ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2015; 6 (3): 29- 32

RESEARCH ARTICLE

Study of the Genotoxic Effect of Polluted Water of Certain Ponds of Narsinghpur in the R.B.Cs. of the Fish *Catla catla*, by Applying Micronucleus Assay

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Received 08 Mar 2015; Revised 02 Jun 2015; Accepted 17 Jun 2015

ABSTRACT

The present study is to evaluate the genotoxic effect of polluted water quality of the ponds on the R.B.Cs. of the fish *Catla catla*. Micronucleus assay were applied to study the genotoxic effect in R.B.Cs. of fish. In the present studies we found formation of micronuclei, binucleated and multinucleated cells, and sticky chromosomes in the cells proving genotoxicity.

Key words: Genotoxic, micronucleus, binucleated, multinucleated, chlorinated, phosphorylated, accumulation, remnants, mutagenicity, proliferation, bioindicator.

INTRODUCTION

The present studies are to determine the genotoxic effects of polluted water on the fish R.B.Cs.The fish selected for following study is a teleost fish named as *Catla catla*. This fish is cultured in the selected ponds and is a surface feeder. The ponds, in which these fishes are cultured, surrounded with fields used for crop culture and for the production of vegetables.

In these fields' crop protection from insect/pests and for better crop production farmers used various chlorinated and phosphorylated insecticides/pesticides and fertilizers.These chemicals and their remnants invade ponds when it rains. Flow off from the fields enters directly into the ponds affecting living organisms.Higher level of BOD results in depletion of available oxygen for aquatic organisms, while high amount of COD in effluent is toxic to biological life (Victoria A. Oriacu *et al*).

The erythrocyte micronucleus test is a preferred bioindicator of environmental mutagenicity. During micronuclei analyses, some authors have observed the occurrence of other nuclear abnormalities, suggesting that they must also be taken into consideration as potential indicators of cytotoxicity (Schroeder, 1970; Tolbert et al., 1991, 1992; Fenechet al., 1999) . The micronucleus assay is a simple and sensitive assay for evaluation of genotoxic properties of various agents. Since teleost erythrocytes are nucleated, micronucleus assay provides information about the measure of clastogenic activities (Nagpure *et al*, 2007).

In fishes chromosomes are small in size but large in numbers. Thus micronucleus in fishes could be smaller in size. The formation of micronucleus depends on the rate of proliferation of the cells, which in turn depends on fish species, environmental conditions and target tissues.

The micronuclei are small, extranuclear bodies that are formed during mitosis from acentric chromosomal fragments, or whole chromosomes that are not included in daughter nucleus.Thus, micronucleus may contain a fragment of chromosome or it may contain a whole body of chromosome that is unable to travel to the spindle pole during anaphase.

MATERIALS AND METHODS

Water samples are collected fromthe ponds and physio-chemical properties of sample water are determined using standard analytical methods.Fishes were captured from the four selected polluted ponds. Blood were collected by the method described below and micronucleus assay was performed using the method described below:

- **Collection of blood samples**: Blood were collected by caudal puncture. For this

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purpose plastic syringes of 1 ml capacity and needle (1.0 inch, 22 gauges) were used. The blood is directly drawn from caudal vein. Mixture of dry ammonium oxalate and potassium oxalate in the ratio of 3:2 is used as blood anti-coagulant.

- **Preparation of MN slides:**
  - **1-** A thin layer of blood is smeared on pre-cleaned glass slides with the help of glass slide.
  - **2-** Slides are air dried over night at room temperature in a dust free moisture free environment.
  - **3-** Slides are then fixed by dipping in absolute methanol for 10 minutes.
  - **4-** Slides are then air dried for 1 hour.
  - **5-** Then the slide are stained with 10% Giemsa stain for 30 minutes.
  - **6-** Then they are washed for 3 times with double distilled water, so as to remove every Giemsa particle.
  - **7-** Slides are dried overnight in dust and moisture free environment.
  - **8-** Then the slides are mounted with DPX to make them permanent.
  - **9-** These permanent blood slides are observed under the microscope with the help of the eye piece 10X and the objective lance of 100X. A drop of immersion oil is used.
  - **10-** In the blood samples collected from different fishes of the different ponds, mean values of the MNi produced were obtained. For this purpose values of the MNi produced per 100 RBCs were observed and tabulated in frequency table. Obtained mean values of MNi produced in the fish RBCs collected from all the four ponds were compared to obtained highest frequency of MNi production within the fishes.

