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Dose- and time-dependent micronucleus induction in peripheral erythrocytes of catfish, *Heteropneustes fossilis* (Bloch) by zinc

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ABSTRACT

As far as the detection of metal genotoxicity in fish is concerned, micronucleus (MN) test is considered an extremely suitable measure. In this study, frequencies of micronucleated erythrocytes were scored in peripheral blood of catfish, *Heteropneustes fossilis* (bloch) after acute in-vivo exposure of zinc at different concentrations (5, 10 and 30 ppm) in the laboratory condition. These three concentrations of zinc were tested at different durations such as 24h, 48h, 72h and 96h respectively. Highly significant ($P < 0.001$) increased values were obtained for MN frequencies in the peripheral erythrocytes of exposed fishes compared to control groups of fishes. These results confirm that dose- and time-dependent micronucleation in the peripheral erythrocytes of fish after short-term exposure to zinc could provide valuable information regarding zinc containing effluent quality and also help in genetic biomonitoring with this test model. In this context safe concentration of zinc vis-a-vis genotoxicity range could be evaluated for future studies.

***Keywords*:** catfish; erythrocytes; *Heteropneustes fossilis*; genotoxicity; micronucleus; zinc

1. INTRODUCTION

Induction of micronucleus (MN) in the living cells indicates genotoxicity in living organisms. The higher frequencies of MN in cells of animals are reported to have the potentiality of structural and/or numerical chromosomal damage (clastogenic / aneugenic effect) in a particular species, which leads to the risk of carcinogenecity when exposed to chemicals. The formation of MN in the peripheral erythrocytes of fish also determine the status of water quality in which pollution by metal or mixture of metals are to be detected and subsequently lead to genetic biomonitoring.

Fish is considered to be a suitable test model for the evaluation of biomarkers at genetic level, which identify sensitive or resistant indicator species for particular heavy metal in aquatic ecosystem. MN inductions by different chemicals have been determined successfully

in several fish species (Hooftman & De Raat 1982; Manna & Sadhukhan 1986; De Floora et al. 1993; Al-Sabti & Metcalfe 1995; Grisolia & Corderio 2000; Rodriguez-Cea 2003; Cavas et al. 2005). MN tests in various fish species exposed to heavy metals have been documented by many workers (Poongothai et al. 1996; Nepomuceno et al. 1997; Castano et al. 1998; Ayllon & Garcia-Vazquez. 2000; de Lemos et al. 2001; Cavas et al. 2005). That the nuclear abnormality can be a tool to detect chromosomal damage induced by genotoxins was established from the work by Umegaki & Fenech (2000).

Zinc pollution occurs in aquatic ecosystem when effluent is discharged from various industrial processes such as electroplating, steel, mining, paints and fungicide factories etc. This metal is an important micronutrient for living organisms and in many countries it is used as a dietary supplement for man with a recommended dose but it has been proven as a toxicant to fish species (Brungs 1969; Benoit & Halocombe 1979; Goel & Gupta 1985; Hemalatha & Banerjee 1993; 1997; Talapatra 2000). Zinc is not only toxic to fishes but at the same time is a potent genotoxin to various animals' cell lines and humans as well (Thompson et al. 1989; Santra et al. 2002; Shuilleabhain et al. 2004). Zinc genotoxicity studies are reported mainly in in-vitro system but in-vivo studies are extremely rare (Gupta et al. 1991).

So far acute in-vivo genotoxicity study is concerned from the available literatures, micronucleus inductions in peripheral erythrocytes of catfish, *H. fossilis* (Bl.) particularly with Zn was not determined previously. Present study is an attempt to detect zinc toxicity at genetic level by dose- and time-dependent micronuclei frequencies in the peripheral erythrocytes. This also proves ability of catfishes to be used as biomarker assessment tool in relation to zinc polluted effluent.

2. MATERIALS AND METHODS

2. 1. Test species and Test chemical

Catfish, *H. fossilis* (Bloch) was used as a test species (length = 7.5-9.5 cm, weight = 5.0-7.5 gm). Metal salt, zinc acetate (CAS registry number = 557-34-6) of analytical reagent (AR) grade. All other chemicals and reagents were used of analytical reagent (AR) grade.

