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Cytogenetic evaluation of cadmium chloride on *Channa punctatus*

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Abstract

The aim of present study was to evaluate the genotoxic effect of heavy metal in *Channa punctatus* through the micronucleus test, chromosomal aberrations and sister chromatid exchange. The fish were kept separately in 0.5, 1.0, 2.0 and 5.0 ppm cadmium chloride for 3 days. For micronucleus test blood was collected from caudal vein and smeared on clean slides fixed in methanol and stained with 2% Giemsa. Mean frequency of micronuclei observed was 0.10, 0.15, 0.24, 0.34 and 0.39 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$ respectively. *In vivo* chromosome preparation from kidney tissues was carried out. The mean frequency of cells with aberrations observed was 0.13, 0.20, 0.34, 0.60 and 0.95 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$ respectively. Likewise the mean frequency of SCE observed was 0.05, 0.16, 0.36, 0.44 and 0.52 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$ respectively. It has been revealed from the results of this study that cadmium produced genotoxic effects in fish.

Key words

Micronucleus test, Chromosomal aberrations, Chromatid exchange, Cadmium chloride

Introduction

Heavy metal contamination in the terrestrial as well as the aquatic environment is a worldwide problem of increasing magnitude. Heavy metals can affect aquatic organisms through water, sediment or food chain (Zyadah, 1995). Of the heavy metals, cadmium is one of the major aquatic pollutants since it is present throughout the ecosystem and is detectable in critical amounts in many parts of the world (Georgudaki and Kotsanis, 2001). Cadmium is recognised as one of the major environmental pollutants and produces toxic effects in living organisms (De Conto Cinier *et al.,* 1998). Phosphorus alleviation of cadmium phototoxicity in plants has also been reported by (Sajwan *et al.,* 2002). Studies have also shown that other metals, vitamins, chelating agents and protein diets, which alter the physiological, biochemical and behavioural aspects in fish, also influence cadmium mobility and toxicity (Shaffi *et al.,* 2001). Environmental exposure to cadmium is mainly from contamination of groundwater from smelting and industrial uses as well as the use of sewage sludge as a food-crop fertilizer. Grains, cereal products, and leafy vegetables usually constitute the main source of cadmium in food. The disease Itai-Itai resulting from consumption of cadmium contaminated rice in Japan is a well known case of cadmium toxicity(Ernest Hodgson *et al.,* 2004).

Cytogenetic endpoints like micronuclei formation, chromosome aberration and sister chromatid exchange are very sensitive genetic assays for detecting genotoxic chemicals and environmental mutagens at sub-toxic levels. Micronucleus is a cytoplasmic chromatin mass with the appearance of a small nucleus that arises from a chromosome lagging behind in the anaphase stage of cell division. Its presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis (Heddle *et al.,* 1991; Fenech *et al.,* 1999; Norppa and Falck, 2003). The binucleation is an indicator of abnormal cell division due to blocking of cytokinesis. This abnormal cell division would result in a genetic imbalance in the cells, which may also be involved in carcinogenesis (Tolga Cavas *et al.,* 2005). A growing interest in genotoxicity caused by environmental pollutants has led to the

development of several biological tests for detecting and identifying genotoxicants in the environment (Grisolia and Cordeiro, 2000). The count of MN has served as an index of chromosome breaks and mitotic spindle dysfunction (Bombail *et al.,* 2001). The advantages of micronucleus test are its simplicity, reliability, and sensitivity. It is widely employed to assess the biological impacts of aquatic pollutants (De Flora *et al.,* 1993; Minissi *et al.,* 1996; Ayllon and Garcia-Vazquez, 2000; Vigano *et al.,* 2002). Some of the advantages of fish as a suitable model for monitoring aquatic genotoxicity owe to their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Corderio, 2000). Heavy metals can affect aquatic organisms through water, sediment or food chain (Zyadah, 1995). Rishi and Grewal (1995) studied the effect of organophosphorous insecticides dichlorvos on chromosomes by using *Channa punctatus* as model species.