Calculation:

$$\text{Mean\%} = \frac{\text{No. of cells find with micronucleus}}{\text{Total no. of cells observed}}$$

RESULTS AND DISCUSSIONS

The result of the physio-chemical analysis of water revealed higher values of most parameters(D.O., B.O.D., C.O.D., Nitrates, Phosphates, Potassium, Chlorides, Fluorides, Iron, Sulfates, Alkalinity, Turbidity, Hardness, pH , and Temperature.) than the standards set by Environmental Protection Agency (table 1).The values obtained for D.O. are showing hypertoxic conditions of ponds. Values obtained for B.O.D. and C.O.D. are showing high level of biological oxygen demand and chemical oxygen demand.Values obtained for dissolved salts like nitrates, phosphates, potassium, chlorides, fluorides, iron and sulfates is also not satisfactory. The alkalinity, turbidity and hardness of pond water are also not matching the standards of Environmental Protection Agency (**Table 1**).

Chlorinated compounds are known to increase acidic conditions (pH below7) while the phosphorylated compounds are known to increase alkaline conditions (pH above 7) of water(Russel E. Train, 1979.).A wide range of pH change also causes alterations in blood parameters (Mahdi Ghanbari&Mansoureh Jami, 2010).

The pH value of pond I and II are 6.9333 and 6.8333 respectively, showing acidic nature of water, while that of pond III and IV are 8.375, and 9.1666, showing alkaline conditions (Table 1).

Fishes had taken as control showing normal RBCs and nucleus in the blood screening (**Fig 1**) of the fish *Catla catla*.In fishes, many nuclear alterations have been reported after exposure to chemical agents or polluted waters (Cavas and Ergene-Gozukara, 2003; Da Silva Souza and Fontanetti, 2006). Fishes are excellent organisms for the study of the mutagenic and carcinogenic potential of contaminants in the aquatic environment because they can metabolize, concentrate and store water borne pollutants (Al-Sabti, 1991). Pollution in aquatic environment impacts on physiology, development, growth or survival of fish, affects human, at the top of the food chain (Lin and Hwang, 1998).

In this research study the result shows the formation ofabnormal nucleus(AN), bilobednucleus(BN)and micronucleus(MN), appeared very clearlyin RBCs of *Catlacatla* collected from pond I with inconsistent physiochemical conditions (**Fig 2**).Blood sample collected from pond II fishes showing multinucleated cells(MNC), destroyed cells(DC), abnormal nucleus(AN), bilobed nucleus(BN), and chromatin oozing out from the cell(COC), enlarged nucleus(EN) in the RBCs (**Fig 3**)

Fishes *Catla catla* collected from pond III showing binucleated cells (BNC), multinucleated cells (MNC), abnormal nucleus (AN), and chromatin oozed out of the cells (COC), destroyed

Table 1: Showing permitted values and obtained values of different physiochemical parameters in gm/l and temperature in ^{O} C

