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MICRONUCLEI AS AN EVIDENCE OF DNA DAMAGE IN FRESH WATER CATFISH *Heteropneustes fossilis* (BLOCH) EXPOSED TO SYNTHETIC SINDOOR

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ABSTRACT

Fish serve as useful genetic models for the evaluation of pollution in aquatic ecosystems. Plenty of commonly available synthetic sindoor is being used in the religious rituals especially during the idol immersions in India, which contains lead (Pb), mercury (Hg) and industrial dyes and can be harmful to the fishes. The present study was an attempt to explore the genotoxicity of synthetic sindoor in freshwater catfish, *Heteropneustes fossilis* (Bloch) by micronucleus assay. Healthy fishes were collected and housed in well aerated aquaria. Different groups of fishes were treated with different concentrations of synthetic sindoor and the toxicity produced was compared with the control group. Significantly increased frequency of micronuclei in the renal and peripheral blood erythrocytes, observed in the present study suggests that synthetic sindoor is a potential clastogen at higher concentrations.

Keywords: synthetic sindoor, *Heteropneustes fossilis*, genotoxicity, micronucleus assay, clastogen.

INTRODUCTION

Fish are excellent subjects for the study of the mutagenic and carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants (Al-Sabti, 1991). Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application for model systems using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment (Al-Sabti and Metcalfe 1995).

Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water (Carins *et al*., 1975; Brugs *et al*., 1977). The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants (Poele and Strik 1975; Koeman *et al*., 1977; Poele 1977; Sloof 1977; Badr and El-Dib 1978).

Plenty of commonly available synthetic sindoor 'Vermillion' is being used in the religious rituals especially during the idol immersion of Hindu's which contains lead (Pb), mercury (Hg) and industrial dyes which can indirectly damage the human beings and other animals through the consumption of polluted water and aquatic food like fishes. Evidence suggests that lead and mercury cause many adverse effects such as cancer, reproductive, liver and thyroid disorders. Fish exposed to high levels of lead and mercury exhibit a wide range of toxicity including muscular and neurological degeneration and destructive growth inhibition, mortality, reproductive problems and paralysis (Tekale, 2003). These toxic substances may indirectly reach to the human system through the food chain and pose a risk to the human health.

Increasing environmental pollution and lack of public awareness in developing countries like India, have forced scientists to study the direct and indirect effects of the disposal of industrial and other pollutants on the aquatic environment (Central Pollution Control Board 2001-02). Varied methods are followed to investigate the genotoxicity of pollutants but micronucleus assay is one of the widely used methods used to unveil the genotoxicity of pollutants in aquatic environments.

The micronuclei test is employed both for laboratory assays of genotoxicity of many compounds and for *in situ* surveys to assess the risk of mutagen-polluted environments. The in-situ detection of environmental contaminants using fish micronuclei assay has been carried out in marine coast waters, rivers and lakes by several authors. However, the clastogenic effects of pollutants can be measured in different target tissues such as erythrocyte, gills, kidneys and liver etc. (Williams and Metcalfe 1992; Hayashi *et al*., 1998), but the erythrocyte micronuclei test has been used with different fish species to monitor aquatic pollution displaying mutagenic features (De Flora *et al*., 1993; Kligerman, 1982) demonstrated that fish inhabiting polluted water have greater frequencies of micronuclei.

The development of *in-vivo* genotoxicity assays using fish as model animal (Powers, 1989) is being enhanced by their easy handling in the laboratory. Micronucleus test detects both clastogenic and aneugenic effects (Mersch and Beauvais, 1997). Therefore, it allows the detection of genotoxicity of wide range of compounds. In addition, results of micronucleus test are rapidly obtained if carried out on haematopoetic tissues. The present study was thus undertaken to elucidate genotoxicity of synthetic sindoor in the freshwater catfish *Heteropneustes fossilis* in laboratory conditions using micronucleus assay.

MATERIALS AND METHODS

The freshwater, air-breathing, stinging catfish, *Heteropneustes fossilis* Bloch (Order: Siluriformes; Family: Heteropneustidae) commonly known as "Singhi" in vernacular "Hindi" are the test organisms for the present study. These were collected from water reservoirs in and around Bhopal, M.P. (India) with the help of local fisherman, brought to laboratory and acclimatized to laboratory conditions for 30 days before the experiments. For toxicity test six aquaria of 50 liter capacity were taken having 30 liters of dechlorinated tap water (Physico-chemical properties, pH = 7.6±0.2; Temp. = 26±20^{0}C; Alkalinity = 65±4.5 mg/L; Total Hardness = 265±2.5 mg/L; D.O. = 7.0±0.2 mg/L). Series of five concentrations *viz*. 50, 100,500, 1000 and 2000ppm were prepared by adding calculated amount of synthetic sindoor in the respective aquaria. One aquarium having diluent water without synthetic sindoor served as control. Adult 5 fishes of similar size and weight (average length 15± 1.5cm and weight 35.5± 2.0gm) were introduced to each test as well as control aquaria. The fishes were dissected on fifteenth day and the target organs *viz*. gills and kidneys were exposed. Blood was drawn from both the organs and smears were prepared on slides and stained with Giemsa stain (Qualigens) after fixing them with absolute methanol. All slides were coded and scored blind. Five slides were prepared for each fish, and 1000 cells were scored from each slide under X100 magnification. Norefractive, circular or ovoid chromatin bodies, smaller than the one-third of the main nucleus and displaying the same staining and focusing pattern as the main nucleus, were scored as micronuclei (Al-Sabti and Metcalfe 1995). The statistical analysis was carried out with the help of GraphPad Prism Version 4 software on PC.

