

GENOTOXIC ALTERATIONS INDUCED BY FLUORIDE IN ASIAN CATFISH, *CLARIAS BATRACHUS* (LINN.)

Nalini Tripathi, Sandeep Bajpai, Madhu Tripathi^a

Lucknow, India

SUMMARY: Genotoxicity induced by fluoride (F) has been observed in kidney cells of freshwater Asian catfish, *Clarias batrachus*. A control group of fish was maintained without any treatment, a positive control group was treated with mitomycin-C (MMC), and two experimental groups were exposed to sub-lethal concentrations of F (35 and 70 mg F/L). After 90 days the mitotic index (MI) in the kidney cells was significantly decreased ($p < 0.05$) in both F-treated groups compared to the untreated control. Chromosomal aberrations were increased in the F treated groups and were greater at the higher F concentration. These findings thus indicate that F is able to induce genotoxic effects in catfish.

Keywords: Asian catfish; Chromosomal aberrations; *Clarias batrachus* (Linn.); Genotoxicity of fluoride; Mitomycin-C; Mitotic index and fluoride.

INTRODUCTION

Chromosomal studies have received considerable attention in recent years, in part from a growing interest in the evaluation of genotoxicity of environmental toxicants and carcinogens. Genotoxic studies in different species of fish using cytogenetic analysis have been reported by a number of workers.¹⁻³ Exposure of fish to pollutants and toxicants for a prolonged period, even at low levels, leads to chromosomal aberrations including gene changes.⁴⁻⁵ An advantage of chromosomal studies is that they reveal a measure of sublethal effects of xenobiotics *in vivo*.

Recently fluoride (F) has been reported to induce chromosomal aberrations in bone marrow cells of mice.⁶⁻⁷ In earlier studies, contrary findings were reported in regard to the effect of F on chromosomal aberrations in human peripheral blood lymphocytes and in human populations of F endemic areas.⁸⁻¹² Although substantial studies of the genotoxic effects of F have been conducted in the mammalian system, yet the available information regarding such effects on fish is very limited. Hence the present study was planned to observe the genotoxic potential of F in freshwater Asian catfish, *Clarias batrachus* (Linn.).

MATERIALS AND METHODS

Two dozen healthy *Clarias batrachus*, 15.0 ± 2.5 cm in length and weighing 65.0 ± 6.5 g were procured from the local Lucknow fish market. They were acclimated under laboratory conditions for 15 days. Physico-chemical properties of the holding water during acclimation were determined by standard APHA methods,¹³ as temperature $26 \pm 2^\circ\text{C}$, pH 6.9 ± 0.2 , dissolved O_2 8.8 ± 2.5 mg/L, hardness as CaCO_3 118–120 mg/L, and alkalinity 90–100 mg/L.

^aFor correspondence: Aquatic Toxicology Research Laboratory, Department of Zoology, University of Lucknow, Lucknow- 226 007 (U.P.) India. E-mail: drmtripathi@gmail.com.

Water in the aquaria was changed every alternate day after feeding. Fish were fed with minced liver and dried prawn pieces. For the experiment, the fish were divided into four groups with six fish in each group. Group I served as the primary control and was maintained under normal conditions. Group II served as a positive control and was administered by intramuscular injection once, (1.0 mg mitomycin-C [MMC]/kg body weight) dissolved in double distilled water. The experimental Groups III and IV were exposed to sublethal concentrations of F in the water, i.e., 35 mg F/L and 70 mg F/L, respectively. After 90 days, fish of all the groups were injected with 0.05% colchicine dissolved in double distilled water (intramuscularly at 1 mL/100 g body weight) 2 hr prior to dissection to arrest the metaphase stage. The fish were anesthetized with ethylene glycol and the kidneys were dissected out for study. The kidneys were subjected to chromosomal analysis using the method of Klingerman et al.⁴ More than 100 well-spread metaphase plates were analyzed for chromosomal aberrations at a magnification of 100 × 10 under oil immersion for all the groups, selecting 10–20 metaphases from each slide.

