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Assessment of Genetic Biomarkers with Special Reference to Micronucleated and Binucleated Erythrocytes in Two Fish Species Grown at Industrial Vicinity of Thermal Power Plants, Kolkata, India

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Abstract: Assessment of biological marker at gene level in fish, micronucleus test as well as the study of abnormal shape of nucleus is a suitable measure, in which the presence or absence of genotoxins can be detected in water. In this study, frequencies of micronucleated (MN) and binucleated (BN) erythrocytes were scored in gill (peripheral) and kidney (renal) blood of two fishes, *Labeo bata* and *Oreochromis mossambica* inhabited in the pond located at industrial vicinity of thermal power plant, Kolkata, India. Two experimental sites were chosen, which were compared with pond located in non-industrial vicinity (far away from this area) as control area. Highly significant differences (P<0.001) were noticed for MN frequencies in gill and kidney erythrocytes of experimental fishes, where kidney erythrocytes showed increased value than gill erythrocytes. The comparison between gill and kidney erythrocytes also showed highly significant differences (P<0.001). The frequencies of BN were also counted separately for gill and kidney erythrocytes, in which significantly increased values (P<0.001) were obtained in comparison to control populations. These results confirmed that micronucleation and binucleation in erythrocytes of fish could provide valuable information regarding water quality status and also the health risk as biomarkers at genetic level of particular test models.

Key words: Biomarkers, cytogenotoxicity, nuclear abnormality, flyash, fish health.

Introduction

Modern civilization always creates industrialization to balance the economic condition of the country. But the by-products and waste products of the industries may cause adverse effects on the environment. The aquatic environment is the ultimate destination for almost all industrial wastes. Water quality has become seriously impacted by these waste products. Thermal power plant generates electricity, which helps to fulfill the need of industries as well as urban area. Flyash is a finely divided residue formed by the combustion of ground or powered coal. It is an important air pollutant containing many inorganic and organic chemicals such as nickel, copper, aluminium, benzene, PCBs etc. (Belpaeme et al., 1997) emitted from this industry and a tendency to deposit onto leaf surface, local water bodies etc.

The extent of impact of water quality can be determined through biological assessment in conjunction

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with chemical and physical analysis. Biomarkers are physiological changes in an organism, which indicate exposure and effect to a chemical or combination of chemicals. Measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) serve as indices for health and physiology related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiological studies, etc. (Perera, 1987). Depending on the specific characteristic, biomarkers can be used to identify the risk of developing an illness (antecedent biomarkers), aid in identifying disease (diagnostic biomarkers), or predict future disease course, including response to therapy (prognostic biomarkers). In genetic biomarkers assessment, some heritable changes occur in gene level, which indicate exposure to a genotoxic compound (Vigano et al., 2001). Genotoxicity is an event where the toxic element affects the genetic complements of organisms. In a single gene (DNA), a mutation, deletion, single nucleotide polymorphism (SNP) or some other feature provides predictive value.

Water quality means the current status or condition of the water in a specific aquatic ecosystem. Generally each water body contains some physical and chemical properties, such as dissolved oxygen content, chloride content, hardness, natural buffering capacity which allow the water to adopt and compensate for normal changes in the environment. Pollution occurs when conditions exceed the watershed's ability to compensate for the changes. Polluted water may be discoloured, possess a high coating on the bottom of the water body or may show no visible sign at all of pollution.

For in situ biomonitoring, the field study of an aquatic ecosystem is very important in which suitable biomarkers are to be known. Field study indicates the health quality of biota in aquatic ecosystem, which determines whether it is polluted or unpolluted. In a polluted aquatic ecosystem the sensitive and resistant species can be identified by field assessment (Sanchez-Galan et al., 1998; Rodriguez-Cea et al., 2003). When the number of resistant species increases and sensitive species decreases the health of the water body becomes precarious. Thus by field study, ecological risk assessment can be predicted because biomonitoring identifies ecological risks which are obvious threats of toxic pollution. Fishes are the most useful bioindicators and erythrocytes of fishes are potential biomarker for in situ monitoring of water quality of an aquatic ecosystem because of their high nutritive value (De Flora et al., 1993). Genotoxic effect in fish is a matter of great concern because of their potential risk on human health after consumption. Among all other tissues, blood is a suitable biomarker where damages can be detected at gene level due to direct contact with toxicants.

The micronucleation (MN) and binucleation (BN) test is one of the simplest, short-term tests for genetic biomonitoring in which the quality of water is known. This is a suitable and effective method to use in fishes because of its simplicity and easy scoring. Micronuclei are small, intracytoplasmic masses of chromatin resulting from chromosome breaks after clastogenic action or whole chromosomes that do not migrate during anaphase as a result of aneugenic effect (Manna and Sadhukhan, 1986; Heddle et al., 1991; Al-Sabti and Metcalfe, 1995; Ferraro et al., 2004, Cavas et al., 2005). In case of binucleation, it is observed that the cells divide abnormally due to blocking of cytokinesis (Cavas et al., 2005) which leads to genetic imbalance in the cells and may be involved in carcinogenesis (Rodilla, 1993). The MN and BN tests have been successfully used as a measure of genotoxic stress in fish, under both laboratory and field conditions.