S. No	Parameters	Permitted value	Obtained value.			
			Pond I	Pond II	Pond III	Pond IV
1	D.O.	5-10	13.7166	13.4416	12.4916	9.7583
2	B.O.D.	6.00	10.575	10.6333	10.375	10.5666
3	C.O.D.	6.00	10.5083	10.425	10.375	10.1666
4	Nitrates	45	69.8333	71.8333	62.8333	96.3333
5	Phosphates	0.05	1.00	0.9416	0.925	0.9333
6	Potassium	<20	45.833	50.6666	29.4166	27.50
7	Chlorides	200	326.25	317.50	228.6666	206.0833
8	Fluorides	0.5	0.175	0.375	0.0	0.05
9	Iron	0.1	0.0583	0.0583	0.075	0.333
10	Sulfate	200	246.6666	239.1666	254.1666	264.1666
11	Alkalinity	200	215.8333	234.1666	434.1666	459.1666
12	Turbidity	10	78	71.8333	76.1666	78.0833
13	Hardness	200	483.3333	632.50	992.50	687.50
14	pH	6.5-7.5	6.9333	6.8333	8.375	9.1666
15	Temperature	13-22 C.	17.0833	16.0416	14.6666	15.00

cells (DC) and clumped RBCs (C RBCs) in their blood cells (Fig4). Multinucleated cells (MNC), abnormal nucleus (AN), extracellular chromatin (EC), chromatin oozed out of cell (COC) and destroyed cells (DC) bring into being in the blood of the fish Catlacatla collected from pond 4(**Fig 5**). The results obtained by applying micro nucleus assay indicates that the pollutants present in all the 4 study ponds have genotoxic and clastogenic effects in the fishes and are harmony with the findings of the researchers who had worked on genotoxic, cytotoxic and clastogenic effects of chemicals and toxins on fishes. The mean % value of micronucleus formation in the RBCs of *Catla catla* is shown in the (**Table 2**).

Table 2: Frequency of MNi in R.B.Cs. of *C. catla*

S. No	No .of RBCs observed/ slide	No .of cells with MNi	Total	Mean%
1	400	15	15	3.75
2	400	16	16	4.00
3	400	16	16	1.00
4	400	6	6	1.50
5	400	12	12	3.00
6	400	15	15	3.75
7	400	20	20	5.00
8	400	20	20	5.00
9	400	12	12	3.00
10	400	20	20	5.00

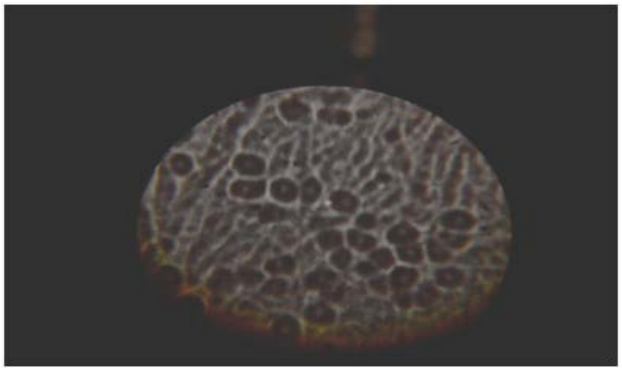

Fig 1:Photomicrograph showing recently divided normal RBCs and nucleus in the blood of the fish *Catla catla*

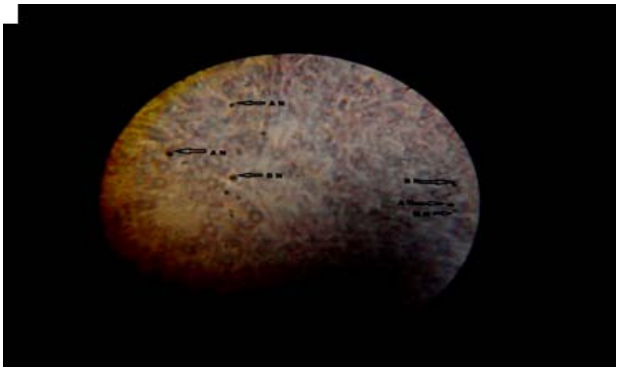

Fig 2: Photo micrograph showing abnormal nucleus (AN), bilobed nucleus (BN) and micronucleus (MN) in RBCs of *Catla catla* collected from pond I