RESULTS

As seen in the Table, the mitotic index (MI), evaluated as the percentage of dividing kidney cells was decreased in the MMC-treated Group II fish in comparison to the control Group I fish.

Table. Frequency of chromosomal aberrations induced by Mitomycin-C and fluoride in kidney cells of *C. batrachus* after 90 days exposure (Values are Mean±SD)

Group	Total no. of metaphases analyzed	Metaphases with abnormalities	A	B	C	D	E	F	G	H	I	Total no. of aberrations	Percent of aberrations	Mitotic Index (MI)
I Control	140	12	1	1	2	3	2	2	1	0	0	10	7.14	4.14±0.22
II Mitomycin-C	160	75	2	2	20	18	11	8	10	3	1	66	41.25	3.77±0.25
III Fluoride (35 mg/L)	152	30	2	1	4	2	1	8	10	1	1	27	17.76	2.65±0.26*
IV Fluoride (70 mg/L)	151	36	1	3	6	3	2	18	2	1	0	32	21.19	2.72±0.07*

*p<0.05 (Compared to control). A: Chromatid gap, B: Chromosome gap, C: Chromatid break, D: Chromosome break, E: Chromatid deletion, F: Fragment, G: Acentric fragment, H: Ring chromosome, I: Di-centric chromosome.

In the F-treated groups III and IV the decrease in the MI was significantly greater (p<0.05) than in the primary control Group I. As expected, chromosomal aberrations were greater in the MMC Group II in comparison to Group I, whereas in the F-treated Groups III and IV they were also greater than in the control group but less in comparison with the MMC positive control. In the two F-treated

groups, chromosomal aberrations increased in a concentration dependent manner (Figures 1–3).

Figure 1. Metaphase spread with arrows showing chromatid deletions (CD), acentric fragments (AF), and ring chromosomes (R) after 90 days in control group I.

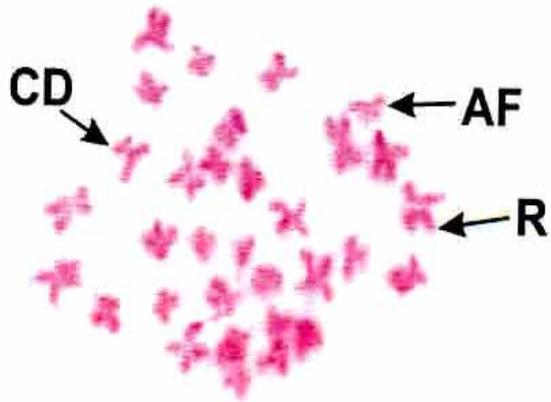


Figure 2. Arrows showing chromatid deletions, acentric fragments, ring chromosomes, and fragments (F) induced by MMC after 90 days in Group II.

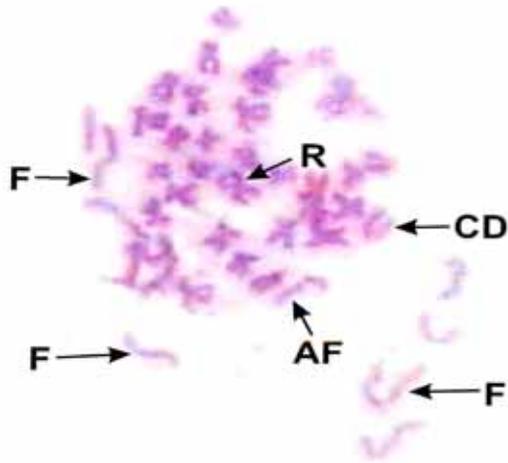
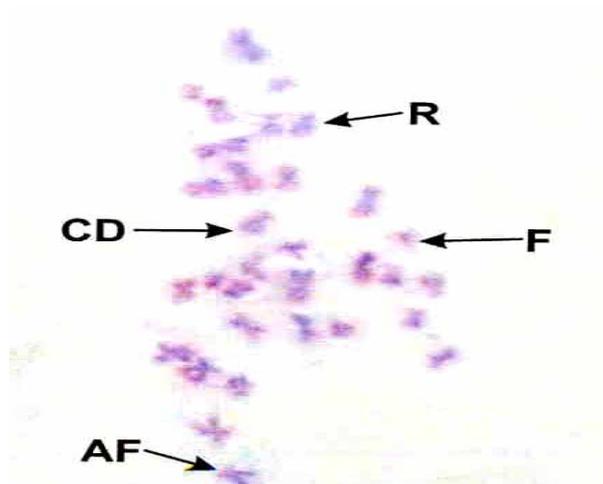