In the present study, an attempt has been made to detect genetic biomarker in two fish species *Labeo bata* and *Oreochromis mossambica* by MN and BN in the gill and kidney erythrocytes. These fishes are grown in flyash depositing pond from thermal power plant emission at Titagarh Thermal Power Plant, Kolkata, India.

Materials and Methods

Study Area

The study area was selected in the industrial vicinity specially Thermal Power Plant at Titagarh. The total area covers 12500 ha and is located between 22°25′ and 22°40′ N and 88°20′ and 88°35′E. This power generating station was created to supply power in Northern region with a capacity of 240 MW.

The effluents after blowdown of this power plant is released into river Ganges, but the flyash emitted from the power plant deposits on the ground, leaves of plants and into the aquatic bodies (ponds) located near this industrial area. These water reservoirs are generally used for commercial pisciculture without drinking water supply and different types of fishes such as Indian major carps, minor carps, tilapia are generally cultivated. In this study area two major ponds were selected namely experimental (close to this industry) and the reference

site (control area far away from industry nearly 6 KM from the industry) at Titagarh area.

Sample Collection

The fish species, *Labeo bata* (12-17 cm in length and 18-25 gm in weight) and *Oreochromis mossambica* (13.5-15.5 cm in length and 62-65 gm in weight) were selected for the detection of genotoxic effect among other major cultivated fish species, which are regularly trapped by the local fish farmers for consumption. These minor carp and tilapia have major demand as edible fishes locally, so they are preferred as test species. These fishes were collected from the fish farmers. After collection from above mentioned sites as an experimental as well as control group the fishes were immediately brought to the laboratory for genotoxicity studies (Hose et al., 1987; Ayllon and Garcia-Vazquez, 2001). Ten fishes were used in each site from above mentioned area. The fishes were sacrificed for drawing blood from gill and kidney for slide preparation.

Slide Preparation and Staining for MN and BN Test

For each fish, two microscopic slides were prepared for each tissue. The clean slides were taken and from each fish gill and kidney blood were smeared onto the slide for experimental as well as control group with proper coding. The coded slides were air-dried for 12 h and then fixed in absolute methanol for 10 min. After fixing, the same slides were stained in aqueous Giemsa (5%) for 10 min (Palhares and Grisolia, 2002).

Slide Analysis

In each fish, 2000 erythrocytes were counted for gill and kidney blood separately from experimental and control group respectively. The frequencies of micronucleation (MN) and binucleation (BN) in erythrocytes were detected under binocular microscope (Olympus) using a 1000 × oil-immersion lens. Frequencies of MN and BN were expressed per 1000 cells.

Statistical 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.

Results

The results indicate that the frequencies of MN and BN are significantly (P<0.001) increased in both fish species collected from pond located at industrial vicinity when compared with the control groups of fishes (Figures 1 and 2).

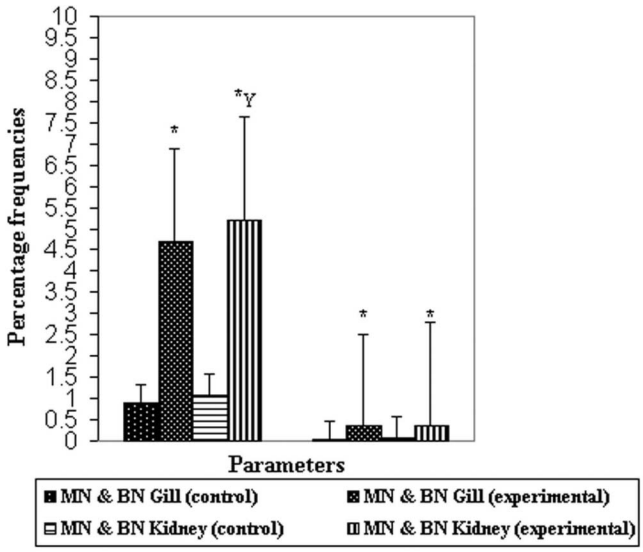

Figure 1: Micronuclie and binuclei frequencies in gill and kidney erythrocytes of fish *labeo bata* *P<0.001 (control and experimental comparison) YP<0.001 (gill and kidney comparison).

