure to varied Cd doses for seven days. Data are presented as means ± SE (n = 6).

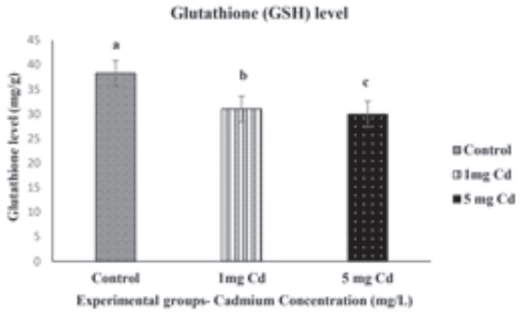

Fig. 3. Effect of cadmium injection on low levels of glutathione (nM GSH/mg protein) in *O. niloticus* liver tissue. Information is presented as means SE (n = 6).

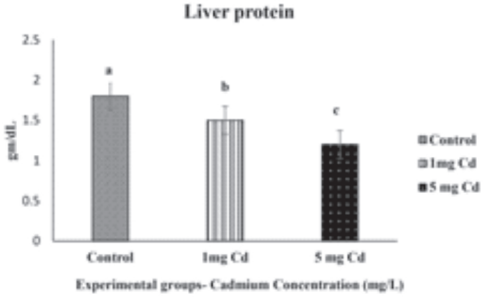

Fig. 4. A seven-day exposure to water-borne cadmium in *O. niloticus* caused changes in the liver protein composition. Information is presented as means SE (n = 6).

Table 1. Experimental protocol for the effect of Cadmium Chloride in *O.niloticus* (7 days)

Sl. No.	Group	Dose (mg/l)	Exposure period
1	Control	Cadmium free	7 days
2	1 mg $CaCl_2$	1 mg/l	7 days
3	5 mg $CaCl_2$	5 mg/l	7 days

Table 2. Average Comet and tail length of liver tissues of tilapia exposed to Cadmium chloride contamination

Groups	Comet Length (AU)	Tail Length (AU)
Control	27	3.7
1 mg $CaCl_2$	51.2	7.5
5 mg $CaCl_2$	69	25.8

group, the levels of glutathione (GSH) in the liver tissue of tilapia exposed to cadmium were considerably reduced (Fig. 3). The level of glutathione peroxidase (GPx) was significantly lower ($P< 0.05$) in the liver tissue of tilapia exposed to cadmium than in the control (Table 3). Compared to the control fish, the liver protein of cadmium-exposed tilapia was significantly decreased ($P< 0.05$). (Fig. 4). Liver histology from control and fish exposed to different dose of cadmium were briefly illustrated in Fig. 5.

Table 3. Effect of waterborne-cadmium (7 days) on the level of Glutathione peroxidase (GPx, U/g tissue) in the hepatocytes of *O. niloticus*

Groups	GPx activity U/g tissue
Control	53.30 ±8.10
1 mg $CaCl_2$	48.93 ±3.71*
5 mg $CaCl_2$	45.74 ±4.03**

Data are represented as means ±SE (n=6). Values with asterisk (*) describes a significant difference between the groups ($P<0.05$)

Heavy metals are ubiquitous in natural water sources and detrimental to aquatic life. This study demonstrated that the genotoxic, histopathological, and antioxidant parameters of Nile Tilapia, *O. niloticus*, treated for 7 days with 1 mg and 5 mg/l cadmium chloride exhibited significant changes. The pathogenicity of the metal toxin was elucidated by histological examination. Heavy metal accumulation in biological tissue systems causes biochemical changes and oxidative stress. Oxygen species with unpaired electrons and its numerous reactive intermediates typically inhibit metabolic reactions. Antioxidant defence mechanisms have evolved in the cellular mechanisms of fish to combat oxidative stress. Heavy metal chelation may disrupt liver tissue by degrading the functional and structural properties of the cells. Heavy metal toxicity causes liver alterations in fish, which can serve as an indicator of heavy metal stress in fish (Couch, 1975). The comet test results showed that the fraction of tail DNA could be enhanced by cadmium chloride in with respect to concentration. This suggests that when exposed to $CdCl_2$, *O. niloticus* is susceptible to breakage of single strand which cause damage in DNA. Similarities exist between our findings and those of Jindal and Verma (2015) who observed a concentration-dependent increase in the proportion of tail DNA in the RBC of *L.rohita* subjected to cadmium

