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RESEARCH ARTICLE

Investigation on the Genotoxicity of Mercuric Chloride to Freshwater *Clarias gariepinus*

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ABSTRACT

The cytogenetic effect of heavy metal was studied in *Clarias gariepinus* using the micronucleus test, chromosomal aberrations and sister chromatid exchange. The fish were kept separately and treated with four different concentrations of mercuric chloride for a period of 7 days. For the micronucleus test blood samples were obtained from the caudal vein. The mean micronuclei frequencies were recorded as 0.20, 0.25, 0.50, 0.65 and 0.79 in controls and 1.0, 2.0, 4.0 and 6.0 ppm in groups treated with mercuric chloride, respectively. Kidney tissues were used for *in vivo* chromosome preparation. The mean frequencies of cells with chromosomal aberrations were 0.21, 0.32, 0.49, 0.70 and 0.97 in the control and 1, 3, 5 and 7 ppm in groups treated with mercuric chloride, respectively. Similarly, the mean frequencies of sister chromatid exchange were recorded as 0.10, 0.28, 0.47, 0.58 and 0.64 in controls and 1, 3, 5 and 7 ppm in groups treated with mercuric chloride, respectively. The findings of present experiment suggest that $HgCl_2$ caused genotoxic effects in fish.

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INTRODUCTION

Trace metal pollution in the terrestrial and aquatic environments is a universal issue of increasing magnitude. Trace metals can disturb aquatic organisms through water, sediment or the food chain (Zyadah, 1995). Heavy metals, such as mercury ($HgCl_2$), selenium, copper and zinc, are essential components of the metabolism of fish, and certain concentrations are required for the health of fish. However, heavy metals have a tendency to bioaccumulate in different fish tissues and continuous exposure to high levels of these metals can induce health hazards (Javed, 2012; Naz and Javed, 2012; 2013). Mercury and cadmium have been analyzed and confirmed as being the most toxic heavy metals, followed by copper, chromium, nickel, aluminum, manganese and zinc. The soluble forms of these metals are most harmful to fish. Some heavy metals, such as copper, iron, nickel and chromium, are important metals due to their essential functions in living systems, whereas cadmium and Pb are non-essential and are toxic even in trace amounts (Fernandes *et al.*, 2008). Fish require a certain amount of essential metals for their metabolism. Essential metals may, however, have hazardous effects when the intake of these metals is greatly increased.

Mercuric cholride is a well known toxicant used as a novel compound to cause toxicity in the kidney (Nagarani *et al.,* 2012). $HgCl_2$ affects the proximal tubule and can rapidly accumulate there. Kyu-Bong Kim *et al.* (2010) reported the main reason of $HgCl_2$-influenced toxicity is the uncoupling of oxidative phosphorylation and reduced respiratory control in the mitochondria of kidney cells. $HgCl_2$ is the only heavy metal that bioaccumulates and is biomagnified through the aquatic food web (Nwani *et al.,* 2010).

$HgCl_2$ is considered to be one of the most hazardous of the heavy metals, due to its high toxicity, bioaccumulative potential, and other harmful effects on fauna and flora, including many genotoxic effects or mutation (WHO, 1990). Among other mutagenic properties, $HgCl_2$ and certain organomercurial compounds exert an adverse effect on tubulin, the structural subunit of the microtubules involved in cytoplasm organisation, and also a component of the spindle fibers. $HgCl_2$ impairs tubulin polymerisation, causing the contraction of metaphasic chromosomes, a delay in centromere division and slower anaphasic movement (Thier *et al.,* 2003).

The examination of cytogenetic endpoints, such as micronucleus formation, chromosomal aberrations and sister chromatid exchange provides sensitive genetic assays for the detection of genotoxic chemicals and environmental mutagens at sub-toxic levels. The micronucleus is a cytoplasmic chromatin mass with a

