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Effect of Mercuric Nitrate on the Chromosomes of *Channa punctatus* (Bloch, 1793)

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The effect of different doses (0.019, 0.038 and 0.075 ppm) of mercuric nitrate on the chromosomes of *Channa punctatus* was studied. The karyotype was done by standard colchicine, potassium chloride, acetomethanol and air-drying geimsa staining technique. It was observed that the percentage of Total Metaphase spreads with Chromosomal Aberrations (T.M.C.A.) increased with the increasing concentration levels. Mercuric nitrate induced structural chromosomal aberrations included breaks, fragments, dicentric and ring type chromosomes. The study revealed that 120 h of exposure in mercuric nitrate at concentration levels as low as 0.019 ppm induced chromosomal aberrations in fish.

Key words: Karyotype, mercuric nitrate, *Channa punctatus*

Studies on chromosomes are essential in fish genetics, taxonomy and environmental toxicology research programmes. The genetic variations can be detected at chromosomal and genomic level and in practice conventional karyological techniques are used as reliable tools. In the absence of direct genotoxic test the karyotyping of fish can be used as a simple test and also as starting point for detailed genotoxic studies.The sublethal mutations do not cause immediate mortality to the fish. However, they have more severe consequences for the development of the entire population. Heavy metals such as lead, mercury, zinc, copper and cadmium are known to cause damage to the genetic material in aquatic animals (Abel, 1989). The LC_{50} for mercuric nitrate was considered as 0.15 mg/l (Sebastian and Gerlach, 1981). The indirect exposure of fishes to Phenyl Mercuric Acetate (P.M.A.) increased the frequency of chromosomal aberrations at all dose levels from 0.01 to 0.05 ppm (Krishnaja and Rege, 1982).

Manna (1986, 1989) reviewed genotoxic studies in fish and opined, tilapia to be a good cytogenetic model for testing genotoxicity. Subsequently Thomas (1990), Mitrofanov *et al.* (1991), Pechkurenkov (1991), Liu *et al.* (1991), Nishimoto *et al.* (1991), Zhang *et al.* (1992), Bailey *et al.* (1992), Yasuhira *et al.* (1992), Reichert *et al.* (1992), Schnitz and Connor (1992), Kubota *et al.* (1992), Adams *et al.* (1992) and Aoki *et al.* (1993) have studied various aspects of fish genotoxicity using different species including, various fresh water carps, rainbow trout, gold fish, tilapia, eastern mud minnow, etc.

The majority of fish has large number of 2n chromosomes and is acrocentric type, which makes it difficult to study the genotoxic effects. However, *Channa punctatus* has small number of 2n chromosomes and majority of them are larger in size, which makes it a suitable species on genotoxic effects (Manna, 1983).The present study was

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envisaged to study the effect of different concentrations of mercuric nitrate on normal chromosomes of *Channa punctatus* and to compare the chromosomes of fish exposed to mercuric nitrate with those of normal *Channa punctatus*.

Material and methods

Channa punctatus weighing 30-50 g were reared in the laboratory and maintained under standard management conditions. The genotoxic effect of mercuric nitrate was studied at 0.019, 0.038 and 0.075 mg/l concentration (Table 1). To obtain high rated mitotic metaphase spreads and preparation of permanent slides, colchicine 0.1% was used at the rate of 0.5ml/mg body weight of fish. After 3 to 4 hours, gills, kidney and liver were collected, immediately ground well and kept in KCl hypotonic solution for 30 min at room temperature. Each tissue sample along with hypotonic solution was centrifuged for 10 min at 1000 rpm. The supernatant was discarded.

A freshly prepared and chilled fixative (methanol: acetic acid (3:1) of about 5.0 ml was added to each tube slowly along the sides and the contents were resuspended and subjected to centrifugation for 10 min at 1000 rpm and the supernatant was discarded. The above step was repeated two or three times until a clear supernatant along with a mass of white cells was obtained as sediment. The final volume of the fixative was adjusted based on a pellet size and a uniform suspension was prepared. Four to five drops of the cell suspension was allowed to fall on a clean cold slide (4°C) from the height of about two to three feet. Then the slides were air dried for one hour. 4% Geimsa stain in Sorenson's phosphate buffer was used for staining the chromosomes. The slides were stained for 30 min in a cuplin jar and washed in distilled water. The slides were air dried and finally mounted with DPX. Each slide was systematically screened to identify different chromosomal abnormalities viz. chromosomal breaks, fragments, dicentric and ring types. Careful attention was taken to discriminate morphological aberrations, which might have occurred due to defective colchicine or hypotonic treatment on staining and washing procedure.