Nuzhat and Shadab (2011) studied the effect of agriculture pesticide malathion on *Channa punctatus* through micronucleus test. Several ecotoxicological characteristics of air-breathing freshwater food fish *Channa punctatus* such as its wide distribution and availability throughout the year, easy maintenance in the aquaria/wet lab, ease of blood collection noninvasively, and the presence of 32 well-differentiated diploid chromosomes make this species an excellent model for toxicity studies (Kumar., *et al.,* 2010). Therefore, the present study was undertaken to assess the genotoxic potential of cadmium chloride on *C. punctatus.*

Materials and Methods

Experimental Protocols : For the present study alive, healthy and disease free fishes (*Channa punctatus* weight 22-50 gm) were collected from local market Rasalganj, Aligarh. After disinfection with a dip of 2% $KMnO_4$ solution fish were acclimatised in aquaria for one month before initiation of experiment. Fish were fed on lab made diet (cooked mixture of fish meal, soybean meal, mustard oil cake in 1:1:1 ratio) on alternate days during the acclimation period. A minimum of 8 fish per group were exposed to each concentration of $CdCl_2$ (0.5, 1.0, 2.0 and 5.0 ppm) for 3 days and each experiment was conducted in triplicate. One group of 8 fish served as control. Water of each tank containing toxicant was changed daily to remove faecal matter.

Micronucleus test : Micronucleus test was performed as per method of Schmid (1975). Blood from fishes of each group was collected from heart puncture in a heparinised syringe to make a thin smear on precleaned slide. Slides were fixed by dipping it in absolute methanol for 5-10 min, air dried for at least 1 hr and stained with Giemsa for 10 min. Slides were washed with distilled water, air dried overnight, mounted with DPX and observed under Nikon microscope using 40/100X objective lenses and were scored for micronucleated cells .

Chromosomal aberration test : Chromosomal aberration test was performed as per method of Bertollo *et al*. (1978). After exposure for specified time of 3 days, colchicine was injected intramuscularly to each fish. After 1 hr of colchicine treatment, the kidney tissue was homogenized in hypotonic solution to prepare cell suspension. The cell suspension was poured in 15 ml centrifuge tube and incubated for 20-25 min at room temperature for optimum swelling of cells. Hypotonic action was stopped by adding freshly prepared chilled Carnoy's fixative. Cell suspension was centrifuged at 1200-1500 rpm for 10 min at room temperature to get cell pellet at the bottom. 2 or 3 washings were required till clear cell suspension was obtained. Slides were prepared by flame drying technique. After 1 hr slides were stained in 5% Giemsa in phosphate buffer (pH 6.8). Detectable and finely spread metaphase chromosome spots were analyzed under 100X oil immersion.

Sister chromatid exchanges : Sister chromatid exchange assay was performed as per the method of Perry *et al*. (1974). BrdU was injected intramuscularly according to the fish body weight for 24 hrs followed by intramuscularly injection of colchicine. After 1 hr of colchicine injection, the kidney tissues was dissected out in a petri dish and the tissue was homogenized in hypotonic solution in a glass tissue grinder to prepare cell suspension**.** Then the cell suspension was poured in 15 ml centrifuge tube in dark and incubated for 20-25 minute at room temperature. Hypotonic action was stopped by adding freshly prepared chilled Carnoy's fixative. Cell suspension was centrifuged at 1200-1500 rpm for 10 min at room temperature to get cell pellet at the bottom. 2 or 3 washings were required till clear cell suspension was obtained. Slides were prepared by flame drying technique in dark. After 1 hr slides were stained in Hoechst stain 33258 dye for 20 min in dark. Slides were exposed to mercury bulb for 10 min and further treated with 5N HCl for 10-15 min then rinsed with double distilled water and dried in air. Slides were stained with 5% Giemsa in phosphate buffer (pH 6.8) for 15-20 min to observe metaphase spreads in bright field microscope to ascertain the quality of staining. SCE's were scored for each individual chromosome.

Statistical Analysis: Data are expressed as mean ±S.E. and statistically analyzed using Student's t-test (two-tailed) with the help of SPSS 18. The level of significance was set at $P < 0.05$.

Results and Discussion

Micronucleus test : A total of 2,000 cells were scored for each group to study the micronuclei. Mean frequency of micronuclei observed was 0.10, 0.15, 0.24, 0.34 and 0.39 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$, respectively (Table 2**).** The result indicated that the percentage of micronuclei increased ($P<0.05$) with increase in concentration of cadmium chloride.

Chromosomal aberration : A dose-dependent significant increase ($P<0.05$) in clastogenic damage was found. Table I represents a summary of the results of chromosomal aberrations as mean frequency of cells with aberrations observed was 0.13, 0.20, 0.34, 0.60 and 0.95 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$, respectively.