small nucleus that is created from a lagging chromosome in the anaphase stage is due to some structural and/or numerical chromosomal aberrations in the cells uring mitosis (Parveen and Shadab, 2012). Binucleation is an indicator of abnormal cell division due to blocking of cytokinesis. This abnormal cell division is considered to result in a genetic imbalance in the cells, potentially leading to carcinogenesis (Tolga Cavas *et al.*, 2005). An increasing interest in genotoxicity due to water pollutants has guided to develop various biological tests for detection and identification of environmental toxicants (Güner and Muranlı, 2011). Bombail (2011) stated that micronuclei (MN) count can serve as an indices of chromosome aberration and non- functioning of spindle in mitosis. The advantages of the micronucleus test are its simplicity, reliability, and sensitivity. This test is widely employed to assess the biological impacts of aquatic pollutants. Fish are a suitable model for monitoring aquatic genotoxicity is their ability to metabolise xenobiotics and accumulate pollutants (Grisolia and Corderio, 2000). Mohamed *et al.* (2008) analysed the cytogenetic damage by measuring the chromosomal aberration in the gill cells under the influence of Cu and Pb in *Oreochromis niloticus*. Mahrous and Abdou (2001) reported that water pollutants caused significant changes in chromosomal structures and centromeric attenuation in *Oreochromis niloticus* and *Clarias lazera.* Parveen and Shadab (2011) studied the effects of malathion on *Chana punctatus* using the micronucleus test. Ecotoxicological potential of *Clarias gariepinus* due to its diverse division and accessibility round the year, easy handling in aquaria/wet laboratories, easy collection of blood by non-invasive and its 32 well-distinguished diploid chromosomes consider this species an excellent biological model for pollution studies (Kumar *et al.,* 2010).

Our study therefore aimed to investigate and evaluate the effect of different concentrations of mercuric chloride on *C. gariepinus* through a piscine micronucleus test, a chromosomal aberration test and sister chromatid exchange, which is considered to be the first biomarker for the effects of water pollution on fish.

MATERIALS AND METHODS

Experimental protocols: In the present study, live, healthy and disease-free *C. gariepinus* (weight: 28.50±1.5 g; total length 6.8±1.05 mm) were collected from an unpolluted commercial fish farm. Fish were brought to the laboratory in a large oxygenated box. After disinfection with a dip of 2% $KMnO_4$ solution, the fish were allowed to acclimatize to in large fiber tanks (210 cm × 120 cm × 2 cm) for 30 days. In the laboratory, the fish were held in 100 L^{-1} glass aquaria (120 cm × 45 cm7 × 80 cm) supplied with tap water for acclimatization for a period of 30 days at 22.0±1.0°C. The physico-chemical characteristics of the tap water were determined using the methods described by the APHA (1998). The water was renewed every day to remove fecal matter and a 12 h photoperiod was continued during the experimental period. The fish were fed with commercial feed (35% crude protein mixture of fish meal, soybean meal, sesame oil cake in a 1:1:1 ratio) daily during the acclimatization period. A minimum of 12 fish per treatment with three replicates were exposed to each concentration of $HgCl_2$ (1.0, 3.0, 5.0 and 7.0 ppm) for 7 days. One group of 12 fish was maintained as a control group.

Nuclear abnormalities: The method described by Ayllon and Garcia-Vazquez (2000) for the piscine micronucleus test was used. The bearing of micronuclei and nuclear changes, manifested as changes in the normal elliptic shapes of the nuclei, were considered nuclear abnormalities and scored together. Differences in the frequencies were tested using the χ^2 test.

The method described by Fenocchio *et al.* (1991) was used for chromosome preparations with short-term cultures of kidney cells. The mitotic index was worked out by evaluating the number of cells in metaphase out of a total of 2 000 cells. The mean and standard error values were calculated using Sigma Stat Software (Jandel Scientific Software- Version 4.0: 2008 integrated with Sigma Plot 11, San Jose, California, USA).

A sister chromatid exchange assay was performed using the method of Perry *et al.* (1974). Metaphase spreads were observed under a bright field microscope to ascertain the quality of staining. Sister chromatid exchanges (SCE's) were scored for each individual chromosome.