2. 2. Acclimatization of fish

Healthy fishes were collected from the local farms. They were treated with very dilute solution (0.01 %) of potassium permanganate for 1-2 minutes. After washing with water the fishes were acclimatized to laboratory conditions (25 °C $\pm$1 °C with 12:12 photoperiod) for 15 days in glass aquaria (16"x 10" x 10") containing sufficient dechlorinated water and were supplied *Tubifex tubifex* as food *ad libitum*. The pH and dissolved oxygen of water were 7.0 $\pm$1.0 and 7.8 $\pm$1.0 ppm respectively. During acclimatization the water was changed daily to remove metabolic waste products. Diseased or dead fishes were removed quickly from the aquaria.

2. 3. Treatment of metal

Stock solution of metal was prepared by dissolving zinc acetate in distilled water. Various concentrations of zinc were prepared after diluting the stock solution with aged tap water, in which test fish were added to observe their reactions for a definite time. As there was no known report of toxicity of zinc acetate to this experimental fish, the test solutions were made over three concentrations i.e. 5, 10, 30 mg/l by adding required volumes of stock

solution in the glass jar (capacity = 5 litre). The experiments were continued for 96h duration (static). Parallel controls with water and without zinc were run under identical conditions. After each acute phase of 24h durations, 10 fish were sacrificed from experimental as well as control group for assessment of micronuclei frequencies. Experiments were carried out thrice for getting three replicas.

2. 4. Slide preparation and staining

In each fish peripheral blood were drawn from gill without mortality of fish and smeared onto the clean slide for experimental as well as control groups with proper coding. The coded slides were air-dried for 12h and then fixed in absolute methanol for 10 min. After fixing the same slides were stained in aqueous Giemsa (5 %) for 10 min (Palhares & Grisolia 2002).

2. 5. Slide analysis

In each fish 2000 erythrocytes were counted for peripheral blood separately from experimental as well as control groups. The frequencies of micronuclei in the erythrocytes were detected under Binocular microscope (OLYMPUS, Model-520313) using a 1000X oil-immersion lens.

2. 6. Stastical analysis

To determine statistically significant differences between experimental and control groups all the mean values of data are analyzed by using Student's t-test at 0.05 level.

3. RESULTS

The results reveal that the basal or spontaneous MN frequencies are very low in the control groups of fish, *H. fossilis* (Bl.) when compared to different concentrations of zinc treated groups. Highly significant ($P < 0.001$) values of MN frequencies are observed in the peripheral erythrocytes of the fish in every 24h durations of dose-dependent exposure of different zinc concentrations such as 5, 10 and 30 ppm respectively (Table 1 and Fig. 2). The photomicrographs of normal and micronucleated peripheral erythrocytes are documented in Fig. 1A and B.

Induction of micronuclei frequencies are increased gradually from lower concentration to higher concentration of zinc but the values are obtained without significant differences at 24h and 48h duration in 5 and 10 ppm as well as 10 and 30 ppm respectively.

In the 72h duration, the values are decreased in 10 ppm than 5 ppm but significantly ($P < 0.001$) increased in 30 ppm whereas in the 96h duration comparison of 5 and 10 ppm do not show any significant differences but in 30 ppm highly significant ($P < 0.001$) value is obtained.

4. DISCUSSION

Induction of MN frequencies in the erythrocytes of fish is a suitable method for genotoxicity detection. The frequencies of MN were induced generally in fish when exposed to physical or chemical agents at in-vivo or in-vitro laboratory condition (Manna &

Sadhukhan 1986; Al-Sabti & Metcalfe 1995; Ayllon & Garcia-Vazquez 2000, Cavas & Ergene-Gozukara 2003; Cavas et al. 2005; Arkhipchuk & Garanko 2005). Acute and chronic exposure of metals induced MN frequencies in fish have been reported by many researchers (Privezentsev et al. 1996; Sanchez-Galan et al. 2001; Cavas et al. 2005). Among other metals, organic and inorganic form of zinc is a potent genotoxin to living organisms, cells and cell lines etc. has been reported (Brooks et al. 1983; Thompson et al. 1989; Zenzen et al. 2001; Shuilleabhain et al. 2004).