Figure 3. Arrows showing chromatid deletions, acentric fragments, ring chromosomes, and fragments induced by fluoride after 90 days in Groups II or III.



DISCUSSION

In this study, metacentric, submetacentric, and acrocentric chromosomes were found. Chromosome number and karyotype detail for *Clarias batrachus* has been worked out by Pandey and Lakra,¹⁴ who found it has 50 chromosomes (25 pairs): 8 metacentric, 5 sub metacentric, 2 telocentric, and 10 acrocentric pairs in male specimens. Female specimens also have the same karyotype except for an additional large submetacentric chromosome and a somewhat smaller acrocentric set.¹⁴ Our findings are in agreement with that report.

The mitotic index (MI) decreased in the MMC group as well as in the two F-treated groups after 90 days. This finding agrees with observations of Podder et al.,⁶⁻⁷ who reported a decrease in the MI percentage in bone marrow cells of Swiss mice exposed to MMC and to NaF. A decrease in the MI may be due to suppression of DNA synthesis by F, since F is known to interfere with protein synthesis, DNA synthesis, DNA repair, etc. (International Programme on Chemical Safety, IPCS).¹⁵ It may also be possible that F may be binding with DNA like MMC and delaying cell cycle.

In regard to chromosomal aberrations, our observations in the present study revealed chromatid breaks, chromosome breaks, chromatid deletions, fragments, acentric fragments, and ring and dicentric chromosomes. An increase in chromatid break, isochromatid break, and chromosomal exchange has been reported by Chaurasia et al.¹⁸ and by Podder et al.⁶⁻⁷ in bone marrow cells of Swiss mice and by Gadhia and Joseph¹¹ in human lymphocytes after exposure to F. Similar changes have been reported by Yadav and Trivedi¹⁶ in *Channa punctatus* after exposure to chromium and by Rita and Milton¹⁷ in *Oreochromis mossambicus* (Peters) after exposure to carbamate pesticide. The mechanism for these aberrations by F may involve disruption of DNA synthesis, DNA repair, or protein synthesis directly by enzymatic inhibition or indirectly by some other mechanism.¹⁵ There are, however, reports that F as NaF does not damage DNA in cultured cells.^{12,19,20} This contradiction can perhaps account for the differences in the action of F *in vivo* and *in vitro* found by Podder et al.⁶

Here we found that chromosomal aberrations in the kidney cells of *Clarias batrachus* increased with increased exposure to F by 17.76% at 35 mg F/L to 21.19% at 70 mg F/L in the tank water compared to the primary control. This dose-dependent enhancement of chromosomal aberrations is supported by the finding of Tripathi et al.,²¹ who found decreased levels of lipid and proteins in a dose dependent manner after exposure of *Clarias batrachus* to F. It may be suggested therefore that the assault on cells by a xenobiotic like F is more pronounced at higher concentration as found here. Thus the present findings suggest that F interferes with cellular activities in fishes, even at a genetic level, inducing chromosomal aberrations.