Table 1 : Chromosomal aberrations in *Channa punctatus* exposed to different concentration of $CdCl_2$

Treatment (ppm)	Chromatid type aberrations			Chromosome type aberrations			No. of cells with aberrations	Aberrations/cell
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
Control	5	3	1	2	0	2	13	0.13±0.06
0.5	7	3	3	3	4	0	20	0.20±0.09*
1.0	12	5	5	6	5	1	34	0.34±0.15*
2.0	18	7	10	11	10	4	60	0.60±0.26*
5.0	26	16	11	21	15	6	95	0.95±0.41*

Values are mean of replicates ±SE; *Significant at p<0.02

Table 2 : Sister chromatid exchange (SCE) and micronuclei frequency induced by $CdCl_2$ in *Channa punctatus*

Treatment (ppm)	No. of metaphases observed	Total SCE count	SCEs scored	Total cells	Total MN count	Micronuclei
Control	200	10	0.05±0.01	2000	200	0.10±0.05
0.5 ppm	200	33	0.16±0.04*	2000	302	0.15±0.06
1.0 ppm	200	72	0.36 ±0.05*	2000	480	0.24±0.11*
2.0 ppm	200	89	0.44 ±0.04*	2000	698	0.34±0.12***
5.0 ppm	200	104	0.52 ±0.06*	2000	799	0.39±0.17**

Values are mean of replicates ±SE. *Significant of SCE at p< 0.001; Micronuclei at p<0.05; p< 0.02, p< 0.01

Sister chromatid exchange : Table 2 shows the mean frequency of sister chromatid exchange values observed was 0.05, 0.16, 0.36, 0.44 and 0.52 in control, 0.5, 1.0, 2.0 and 5.0 ppm $CdCl_2$, respectively. The values showed a significant increase when compared to the control (P<0.05).

Fishes are very sensitive to a change in their environment and can play significant role in assessing potential risk associated with contamination in aquatic environment (Lakra and Nagpure, 2009). Cadmium has been frequently used in industries and is among the significant metal pollutant in the effluents (Das *et al.,* 1997). Several pathological examinations suggest that cadmium can cause aneuploidy, and it poisons the mitotic spindle (Berces *et al.,* 1993). The mitotic spindle has a great importance in the formation of micronuclei. Fishes can respond to mutagens at low concentration of toxicants in a manner similar to higher vertebrates (Weis 1989; Wisk and Cooper 1990; Yang *et al.,* 1990). As compared to mammals, the DNA repair was reported to be slower in fishes (Espina and Weiss, 1995). Therefore, fishes might be used as sentinel or surrogate species for the evaluation of genotoxic chemicals in the environment and their risk to human health (Sklarew 1993). Metallic compounds present in effluents may accumulate in various organs of animals or cause huge modification in plant and fish biomass production (Barman and Lal, 1994). After entering into the organs of freshwater fishes through the gills, cadmium binds to albumins and erythrocytes in the blood and then is transferred into tissues and organs where it is bound to proteins of low molecular mass producing metallothioneins by the induction of metallothionein mRNA synthesis (George *et al*., 1996). It was suggested that the mechanism of Cd genotoxicity is mainly conditioned by single strand breaks in DNA through the direct cadmium-DNA interactions as well as by the action of incision nucleases and/or DNA-glycosylase during DNA repair (Privezentsev *et al.,* 1996). Correspondingly, most of the toxic chemicals that produce genotoxic effects have been known to form reactive oxygen species as well as electrophilic free-radical metabolites that interact with DNA to cause disruptive changes. It has been suggested that during the heavy metal exposure, electrophilic ions and radicals are produced, interacting with nucleophilic sites in DNA and leading to breaks and other related damage in the latter. The micronucleus test (MNT) in fish erythrocytes has increasingly been used to detect the genotoxic effects of environmental mutagens and its frequency is considered to reflect the genotoxic damage to cells, mainly the chromosomes. Besides, morphologically altered erythrocyte is taken as an index of cytotoxicity. Castano *et al.* (1998) used MNT to study the effect of cadmium on rainbow trout. Campana *et al.* (1999) evaluated genotoxicity of the pyrethroid lambda- cyhalothrin using the micronuclei test in erythrocytes of the fish *Cheridon interruptus interruptus.* Gustavino *et al.* (2001) has reported dose dependent increase in the micronucleus frequency in fish *Cyprinus carpio* when exposed to X- rays and colchicine. Sandra *et al.* (1996) used MNT for the *in situ* mutagens in freshwaters in erythrocytes of

Barbus plebejus from two natural environments. In the present study there is an increase in micronuclei frequency with the increase in the concentration of cadmium chloride.

The present study reveals that cadmium is a clastogenic chemical inducing various chromosomal abnormalities and increased micronucleated cells in fish. Thus it can be used as a biomarker for monitoring pollution in aquatic environment.

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