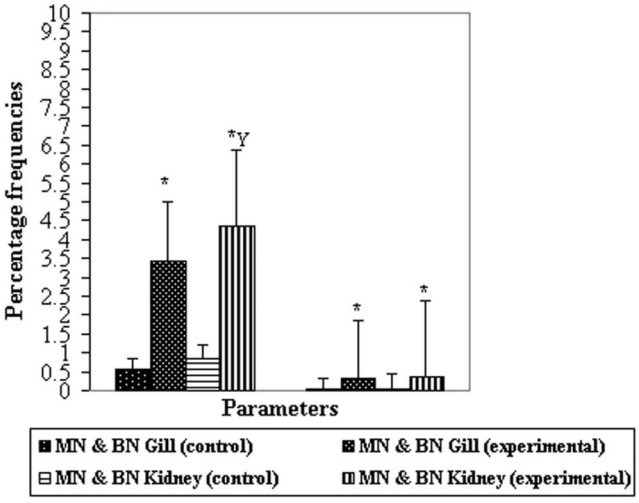

Figure 2: Micronuclei and binuclei frequencies in gill and kidney erythrocytes of fish *Oreochromis mosambica* *P<0.001 (control and experimental comparison) YP<0.001 (gill and kidney comparison).

As shown in Figure 1, significantly higher frequencies of MN and BN in the erythrocytes of *Labeo bata* grew in experimental pond when compared with control pond. The increased frequencies of MN in both gill and kidney erythrocytes are significant at P<0.001 level. Highly significant (P<0.001) frequencies are observed in case of binucleated cells (BN) in both gill and kidney erythrocytes. The MN frequencies are significantly

(P<0.001) increased in kidney erythrocytes when compared to gill erythrocytes but do not observe any significant differences for BN.

In case of *Oreochromis mossambica* the increased frequencies of MN in both gill and kidney erythrocytes are significant at P<0.001 level. Highly significant (P<0.001) frequencies are also observed in BN in both gill and kidney erythrocytes. All comparisons are made between the fishes of experimental and control groups. When compared with gill and kidney erythrocytes the micronuclei frequencies are significantly (P<0.001) increased in kidney erythrocytes but do not observe any significant differences for BN.

Discussion

The Piscine Micronucleus Test (PMT) is the best method for biomonitoring of water quality. The frequencies of micronuclei and binuclei were induced in fish when exposed to physical or chemical agents in in-situ as well as laboratory condition (Al-Sabti and Metcalfe, 1995; Minissi et al., 1996; Ayllon and Garcia-Vazquez, 2000, Cavas and Ergene-Gozukara, 2003; Cavas et al., 2005; Arkhipchuk and Garanko, 2005).

The frequencies of micronuclei and binuclei in the erythrocytes of fish from pond located at industrial vicinity were significantly (P<0.001) higher than that of the control groups. Several authors (Bahari et al., 1994; Campana et al., 1999; Anitha et al., 2000; Al-Sabti, 2000, Gustavino et al., 2001; Atteq et al., 2002; Abul Farah et al., 2003; Buschini et al., 2004; Takai et al., 2004; Porto et al., 2005) have suggested that some physical and chemical agents are responsible for causing genotoxic effects in fish. Genotoxicity study with special reference to micronucleus test in fish erythrocytes by different industrial pollutants have been investigated and bioindicators could be identified easily (Carrasco et al., 1990; Bombail et al., 2001; Cavas and Ergene-Gozukara, 2003).

It is very interesting to note that flyash contains various chemicals and other pollutant loads, which has toxic effect in the tissues of plants and animals (Patri and Naik, 1994; Barman et al., 1999; Dharmlata, 2002). Although not only toxicity, the present study shows genotoxicity in the gill and kidney erythrocytes of these fish species after chronic exposure to pond water containing flyash. In this respect flyash containing genotoxins induced genotoxicity in root meristem cells of *Vicia faba* (Jain et al., 2004). The present result clearly supported that two fish species responded with increased micronuclei frequencies at significant level (P<0.001). This finding has also an evidence that frequencies of micronuclei were higher in kidney erythrocytes than gill erythrocytes in both fish species (Manna and Sadhukan, 1986). *L. bata* showed higher frequencies of MN and BN in gill and kidney erythrocytes compared to *O. mossambicus*. In this study the first species is more sensitive than second species; some similar results have been documented by other authors (Hayashi et al., 1998; Ayllon and Garcia-Vazquez, 2000; Takai et al., 2004) when sentinel comparisons have been made of different species to a common genotoxic agent.

The induction of binucleation (P<0.001) is also observed in both the fishes at field condition. Many laboratory tests have documented binucleated cell in fish after exposure with genotoxic agents and this binucleation is a nuclear abnormality (Hooftman and De Raat, 1982; Cavas et al., 2005; Arkhipchuk and Garanko, 2005) but field study is rare.

In conclusion, the result is clearly indicating the genotoxic effects in both the fishes *Labeo bata* and *Oreochromis mossambica* grown in ponds at thermal power industrial vicinity. The first fish species is showing more sensitive indicator than the second one in respect to their short life cycle. MN and BN tests are confirming that these fish species can be used in genetic biomonitoring for detection of water quality status and erythrocytes are a suitable biomarker for these flyash-depositing fishponds. This is a first time observation with these test species but further research is needed particularly in these areas with other cultivable fish species.

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