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Research Article

Monitoring seasonal changes in micronuclei in *Catla catla* from a natural habitat

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ABSTRACT

Cytogenetic biomarkers play a key role in assessing the impact of pollutants in apparently healthy sentinel aquatic organisms such as fishes. The study of erythrocyte micronuclei is an important biomarker. The frequency of micronuclei was assessed in peripheral blood cells of *Catla catla*, collected from a natural water body during different seasons. The results showed that there was a seasonal variation in frequency of micronuclei in *Catla catla*. Micronuclei frequency is a biological indicator which reflects the extent of genotoxicity in fish population and thus helps in stock assessment. It was highest during spring season and nil in monsoon season.

INTRODUCTION

The surface and subsurface resources of water are contaminated by industrial effluents, agricultural runoff and municipal wastewaters which contain mixture of various known as well as unknown substances. Compounds present in polluted water are main cause of biological alterations that can affect particular population and entire aquatic ecosystem (Mekonnen and Hoeskstra, 2018). As aquatic animals, freshwater fishes are most susceptible to genotoxic effects caused by pollutants. Therefore, they present a sensitive model to monitor the genotoxic effects. Apart from adverse effects on fish germplasm resources, these pollutants may indirectly risk human health through fish consumption. Fish play a major role in energy-flow chain in aquatic ecosystems as well as constitute an important part of human diet in many populations all over India. Moreover, fishes play a number of roles in trophic web. But they are continuously exposed to contaminants in their natural habitats. As a result, they bio-accumulate toxic substances and generally seen to respond to low concentrations of mutagens. Therefore, fishes are often used as sentinels for monitoring genotoxicity in the environment (Al-Sabti and Metcalfe, 1995; Cavas and Konen, 2007).

The genotoxicity bioassays are diverse in nature and their utility has been assessed before by several scientists worldwide (Rajguru *et al.*, 2003; Bolognesi *et al.*, 2006; Anbumani and Mohankumar, 2011). Among many such *in situ* tests, the micronuclei test has been widely applied because of its reliability, sensitivity, and simplicity. Micronuclei test is also relatively independent of karyotypic characteristics of the organism (Heddle *et al.*, 1991). In fish erythrocytes, it seems to be a promising test for environmental mutagenesis investigations. The micronuclei survey has been demonstrated to be a sensitive bioassay for detecting mutagenic pollution in freshwater environments (Utani *et al.*, 2010).

Various studies on experimental induction of micronuclei in fishes which focused on different aquatic pollutants like agricultural pesticides, chemicals and heavy metals in fishes (Polard *et al.*, 2011) have shown micronuclei test to be useful indicator of genotoxicity in fishes. However, there is still a paucity of information on micronucleus test in fishes from natural habitats, especially on the influence of some factors like season, water availability in ponds, rivers and canals on occurrence of micronuclei in fishes. Such information would be valuable in determining the level of aquatic pollution and for planning remedial measures to protect fish germplasm from further deterioration and depletion. The present investigation was undertaken to study occurrence of micronuclei in peripheral blood cells of an important South Asian fish species *C. catla* collected from its natural habitat during different seasons.

MATERIALS AND METHODS

Freshwater fish *Catla catla* (Hamilton, 1822; Family: *Cyprinidae*) was chosen for study, as it is commonly available throughout the year. *C. catla* specimens were procured from water canals in Etawah agricultural fields

during August, October, January and March months of 2017-18; which corresponds to monsoon, autumn, winter and spring respectively. The micronuclei (MN) test was performed according to published protocols (Al-Sabti and Metcalfe, 1995; Bolognesi *et al.*, 2006). In each season, peripheral blood samples were collected individually from caudal veins of six specimens on the same day. Blood smears were made onto grease-free pre-cleaned slides; the samples were fixed in ethanol for 10 min. After fixation slides were allowed to air-dry and stained for 15 min with May-Grunnwald (Merck, India) followed by 10% Giemsa for 30 min. The slides were then rinsed with distilled water, air dried and examined using optical microscopy under 100x objective for the presence of micronuclei. Micro-nucleated red blood cells (RBCs) were scored among 2000-2500 RBCs per specimen and frequency of micronuclei was calculated. Frequency (mean ± SD) of micronuclei observed during four seasons was compared by independent sample t-test assuming equal variance (Pagano and Gauvreau, 2000).

RESULTS AND DISCUSSION

The frequency of micronuclei for four seasons observed in peripheral blood of *C. catla* is summarized in Table-1 below.

Table 1: Frequency of micronuclei in peripheral blood of *C. catla*

Month (Season)	RBCs per Specimen	MN Frequency (Mean ±SD)
August (Monsoon)	2317	0.00
October (Autumn)	2403	0.18 ±0.08
January (Winter)	2089	1.02 ±0.13*
March (Spring)	2195	2.21 ±0.7*

***** Significant at $p \leq 0.05$

The specimens collected in monsoon season, micronuclei were not observed in peripheral blood cells. The frequency of micronuclei in post-monsoon collection was found to range from 0.18 to 2.21. In specimen collected during autumn, *i.e.*, in the month of October the frequency of micronuclei was 0.18 ±0.08, which was statistically insignificant. The frequency of micronuclei observed in January (winter) was 1.02 ±0.13, which is significantly higher ($p \leq 0.05$) than those in August and October. The maximum frequency of micronuclei (2.21 ±0.7) was observed during March (spring) collection, which was significantly different ($p \leq 0.05$) from other three collections (Fig. 1).

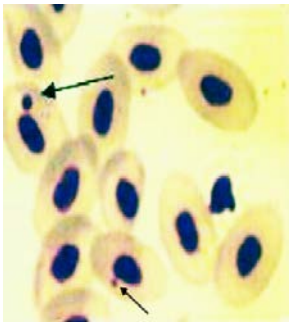

Fig.1: The micronuclei (*marked by arrows*) formed as a result of mutagenesis in peripheral blood cells of *C. catla.*

Micronucleus is a sign of chromosomal instability which indicate genomic damage events that can increase the risk of developmental and degenerative diseases in fishes. Micronucleus is thought to form as a result of misrepair of DNA double-strand breaks leading to symmetrical and asymmetrical chromatid and chromosome exchanges or fragments that fail to be included in the daughter nuclei at the completion of telophase during mitosis. This occurs due to lack of spindle attachment during the segregation process in anaphase (Fenech, M., 2011). Our results demonstrated an increase in the frequency of micronuclei (Table 1) with respect to the change in season from monsoon to spring through autumn and winter. The seasonal variations in the frequency of micronuclei might be due to variation of pollutant concentration in the freshwater channels attributable to the availability of water. Due to heavy influx of rain-water in monsoon season and inundated water canals result in lower concentration of pollutants. This is reflected by absence of micronuclei in *C. catla* as observed in the present study. However, during post-monsoon period, water level in inland water bodies gradually recedes resulting in proportionate increase in pollutant concentration and hence the higher micronuclei frequency.

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