RESULTS

Fishes showed increased swimming, surfacing and hyperactivity just after the introduction to the test solution. Restlessness and color fading was prominent after 72 hr of exposure. After 7th day ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. The fin hemorrhage was also noticed in some fishes. Test fishes lost there natural coloration and became almost reddish in color.

The frequency of micronucleus was increased in the exposed groups and increased further with the increase in the concentration as shown in Table-1.

Table-1. Frequency of micronuclei in study groups and controls.

Group	Concentration of Sindoor (ppm)	Micronucleus (Renal erythrocytes) Mean ± S.E	Micronucleus (PB erythrocytes) Mean ± S.E
H	00	5.00 ± 1.225	5.00 ± 0.577
H1	50	5.500 ± 0.645	5.00 ± 0.707
H2	100	10.00 ± 1.414	9.00 ± 0.577
H3	500	12.00 ± 0.816	9.00 ± 0.577
H4	1000	14.00 ± 0.816	13.00 ± 0.408
H5	2000	29.00 ± 0.912	30.75 ± 1.493

Micronuclei were significantly increased in the groups treated with 100, 500, 1000 and 2000ppm of synthetic sindoor as compared to the controls (P = 0.0265, P = 0.374, P = 0.0010 and P = 0.0001 for renal erythrocytes, respectively and P = 0.0163, P = 0.0163, P = 0.0031, P = 0.0010 for peripheral blood erythrocytes, respectively). However, no significant difference was found in the group treated with lower dose. The results reveal a dose related increase in the frequency of MN as observed in both renal erythrocytes (Figure-1) as well as peripheral blood lymphocytes (Figure-2).

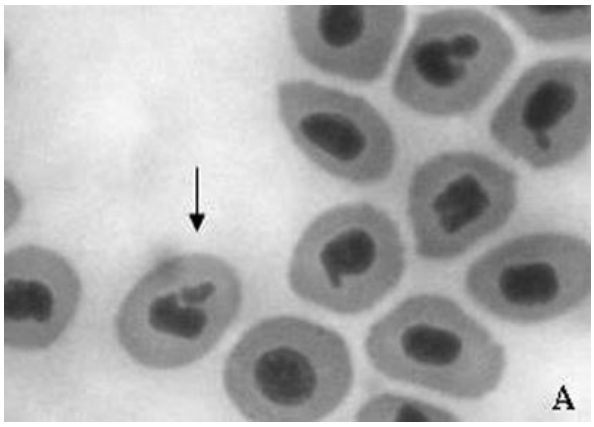

Figure-1. Showing micronucleus in renal erythrocytes.

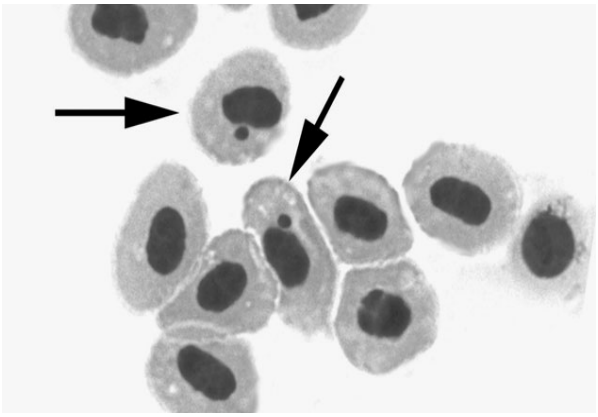

Figure-2. Showing micronucleus in peripheral blood erythrocytes.

DISCUSSION AND CONCLUSIONS

Behavioral alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms (Olla 1980; Sharma and Shukla 1990; Agarwal 1991). Behavioral alterations like erratic swimming, restlessness and surfacing, observed in present study may be an avoiding reaction to the heavy metals as also observed by various workers (Sprague 1969; Giattina *et al*., 1982; Black and Bing 1989).

Fish serve as useful genetic models for the evaluation of pollution in aquatic ecosystems (Mitchell and Kennedy 1992; Park *et al*., 1993). The erythrocyte micronucleus test has been used with different fish species to monitor aquatic pollutants displaying mutagenic features (De Flora *et al*., 1993; Saotome and Hayashi 2003; Pantaleao *et al*., 2006). Our findings are in accordance with Fagr Kh. Ali 2008, stating that the micronucleus assay is a sensitive monitor for aquatic pollution. The obtained results support the fact demonstrated by Kligerman (1982) that fish inhabiting polluted waters have greater frequencies of micronuclei.

The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. In fish, the kidney is responsible for erythropoiesis as well as filtration. Upon fish exposure to toxins, defective erythrocytes undergo passage from the kidney into the peripheral blood, from where they are removed by the hemocatheresis organs (Rabello-Gay 1991). Therefore, we attempted to elucidate the micronucleus frequency in renal as well as peripheral blood erythrocytes. The increased frequency of micronuclei in renal erythrocytes as well as the peripheral cells observed in the present study can be attributed to the heavy metal components of synthetic sindoor mainly lead (Pb) and mercury (Hg). This is supported by the fact that Pb significantly increases DNA damage, principally by inducing single strand breaks that could possibly initiate double strand breaks, as result of the inactivation or alteration of repair mechanisms (Obe *et al*., 2002).

The observations of the present investigation are also supported by the results of our previous study on cytogenetic and tissue toxicity of synthetic sindoor, which revealed increased incidence of various chromosomal aberrations including fragments and acrocentric associations and also histopathological changes in *H. fossilis* at higher doses (Malla and Ganesh, 2009). The end point analysed in this paper shows that synthetic sindoor, being having heavy metal components, is a clastogen and produces stress in fishes at higher concentrations.

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