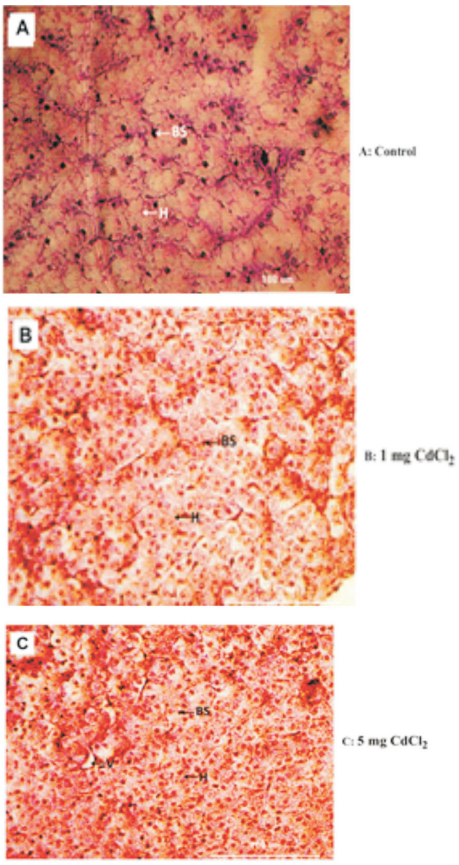

Fig. 5. Photomicrograph of liver of *O. niloticus* after 7 days exposure to water-borne cadmium. (A) Control (B) 1 mg $CdCl_2$ (C) 5 mg $CdCl_2$. (Haematoxylin & Eosin staining, 20X) Scale bar: 100 µm. H-Hepatocytes, BS-Blood Sinusoids, V-Vacuolation

for hundred days. In the current study, exposure to cadmium at low and high dose caused damage in DNA as evidenced by a noticeably higher percentage of DNA in the comet tail.

Enhanced CAT, SOD, and LPO activities in liver tissue may indicate their essential function in cell protection against the toxic effects of cadmium, thereby forming a creation of defence mechanism against the harmful properties of the metal. Superoxide dismutase is an essential antioxidant enzyme that converts superoxide radicals into hydrogen peroxide. Li *et al.* (2018) reported elevated activities of oxidative system enzymes, such as CAT and SOD, in various fish species exposed to Cd and other metals, indicating a possible shift toward a detoxification mechanism and an antioxidant system response to metal stress. A drop in GSH levels could be the cause of increased lipid peroxidation in the liver.

The cadmium treated group's GSH level were suppressed, which indicated that GSH was essential for maintaining the fishes' stress tolerance and preventing mortality during the experiment. The decreased GPx activity in the liver of the fish exposed to cadmium suggests increased free radical harm. The decrease in protein content observed in the liver tissues of this study may be a result of the ketoacids-to-gluconeogenesis metabolic pathway being utilised for glucose synthesis. The liver histopathology of affected fish was significantly altered compared to its reference fish, which negatively impacted the quality and quantity of fish stocks. The consumption of these fish by the local population raises grave concerns for human health.

This study provides conclusive evidence that cadmium exposure causes oxidative stress in fish. The reaction of *O.niloticus* to the cadmium chloride suggested its fitness and susceptibility as a model organism for the studies on the effect of environmental genotoxicity. It is a useful tool for assessing pollution stress in aquatic ecosystems. Therefore, *O. niloticus* utilised antioxidant defence mechanisms to resist the oxidative damage brought on by these heavy metals.

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Conflict of interest: The author has no conflict of interest.

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