



ORIGINAL RESEARCH PAPER

Zoology

GENOTOXIC EFFECT OF CARBARYL ON GILL CELLS OF CHANNA PUNCTATUS

KEY WORDS: Genotoxicity, Carbaryl, Channa punctatus, Chromosomal Aberration Test, Chromosomal Aberrations.

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ABSTRACT

The genotoxicity of carbaryl was evaluated through Chromosomal Aberration Test on gill cells in freshwater fish Channa punctatus. Fishes were acclimatized in laboratory and divided into control and experimental groups. Two sublethal concentrations of carbaryl (0.1ppm and 0.5ppm) were identified and experimental fishes were exposed to these concentrations for a period of 144 hrs. Chromosomal aberrations were increased in carbaryl treated group, both were greater at higher concentration of carbaryl. These finding indicate that carbaryl is able to cause genotoxic effects in Channa punctatus.

INTRODUCTION

Fishes are excellent material for the study of mutagenic and carcinogenic potential of contaminant present in the water since they can metabolize, concentrate, and store water borne pollutants. Genotoxic effects in fish is a matter of great concern because of their potential risk on human health after consumption. The genotoxic effect of environmental pollutants can be monitored using a wide range of biomarker assays.

The chromosomal aberration test is a sensitive and extensively used tool for detecting mutagenic and genotoxic effects of chemicals in the environment (Tucker and Preston, 1996). Genotoxic study in different species of fish using cytogenetic analysis have been reported by several workers (Das and Nanda, 1986; Al-sabti and Metcalfe, 1995 and Kushwaha et al, 2003). Exposure of fish to pollutants and toxicants for a prolonged period, even at low levels, lead to chromosomal aberrations including gene changes (Klingerman et al. 1975; Barker and Rackham, 1979).

Genetic damage at the chromosomal level entails an alteration in either chromosome number or chromosome structure. Such alterations can be measured as chromosomal aberration.

In present study chromosomal aberration test was performed on gills cells of Channa punctatus to evaluate the genotoxicity induced by carbaryl.

MATERIAL AND METHOD

Specimens of freshwater fish Channa punctatus (Bloch; Family: Channidae and Order: Channiformes) were collected from local water bodies. The fishes were acclimatized for one week under laboratory conditions before carbaryl exposure. After acclimation, the fishes were divided into experimental and control groups. The experimental groups of fishes were exposed to three sub lethal concentration of carbaryl for a period of 144 hrs. Tissue sampling was done at interval of 48, 96 and 144 hrs at the rate of five fishes per duration. On each sampling day, the gills were collected immediately and processed for chromosomal aberration test.

CHROMOSOMAL ABERRATION TEST

Chromosomes were prepared according to the method of Ojima (1982) and Asano et. al. (1998) as follows:

After exposure, the fishes were injected 0.05% Colchicine (1 ml per 100 gm body wt.). These fishes were left for 3-4 hrs. After this time duration. The gills were excised from the treated fish and immediately transferred to petri dishes containing freshly prepared 0.56% KCl (hypotonic solution)

After processing the tissue, the suspension was prepared and centrifuged at 1000 rpm for 10 minutes and supernatant was discarded, remaining part was fixed with methanol acetic acid fixative and slides were prepared by dropping method. These cells were stained with Giemsa stain and covered with DPX.

RESULTS

In Channa punctatus 32 chromosomes were observed in diploid cells in both sexes. No sex chromosomes were observed in this fish species. The frequency of chromosomal aberrations developed due to exposure to carbaryl was recorded for different exposure period. It was observed that the frequency of chromosomal abnormalities was increased against control with increase in exposure period. Fig. 1 & 2 and Table 1 show different types of chromosomal abnormalities obtained in gill cells of fish. These types were chromatid separation, fragmentation and condensation.

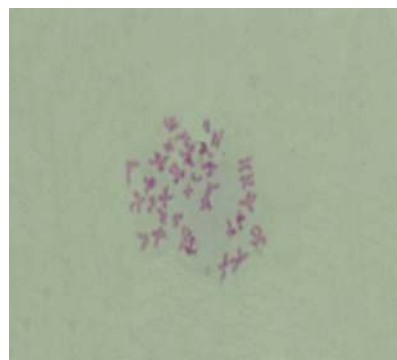


Fig.1: Photomicrograph showing chromosomes before treatment of carbaryl.

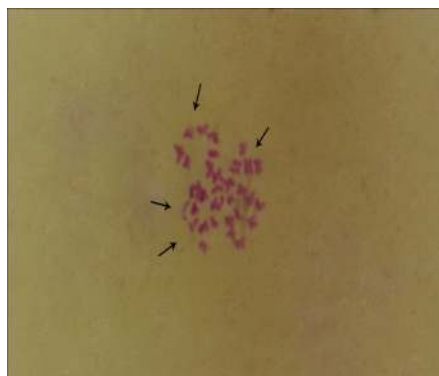


Fig.2: Photomicrograph showing chromosomes after treatment of carbaryl.