Statistical analysis: The obtained data were described as the mean±SD and using Student's t-test (two-tailed) with the help of SPSS 18 (Statistical Package for the Social Sciences, University of Bristol, UK). The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

A total of 4 000 cells was scored for each group to examine the micronuclei. The mean frequencies of micronuclei (MN) in *C. gariepinus* were recorded as 0.20, 0.25, 0.50, 0.65 and 0.79 in the control group, 1.0, 3.0, 5.0 and 7.0 ppm mercuric chloride ($HgCl_2$), respectively. The micronucleus frequency was increased with rise in the concentration of mercuric chloride in all the experimental groups (Table 1). The numbers of micronuclei were increased significantly in the fish treated with 3, 5 and 7 ppm of $HgCl_2$ in comparison to the control fish. There was a non-significant difference in the controls and the group treated with 1.0 ppm (low dose) mercuric chloride. Figures 1 and 2 shows the dose-dependent increase in the frequency of MN in renal erythrocytes and in peripheral blood lymphocytes. The micronucleus test (MNT) in fish erythrocytes has been employed extensively to study the genotoxic effects of different mutagens. Furthermore, the morphologically alterations detected in erythrocytes serves as an index of cytotoxicity. Porto *et al.* (2005) found significantly higher mean frequencies of MN in *Prochilodus nigricans* (detritivorous), *Mylossoma duriventris* (omnivorous) and *Hoplias malabaricus* (piscivorous) from the Madeira River compared to the frequencies observed in the same species in the Solimões River. The Madeira River has been highly impacted by artisanal gold mining areas that used mercury during the amalgamation process; mercury being the most significant toxic metal present in this environment,both quantitatively

and qualitatively. In addition, the mean frequencies of MN in piscivorous species have been shown to be almost five-fold higher in the detrivorous and/or omnivorous species (Porto *et al.,* 2005). Nagarani *et al.* (2009) reported micronuclei formation under the influence of $HgCl_2$. They observed that micronuclei formations varied significantly with exposure concentrations. The MNT has been employed successfully in various fish species to detect mutagenic changes caused by aquatic pollutants (Pantaleao *et al.*, 2006). Our results gave similar results to those obtained by Nagarani *et al.* (2009) and Parveen and Shadab (2012). They concluded that the micronucleus assay is a one of the monitoring tools for the examination of the effects of water pollution in fish. The frequencies of micronuclei may vary with season, type of pollutants engaged and species of fish. It is opined that the kidney may be the vital tissue for erythropoiesis and filtration in *C. gariepinus*. Authors are of the opinion that fish on exposure to $HgCl_2$ have caused damage in erythrocytes and move from the kidney into the blood, then they are discharged from the hemocatheresis tissues. Therefore, in the present study it was an effort to demonstrate the micronucleus frequency was noted in renal and in peripheral blood erythrocytes. The increase in the micronuclei frequency with an increase in the concentration of mercuric chloride. The findings of our study reinforce the evidence of mercury genotoxic action in fish. Porto *et al.* (2005) have proposed that some chemical mutagens are responsible for producing genotoxic effects in fish. Behavioural changes were noticed in the *C. gariepinus* during the experimental succeeded by death which was probably due to the toxic effect of mercuric chloride which might be mediated by disturbances in the nervous and the enzyme system of the fish which affected the respiratory function and is responsible for activities of vital organs. Our findings confirmed those of Nagarajan *et al.* (2009).

A significant dose-dependent increase ($P<0.05$) in clastogenic damage was observed in *C. gariepinus* in all groups treated with mercuric chloride. Table 2 gives a summary of the results of chromosomal aberrations as the mean frequency of cells with aberrations. The recorded values of this index were 0.21, 0.32, 0.49, 0.70 and 0.97 in controls, 1.0, 3.0, 5.0 and 7.0 ppm mercuric chloride ($HgCl_2$), respectively.

The mean frequencies of sister chromatid exchange observed were 0.10, 0.28, 0.47, 0.58 and 0.64 in control, 1.0, 3.0, 5.0 and 7.0 ppm mercuric chloride, respectively (Table 1). The values depicted a significant increase ($P<0.05$) as compared to the control fish. Fishes are easily affected by a change in their habitat and can be used as a model animal in detection potential risk combined with pollution in aquatic environments (Lakra and Nagpure, 2009). The mitotic spindle displays a key function in the formation of micronuclei. Fishes can respond to mutagens at a low concentration of toxicants in a manner similar to higher vertebrates. DNA repair has been reported to be slower in fish than in mammals. Therefore, fishes might be used as scouting or spotter species for the detection of genotoxic compounds in the aquatic habitat and the risk of these pollutants to human health. Metallic compounds that are found in wastewater may deposit in different organs in animals and can significantly modify plant and fish biomass production. Most of the toxicants that induce genotoxic effects have been observed to form reactive oxygen species (RO) as well as electrophilic free-radical metabolites that interact with DNA to induce consequential changes. It has been suggested that mercuric chloride is a clastogen and may induce stress in fish at higher concentration. Malla and Ganesh (2009) reported the cytogenetic and tissue toxicity of mercuric chloride with higher doses in *H. fossilis*. They further mentioned that $HgCl_2$ stress has caused the different chromosomal aberrations including fragments and acrocentric associations and along with histopathological changes in *H. fossilis*. It has been proposed that during the trace metal treatment, electrophilic ions and radicals are produced, that interacts with nucleophilic sites in DNA and resultantly caused to chromosomal aberration and other associated DNA damage. The present study showed that mercuric chloride is a clastogenic chemical that induces various chromosomal abnormalities and increased the frequency of micronucleated cells in fish. Thus it can be successfully used as a biomarker for monitoring water pollution.