The following criteria were adapted to choose suitable metaphase spreads:

i) Minimum chromosomal overlapping

ii) Chromosomes of sufficient length

iii) A distinct sharp banding pattern.

The metaphase spreads were photographed under oil immersion objective with a final magnification of 1000x. Individual chromosomes were cut from photo prints. The homologous pairs were made and arranged in the order of metacentric, submetacentric, subtelocentric and acrocentric. Chi-square test of significance was applied to detect the significant genotoxic effect of heavy metal at different doses, sex and time intervals (Pillai and Rajagopalan, 1979).

Results and discussion

The model number of chromosomes in the present study is 2n = 32 consisting of 14 metacentric, 8 submetacentric, 6 subtelocentric and 4 acrocentric chromosomes (Plates 1 and 2). The absence of difference between male and female karyotype indicates that the heteromorphic chromosomes are absent in this species. The study revealed that mercuric nitrate at the

Table 1. Analysis of chromosomal abnormalities induced in *Channa punctatus* after exposure to various doses of mercuric nitrate for 120 h

Treatment group (mg/l)	Total number of spreads studied	Metaphase with chromosomal abnormalities	Number of spreads with three or more aberrations	two aberrations	at least one aberration	χ^2 value	Percentage of spreads with chromosomal abnormalities
0.019	134	86	20	19	52	39.8	67.9
control	134	49	0	0	08		34.6
0.038	127	91	32	22	32	58.9	77.7
control	110	39	0	0	13		35.5
0.075	116	99	37	23	39	102.5	85.4
control	123	31	0	0	12		35.2

Value ($P \leq 0.01$)

concentration of 0.019 mg/l induces highly significant chromosomal abnormalities in *Channa punctatus* (Table 1). The structural abnormalities in the chromosome induced by mercuric nitrate consist of chromosomal breaks, fragments, dicentric chromosomes and ring type of chromosomes (Plate 3). With the increase in concentrations the percentage of Total Metaphase spreads with Chromosomal Abnormalities (T.M.C.A.) increased (Table 1).

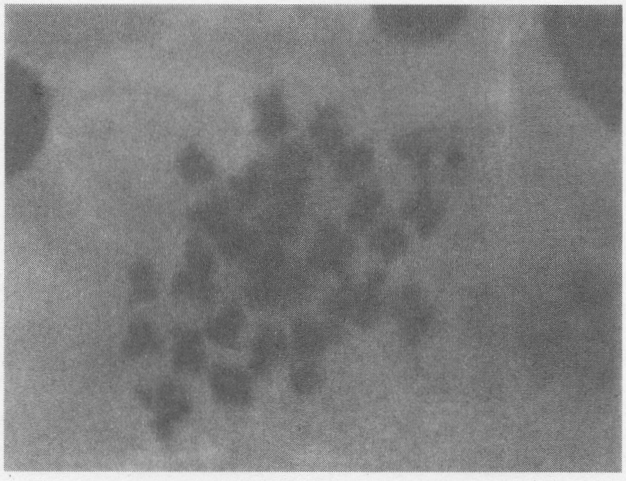

Plate 1. Normal metaphase spread of *Channa punctatus*

Mercuric nitrate at the concentration of 0.019 mg/l was clastogenic. The percentage of metaphase spreads with chromosomal abnormalities increased gradually as the concentration increased. It was 67.9 percent when the exposure time was 120 h (Table 1). In all the concentrations, metaphase spreads, which contained three or more type of chromosomal abnormalities, were observed (Table 1). The percentage of metaphase spreads when the fish were exposed to mercuric nitrate (0.038mg/l, 0.075mg/l) for 120 h was 77.7 and 85.4 (Table 1). At all the concentrations, chromosomal breaks were more in number followed by fragments (Plates 3,4,5). The dicentric and ring chromosomes were in equal percent. Both male and female fish were affected equally at all three exposures. The difference between the percentages of metaphase spreads with chromosomal abnormalities between the two sexes