The present study is shown the dose- and time-dependent induction of micronuclei frequencies in the peripheral erythrocytes of fish, *H. fossilis* (Bl.) by zinc. After acute in-vivo exposure of zinc gets supports from the work of other researchers. When fishes are exposed to EMS (Ethyl methanesulphonate) they give dose and time-dependent MN inductions in erythrocytes (Hooftman & De Raat 1982). Physical genotoxin like γ-irradiation and chemical genotoxin like mitomycin-C have shown linear and dose-dependent MN inductions in the erythrocytes of fish within 48h to 96h duration (Bahari et al. 1994). In the acute phase i.e. one to five days post-exposure a maximum MN formation is found in the cells, which supports the present findings (Al-Sabti & Metcalfe, 1995).

Acute in-vivo MN test in the peripheral erythrocytes of fish is a suitable method for assessing sensitive biomarkers of genotoxicity. Because of the short-term exposure periods ranging between 24h to 96h in animal models and considered to be an enough time to induce MN in the erythrocytes or other cells (Cavas et al. 2005). In the present study significant ($P < 0.001$) induction of micronucleated erythrocytes of the peripheral blood in fish, *H. fossilis* (Bl.) were found between the periods of 24h to 96h exposed to various concentrations such as 5, 10 and 30 ppm respectively.

Zinc is shown dose- and time-dependent positive genotoxic effects in this particular species. Similarly, in this study of zinc genotoxicity, it was observed that zinc acetate has positive dose-related responses in L5178Y mouse lymphoma test and in-vitro cytogenetic test in Chinese hamster ovary cells (Thompson et al. 1989), which finds an agreement with the present results. Unlikely, instead of in-vivo genotoxicity, zinc metal salts have potent cytotoxic effects at in-vitro investigation with three fish cell lines such as EPC, CHSE and RTG-2 respectively (Shuilleabhain et al. 2004). In another experiment, it was also reported that induction of MN in the cultured human leucocytes by cytokinesis block micronucleus (CBMN) assay with two different concentrations of zinc, which has shown dose-dependent genotoxic effect (Santra et al. 2002). Gupta et al. (1991) have reported that cytotoxic effect of zinc chloride in mice after in-vivo exposure. Different available literatures clearly reveal that major work has been done on acute in-vitro genotoxicity study of zinc compared to in-vivo study with fish as a test model.

It is very interesting to note that the established permissible limit for zinc is 1-5 ppm as drinking water quality standard has been proposed by WHO (1971); USEPA (1975); USSR (1979); Canada (1978) and ISI (1982). Few studies are reported in fish species regarding safe concentration of zinc (Abbasi & Soni 1986) and the safe concentration for zinc genotoxicity in fish is lacking. These findings clear indicate that zinc concentration is very close to permissible limit 5ppm that induces micronucleation significantly ($P < 0.001$) in the erythrocytes of the test species. In a previous study, some disparity of safe concentration and permissible limit of zinc has been documented and proposed for revision (Abbasi & Soni 1986).

In conclusion the result is clearly indicating that genotoxicity is resulted in the peripheral erythrocytes of fish *H. fosslis* (Bloch), after acute in-vivo exposure of zinc. This test fish species is showing more sensitive indicator in respect to their short life cycle and

lower age group. MN tests are confirming that this fish model can be used in genetic biomonitoring for detection of water quality status. Erythrocytes are a suitable biomarker for zinc containing effluent water. This is an observation of dose- and time- dependent zinc genotoxicity after acute in-vivo exposure with *H. fossilis* (Bl.) but further research is very much needed particularly in relation to the safe concentration of this genotoxic response with water quality standard.

Table 1. Frequencies micronuclei in peripheral erythrocytes of *H. fossilis* (Bl.) after acute in-vivo zinc exposure at different durations.