Table 1: Sister chromatid exchange and micronuclei frequency induced by mercuric chloride in *Clarias gariepinus*

Treatment (ppm)	Total SCE count	SCEs scored	Total MN count	Micronuclei
Control	20	0.10±0.02	400	0.20±0.02
1.0	56	0.28±0.05*	500	0.25±0.06
3.0	84	0.47±0.09*	1000	0.50±0.07*
5.0	106	0.58±0.11*	1300	0.65±0.09*
7.0	128	0.64±0.12*	1580	0.79±0.11*

Sister chromatid exchange (SCE) and micronuclei (MN) were search out of 300 metaphases and 4000 cells/group, respectively. Values (mean±SE) bearing asterisk in a column differ significantly ($P<0.01$) than control.

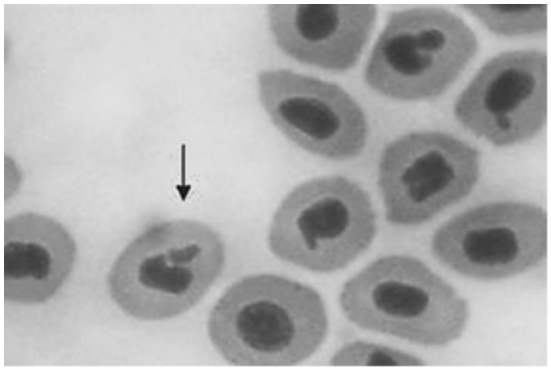

Fig. 1: Micronucleus in renal erythrocytes in *Clarias gariepinus*

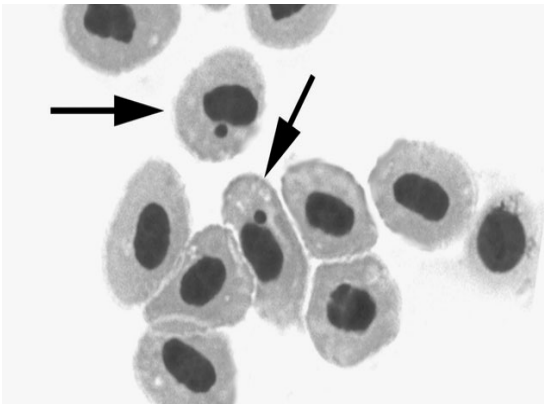

Fig. 2: Micronucleus in peripheral blood erythrocytes in *Clarias gariepinus*

Table 2: Chromosomal aberrations in *Clarias gariepinus* exposed to different concentration of mercuric chloride ($HgCl_2$)

Treatment (ppm)	Chromatid type aberrations			Chromosome type aberration			No. of cells with aberrations	Aberrations /cell
	Gaps	Breaks	Exchange	Breaks	Dicentric	Rings		
Control	7	5	3	4	0	3	21	0.21±0.03
1.0	13	6	6	9	6	0	34	0.34±0.08*
3.0	22	10	13	16	9	2	49	0.49±0.12*
5.0	28	18	15	21	16	7	70	0.70±0.23*
7.0	37	23	18	27	22	9	97	0.97±0.33*

Values (mean±SE) bearing asterisk in a column differ significantly (P<0.02) than control.

Conclusion: This study confirmed that mercuric chloride is a clastogenic chemical that induces various chromosomal abnormalities and increases the numbers of micronucleated cells in fish. Mercuric chloride produced genotoxic effects in fish and can thus be used as a biomarker to monitor pollution in aquatic environments.

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