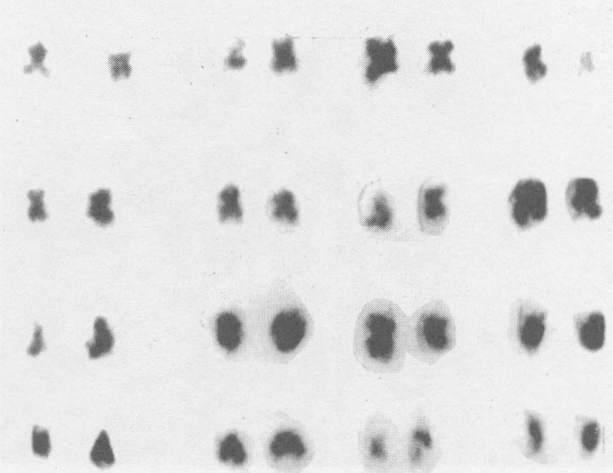

Plate 2. Normal karyotype of *Channa punctatus*

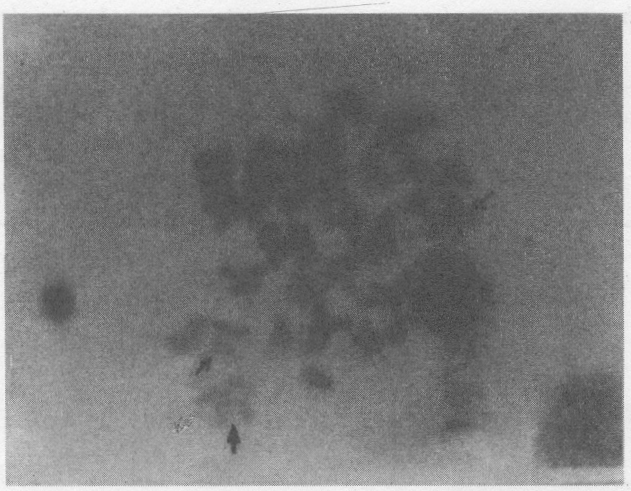

Plate 3. Metaphase spread showing chromosomal break and fragment at 0.019 mg/l for 120 h

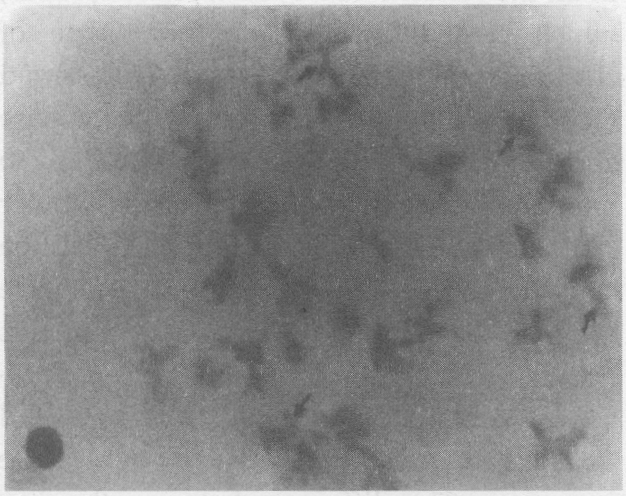

Plate 4. Metaphase spread showing chromosomal break and fragment at 0.038 mg/l for 120 h

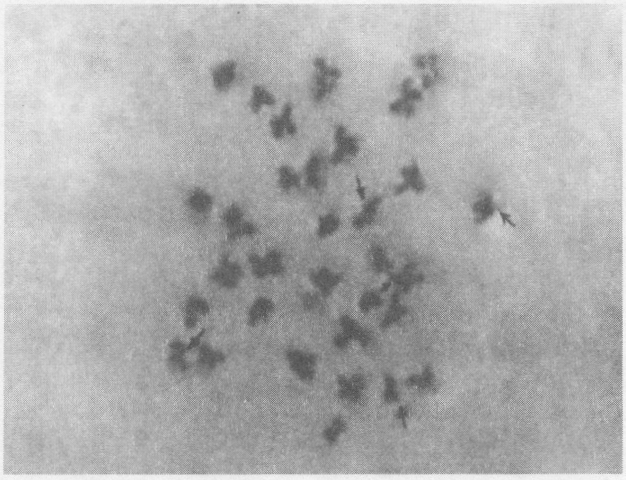

Plate 5. Metaphase spread showing chromosomal break and fragment at 0.075 mg/l for 120 h

(male and female) was not significant ($P \leq 0.01$). The results also indicated that both the sexes were equally susceptible to mercuric nitrate poisoning. The highly significant percentage of metaphase spreads with chromosomal abnormalities observed in fish exposed to mercuric nitrate as compared to control groups indicates that mercury acts as a potential clastogen even at a very low concentration (0.019mg/l). Therefore, it is important that fish should be reared in mercuric nitrate free water. Extreme care should be taken while selecting the brood stock. The brood stock should be selected from a pollution free environment.

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