Concentrations (in ppm)	**MN frequencies in different durations[a]**			
	24h	**48h**	**72h**	**96h**
Control	0.36 ±0.05	0.32 ±0.02	0.42 ±0.02	0.41 ±0.01
5	0.85 ±0.08*****	0.93 ±0.05*****	1.13 ±0.03*****	1.16 ±0.08*****
10	1.18 ±0.02*****	1.29 ±0.05*****	1.12 ±0.06*****	1.18 ±0.08*****
30	1.20 ±0.03*****	1.32 ±0.06*****	1.91 ±0.01*$^{\gamma}$	2.16 ±0.04*****$^{\gamma}$

[a]Mean ± S.D; n = 10. *P < 0.001 (compared between control vs. all zinc concentrations) $^{\gamma}$ P < 0.001 (compared between 10 ppm vs. 30 ppm zinc concentration).

A

Figure 1. A. Normal and B. Micronucleated (indicated arrow) peripheral erythrocytes of catfish, *H. fossilis* (Bl.) after acute in-vivo exposure of zinc.

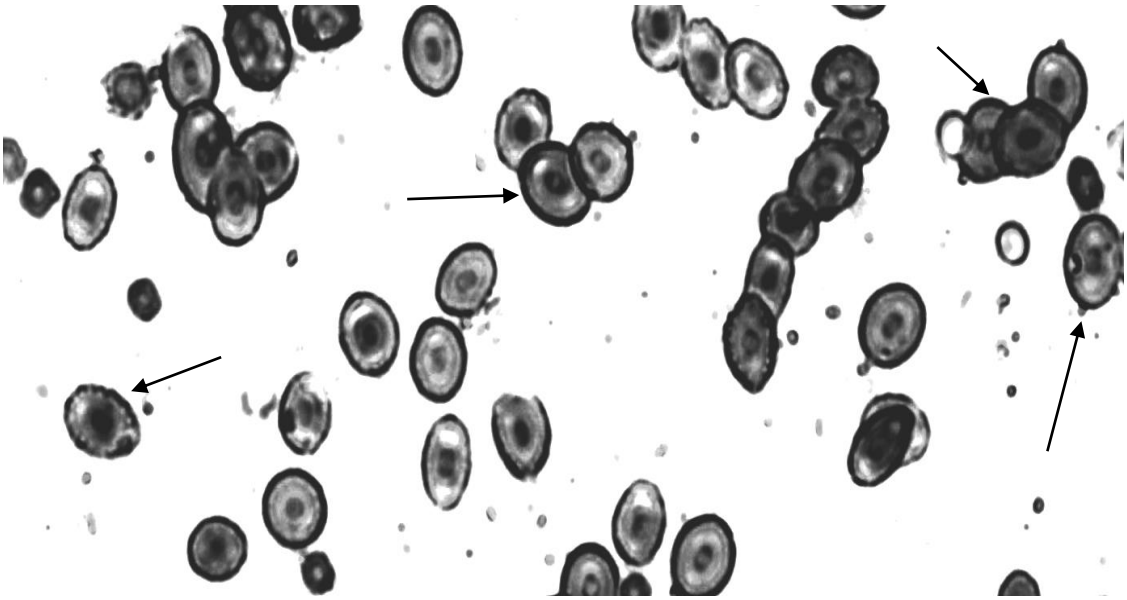

Figure 1(continue). A. Normal and B. Micronucleated (indicated arrow) peripheral erythrocytes of catfish, *H. fossilis* (Bl.) after acute in-vivo exposure of zinc.