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REFERENCES

- 1 Das RK, Nanda NK. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustes fossilis* by Mitomycin-C and paper mill effluent. *Mutat Res* 1986;175:67-71.
- 2 Al-Sabti K, Metcalfe CD. Fish micronuclei for assessing genotoxicity in water. *Mutat Res* 1995;343:121-35.
- 3 Kushwaha B, Nagpure NS, Srivastava SK, Kumar R, Verma MS. Variations of micronuclei in peripheral blood cells of *Channa punctatus*. *Ind J Animal Sci* 2003;73:1192-3.
- 4 Klingerman AD, Bloom SE, Howell WM. *Umbra limi*: a model for the study of chromosome aberration in fish. *Mutat Res* 1975;31:225-33.
- 5 Barker CJ, Rackham BD. The induction of sister chromatid exchanges in cultured fish cells (*Ameioba splendens*) by carcinogenic mutagens. *Mutat Res* 1979;68:381-7.
- 6 Podder S, Chattopadhyay A, Bhattacharya S, Ray MR. Differential *in vivo* genotoxic effects of lower and higher concentrations of fluoride in mouse bone marrow cells. *Fluoride* 2008;41(4):301-7.
- 7 Podder S, Chattopadhyay A, Bhattacharya S. *In vivo* suppression by fluoride of chromosome aberrations induced by mitomycin-C in mouse bone marrow cells. *Fluoride* 2008;41(1):40-3.
- 8 Obe G, Slacik-Erben R. Suppressive activity by fluoride on the induction of chromosome aberrations in human cells with alkylating agents *in vitro*. *Mutat Res* 1973;19:369-71.
- 9 Slacik-Erben R, Obe G. The effect of sodium fluoride on DNA synthesis, mitotic indices and chromosomal aberrations in human leukocytes treated with trenimon *in vitro*. *Mutat Res* 1976; 37(2-3):253-66.
- 10 Khalil AM. Chromosome aberrations in cultured rat bone marrow cells treated with inorganic fluorides. *Mutat Res* 1995;343(1):67-74.
- 11 Gadhia PK, Joseph S. Sodium fluoride induced chromosome aberrations and sister chromatid exchange in cultured human lymphocytes. *Fluoride* 1997;30(3):153-6.
- 12 Riberio DA, Scolastici C, Marques MEA, Salvadori DMF. Fluoride does not induce DNA breakage in Chinese hamster ovary cells *in vitro*. *Braz Oral Res* 2004;18:192-6.
- 13 APHA, AWWA, WPCF. Standard methods for examination of water and waste water. 20th ed. Washington DC: American Public Health Association; 1998.
- 14 Pandey N, Lakra WS. Evidence of female heterogamety, B-chromosome and natural tetraploidy in the Asian catfish, *Clarias batrachus*, used in aquaculture. *Pub Elsevier Sci* 1997;149:31-7.
- 15 International Programme on Chemical Safety. Environmental Health Criteria for fluorides 2002:227.
- 16 Yadav KK, Trivedi SP. Evaluation of genotoxic potential of chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations. *Asian Pac J Cancer Prev* 2006;7(3):472-6.
- 17 Rita AJJ, Milton JMC. Karyomorphological analysis of the fresh water cichlid *Oreochromis mossambicus* (Peters) exposed to carbamate pesticide methomyl (Lannate). *J Adv Zool* 2008;29(1):57-61.
- 18 Chaurasia OP, Kumari C, Ankita, Sangita. Genotoxic effect of ground water salts rich in fluoride. *Cytologia* 2007;72(2):141-4.
- 19 Li Y, Dunipace AJ, Stookey GK. Lack of genotoxic effects of fluoride in the mouse bone-marrow micronucleus test. *J Dent Res* 1987;66(11):1687-90.
- 20 Ribeiro DA, Marques MEA, Salvadori DMF. Lack of effect of prior treatment with fluoride on genotoxicity of two chemical agents *in vitro*. *Caries Res* 2007;41:239-43.
- 21 Kumar A, Tripathi N, Tripathi M. Fluoride induced biochemical changes in fresh water catfish (*Clarias batrachus*, Linn). *Fluoride* 2007;40(1):37-41.