Table 1: Frequency of chromosomal aberrations in gill cells induced by carbaryl in *Channapunctatus*.

Concentration of carbaryl	Exposure period in hours	No. of fishes dissected	No. of spreads studied	No. of metaphase spread with chromosomal abnormalities	% of metaphase spread with chromosomal abnormalities
0.0000 (Control)	0	5	50	0	0
	48	5	50	2	4
	96	5	50	1	2
	144	5	50	2	4
0.1ppm	48	5	50	3	6
	96	5	50	7	14
	144	5	50	16	32
0.5 ppm	48	5	50	6	12
	96	5	50	18	36
	144	5	50	29	58

DISCUSSION

The present result obtained from in vivo exposure of *Channa punctatus* to carbaryl showed the genotoxic effects such as chromatid separation, fragmentation, condensation and sticky plates. These results are similar to those observed in *Channa punctatus* treated with dichlorovos (Rishi and Grewal, (1995), *Oreochromes mossambicus* treated with pyrethroid fenvalerate (Arokia Rita and Selvanayagam, 1998) and *Etroplus suratensis* exposed to organophosphorus pesticides (Das and John, 1999). Our results clearly showed that carbaryl causes chromosomal aberrations even when the fishes kept in the water containing carbaryl¹.

The erythrocytes micronucleus test has been used with different fish species to monitor aquatic pollutants (De flora et al., 1993; Saotome and Hayashi, 2003; Pantaleao et al., 2006). The results obtained support the fact demonstrated by Klingerman (1982) that fish inhabiting in polluted waters have greater frequencies of micronuclei.

The results obtained clearly show that this compound induces chromosomal aberrations and micronuclei even when the fishes are kept in the water containing its sub lethal concentrations. This study also proved the efficiency of fish for use as a model in carrying out genotoxic investigations related to water pollutants.

REFERENCES

1. Al-Sabti, K. and Metcalfe, C.D. Fish micronuclei for assessing genotoxicity in water. *Mutat. Res.* 1995, 343: 121-135.
2. Arokia-Rita, J.J. and Selvanayagam, M. Genotoxic effect of fenvalerate on the chromosomes of fish *Oreochromis mossambicus* (Peter). *Poll. Res.* 1998, 17(2): 119-122.
3. Asano, Y.F.N., Sifuni, T. and Ojima, Y. Development of genotoxic assay systems that use aquatic organisms. *Mutat. Res.* 1998, 399: 125-133.
4. Barker C. J, Rackham B. D. The induction of sister chromatid exchange in cultured fish cells (*Ameia splendens*) by carcinogenic mutagens. *Mutat. Res.*; 1979, 68: 381-87.
5. Das, P. and John, G. Induction of sister chromatid exchanges and chromosome aberrations in vivo in *Etroplus suratensis* following exposure to organophosphorus pesticides. *Toxicol. Lett.*, 1999, 104(1-2), 111-116.
6. De Flora S., Vigario L., D' Agostini F., Camoirano A., Bagnasco M., Bennecelli C., Melodia F., Arillo A. Multiple biomarkers in fish exposed in situ to polluted river water. *Mutat. Res.* 1993, 319: 167-177.
7. Klingerman, A.D., Bloom, S.E., Howell, W.M. Umbra limi: a model for the study of chromosomes aberration in fishes. *Mutat. Res.* 1975, 31, 225-2
8. Klingerman, D. Fishes as biological detectors of the effects of genotoxic agents. In: *Mutagenicity: New Horizons in Genetic Toxicology*, Heddl J (ed) Academic Press, New York., 1982, 435-456.
9. Pantaleao Sde M., Alcantara A.V., Alves Jdo P, Spano M. A. The piscine micronucleus test to assess the impact of pollution on the Japarutuba river in Brazil. *Environ. Mol. Mutagen.* 2006, 47(3): 219-224.
10. Kushwaha B., Nagpure N.S., Srivastava S.K., Kumar R., Verma M.S. (). Variation in micronuclei in peripheral blood cells of *Channa punctatus*. *Ind. J. Animal Sci.*; 2003, 73: 1192-1193.
11. Ojima, Y. Method in fish cytogenetics. *Nucleus.* 1982, 25: 1-7.
12. Rishi, K. K. and Grewal, S. Chromosomal aberration test for the insecticide Dichlorovos on fish chromosomes. *Mutat. Res. Genet. Toxicol.* 1995, 344 (1-2): 1-4.
13. Saotome K., Hayashi M. Application of a sea urchin micronucleus assay to monitor in aquatic pollution: influence of sample osmolality. *Mutagenesis.* 2003, 18(1): 73-6.
14. Tucker, J. D., Preston, R. J. Chromosome aberrations, micronuclei, aneuploidy sister chromatid exchanges and cancer risk assessment. *Mutat. Res.* 1996, 365, 147-159.