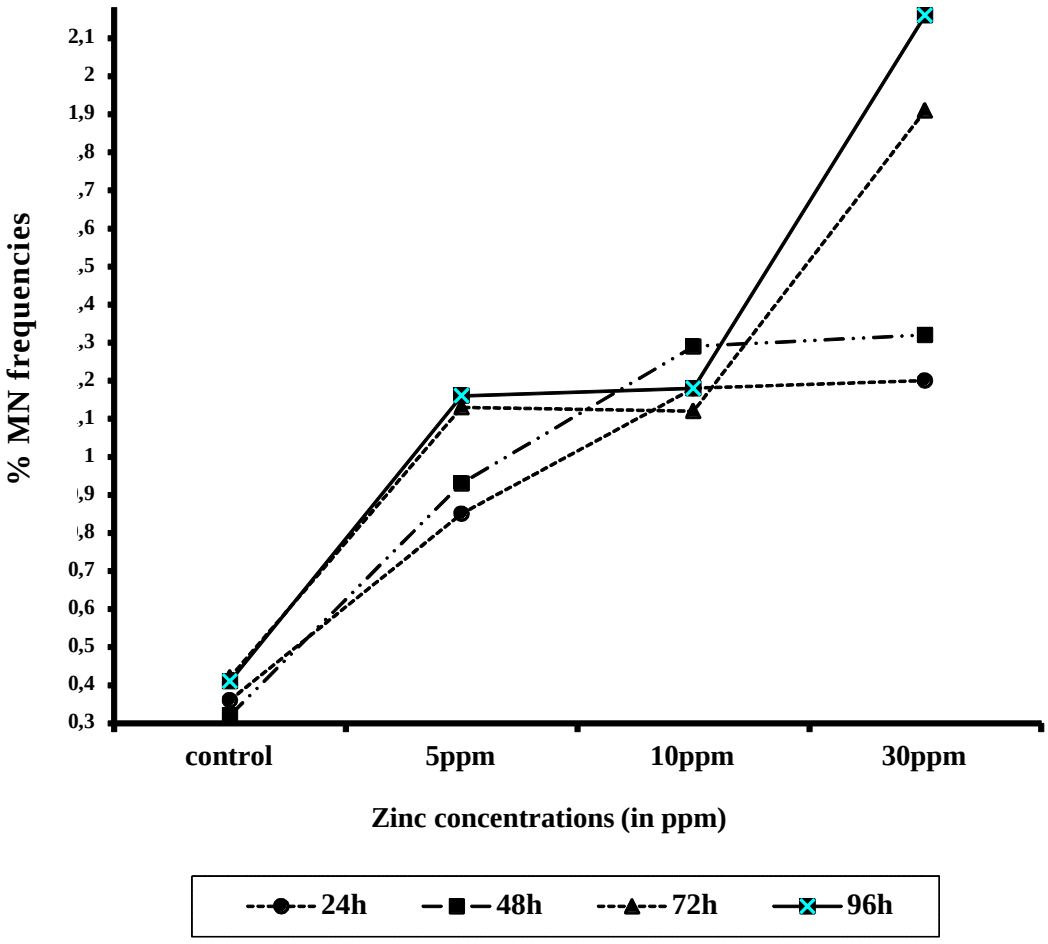

Figure 2. Induction of MN frequencies in the peripheral erythrocytes of fish, *H. fossilis* (Bl.) at different zinc concentrations and durations.

5. CONCLUSION

The current study reports for the first time the acute in-vivo genotoxicity in peripheral erythrocytes of catfish, *H. fossilis* (Bl.) with Zinc (Zn) at genetic level by dose- and time-dependent micronuclei frequencies in the peripheral erythrocytes. In this context, we propose that catfish, *H. fossilis* (Bl.) might be used as a model biomonitoring system for detecting Zn contamination in water. However, further research is in prospect to determine of Zn susceptibility of C. dubia when in combination with other aquatic toxicants.

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References

[1] Abbasi S. A., Soni R., *Environ. Poll.* 40 (1986) 37-51.

[2] Al-Sabti K., Metcalfe C. D., *Mutat. Res.* 343 (1995) 121-135.

[3] Arkhipchuk V. V., Garanko N. N., *Ecotoxicol. Environ. Saf.* 62 (2005) 42-52.

[4] Ayllon F., Garcia-Vazquez E., *Mutat. Res.* 467 (2000) 177-186.

[5] Bahari I., Noor F., Daud N. M., *Mutat. Res.* 313 (1994) 1-5.

[6] Benoit D. A., Halocombe G. W., *J. Fish Biol.* 13 (1979) 701-708.