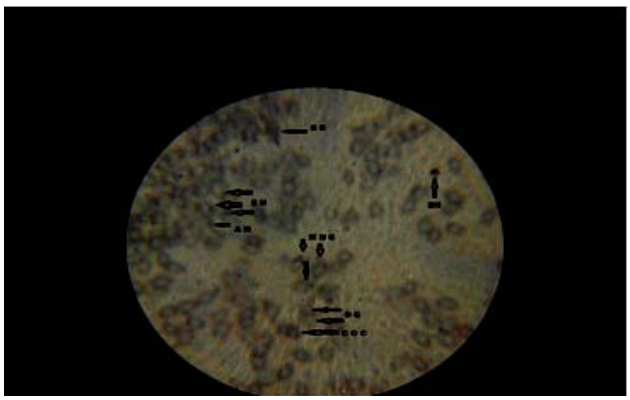

Fig 3: Photomicrograph showing multinucleated cells(MNC), destroyed cells(DC), abnormal nucleus(AN), bilobed nucleus(BN), chromatin oozed out of the cell(COC), enlarged nucleus(EN) in the RBCs and blood of the fish Catlacatla collected from pond II

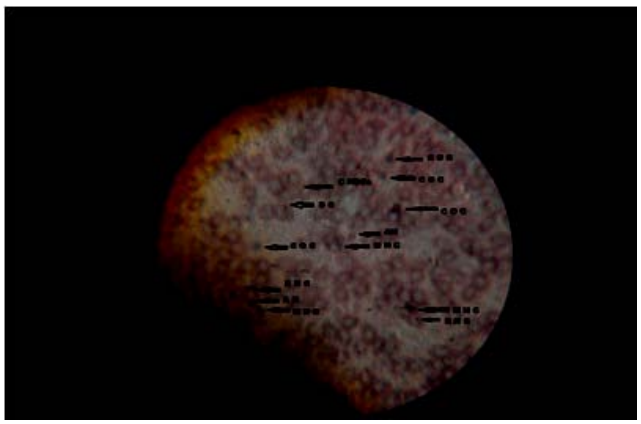

Fig 4: Photograph showing binucleated cells(BNC), multinucleated cells(MNC), abnormal nucleus(AN), chromatin oozed out of the cells(COC), destroyed cells(DC), clumped

RBCs(C RBCs) in the blood of the fish *Catla catla* collected from pond III

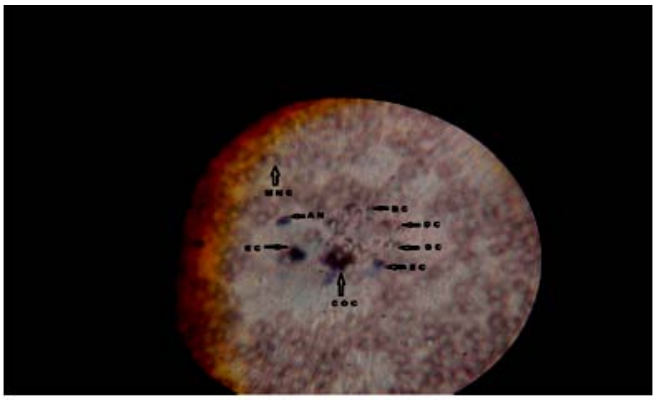

Fig 5: Photograph showing multinucleated cells (MNC), abnormal nucleus (AN), extracellular chromatin (EC), and chromatin oozed out of cell (COC), destroyed cells (DC) in the blood of the fish *Catla catla* collected from pond IV

CONCLUSION

The results obtained from this study indicate that the water of selected 4 ponds is highly polluted and it has genotoxic potential. Nuclear anomalies and formation of micronucleus in blood erythrocytes of the fish *Catla catla* were identified as good genotoxic biomarker. In a broad view, these genotoxic and cytotoxic effects may ultimately induce physiological damage and increasing levels of mutation in fish species studied, leading to an ecological imbalance.

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