[7] Brooks T. M., Meyer A. L., Dean B. J., *Mutat. Res.* 124 (1983) 129-143.

[8] Brungs W. A., *Trans. Am. Fish. Soc.* 98 (1969) 272-279.

[9] Castano A., Carbonell G., Carballo M., Fernandez C., Boleas S., Tarazona J. V., *Ecotoxicol. Environ. Safe.* 41 (1998) 29-35.

[10] Cavas T., Ergene-Gozukara S., *Mutat. Res.* 538 (2003) 81-91.

[11] Cavas T., Garanko N. N., Arkhipchuk V. V., *Food Chem. Toxicol.* 43 (2005) 569-574.

[12] De Floora S., Vigano L., D'Agostini F., Camoirano A., Bagnasco M., Bennicelli C., Melodia F., Arillo A. *Mutat. Res.* 319 (1993) 167-177.

[13] Fenech M., *Drug Dis. Today* 7 (2002): 1128-1137.

[14] Goel K. A., Gupta K., *Indian J. Fish* 32 (1985) 256-260.

[15] Grisolia C. K., Corderio C. M. T., *Gen. Mol. Biol.* 23 (2000) 235-239.

[16] Guidelines for Canadian Drinking water Quality. 1978. Ministry of National Health and Welfare, Canada.

[17] Gupta T., Talukder G., Sharma A., *Biol. Trace Elem. Res.* 30 (1991) 95-101.

[18] Indian Standards Institution. 1982, New Delhi, India.

[19] Hemalatha S., Banerjee, T. K., *J. Freshwat. Biol.* 5 (1993) 191-196.

[20] Hemalatha S., Banerjee T. K., *Biol. Res.* 30 (1997) 11-21.

[21] Hooftman R. N., De Raat W. K., *Mutat. Res.* 104 (1982) 147-152.

[22] de Lemos C. T., Rodel P. M., Terra N. R., Erdtmann B., *Environ. Toxicol. Chem.* 20 (2001) 1320-1324.

[23] Manna G. K., Sadhukhan A., *Curr. Sci.* 55 (1986) 498-501.

[24] Nepomuceno J. C., Ferrari I., Spano M. A., Centano A. J., *Environ. Mol. Mutagen.* 30 (1997) 293-297.

[25] Palhares D., Grisolia C. K., *Gen. Mol. Biol.* 25 (2002) 281-284.

[26] Poongothai K., Shayin S., Usharani M. V., *Cytobios.* 86 (1996) 17-22.

[27] Privezentsev K. V., Sirota N. P., Gaziev A. I., *Tsitol Gen.* 30 (1996) 45-51.

[28] Rodriguez-Cea A., Ayllon F., Garcia-Vazquez E., *Ecotoxicol. Environ. Saf.* 56 (2003) 442-448.

[29] Sanchez-Galan S., Linde A. R., Ayllon F., Garcia-Vazquez E., *Ecotoxicol. Environ. Saf.* 49 (2001) 139-143.

[30] Santra M., Das S. K., Talukder G., Sharma A., *Biol. Trace Elem. Res.* 88 (2002) 139-144.

[31] Shuilleabhain S. N., Mothersill C., Sheehan D., O'Brien N. M., O'Halloran J., Van Pelt, F. N. A. M., Davoren M., *Toxicol. in Vitro* 18 (2004) 365-376.

[32] Talapatra S. N., 2000. Ph.D. Theses, Ranchi University, Ranchi, India.

[33] Thompson E. D., McDermott J. A., Zerkle T. B., Skare J.A., Evans B. L. B., Cody D.B., *Mutat. Res.* 223 (1989) 267-272.

[34] Umegaki K., Fenech M., *Mutagen*. 15 (2000) 261-269.

[35] United State Environmental Protection Agency. 1975. EPA, Washington, D.C.

[36] USSR. 1979. Springer Verlag, Berlin, Heidelberg, New York, pp. 26-30.

[37] World Health Organization. 1971. WHO, Geneva.

[38] Zenzen V., Fauth E., Zankl H., Janzowski C., Eisenbrand G., *Mutat. Res.* 497 (2001) 89-99.

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