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# Evaluation of Genotoxicity in Juveniles and Adults Fish Labeo bata Inhabiting Sewage Fed Fishponds **During Pre-Monsoon**

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Abstract: The objective was to detect genotoxic risk in the peripheral erythrocytes of fish specimens (Labeo bata) of juveniles and adults inhabiting sewage-fed waterbodies during pre-monsoon. The study was conducted in a sewage-fed fishponds inhabiting fish specimens (Labeo bata) during pre-monsoon season at Kolkata. For genotoxicity studies in peripheral erythrocytes of fishes of two age groups viz. juvenile and adults (5 nos. of fishes in each group). The micronucleus (MN) and nuclear abnormalities (NA) were detected as per standard protocol. In the juveniles, the significantly (P<0.001 and P<0.01) increased frequency (%) of MN (0.69  $\pm$  0.06), BN (0.65  $\pm$  0.05), NN  $(0.55 \pm 0.05)$  and RN  $(0.79 \pm 0.01)$  when compared to adults  $(0.47 \pm 0.04, 0.46 \pm 0.05, 0.40 \pm 0.07)$  and  $0.51 \pm 0.04$ . It is concluded that there was a tendency of genotoxic risk in the juveniles of L. bata compared to adult's specimens but not alarming. The cause for genotoxicity is unexplored. It is suggested that regular monitoring of genotoxins in the waterbodies of fishponds.

Keywords: Genotoxicity, Micronucleation, Nuclear abnormalities, Fish species, Labeo bata

#### 1.Introduction

To date, the pollution of heavy metals and organic compounds [1,2,3] and microplastic pollution in water is a matter of great concern, which are causative agents for genotoxic risk aquatic organisms, also fish species. [3-7]

Moreover, these in combinations or individually pose genotoxic impact on fish. [2,3,8] In other words, genotoxicity revealed the induction of damage to DNA strand following cancer via various modes of actions.<sup>[9,10]</sup> Heavy metals are common genotoxic agents that may lead to single- or double-strand (SSB/DSB) DNA breaks causing DNA damage.[11] These also induce the creation of reactive oxygen species (ROS) posing oxidative stress, make several cellular changes, which did not help the repair mechanisms, and alter the recompense of DSB. These induce the "false" repairing of DSBs, proliferate DNA mutations and increase the risk of carcinogenesis.<sup>[12]</sup>

An easy screening to detect DNA damage in cells, the testing of micronuclei (MN) and different nuclear abnormalities (NA) in fish cells is a potential tool to know the presence of genotoxic agents in waterbodies.<sup>[13]</sup> Many studies indicated the induction of MN and NA in the peripheral erythrocytes of different fish species inhabiting waterbodies. [14-16]

This study was attempted to detect genotoxic risk in the peripheral erythrocytes of fish specimens (Labeo bata) of juveniles and adults inhabiting sewage-fed waterbodies during pre-monsoon.

# 2. Materials and Methods

The study was conducted in a sewage-fed fishponds inhabiting fish specimens (Labeo bata) during premonsoon season at Kolkata.

For genotoxicity studies in peripheral erythrocytes of fishes of two age groups viz. juvenile and adults (5 nos. of fishes in each group). The smear was prepared after drawing blood immediately from heart of just died fish specimens. For each fish, two microscopic slides were prepared. The clean slides were used with proper coding. The coded slides were air-dried for 12 hrs and then fixed in absolute methanol for 10 min. After fixing the same slides were stained in Giemsa stain for 10 min. as per earlier study.[17]

In this genotoxicity experiment, micronucleation (MN) and nuclear abnormalities (NAs) in slides identified under brightfield microscope (400x). The frequency (%) micronuclei (MN) and NAs such as Binuclei (BN), Notch nucleus (NN) and Retracted nucleus (RN) was separately evaluated for juveniles and adults.<sup>[14]</sup> A comparative analysis was performed through student 't' test between juveniles and adults to determine significant (P<0.05) induction by using PAST tool (version 3.26).<sup>[15]</sup>

#### 3.Results

A comparative analysis was performed for the frequency (%) induction of MN and NA in the peripheral erythrocytes of juveniles and adults fish (L. bata) (Fig 1). In the juveniles, the significantly (P<0.001 and P<0.01) increased frequency (%) of MN (0.69  $\pm$  0.06), BN (0.65  $\pm$ 0.05), NN (0.55  $\pm$  0.05) and RN (0.79  $\pm$  0.01) when

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compared to adults  $(0.47 \pm 0.04, 0.46 \pm 0.05, 0.40 \pm 0.07)$ and  $0.51 \pm 0.04$ ). Different nuclear abnormalities are exhibited in Fig 2.

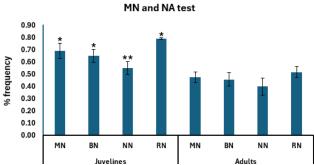


Fig 1: MN and NA test in two age group of fish (n = 5) in each group; \*P<0.001; \*\*P<0.01)

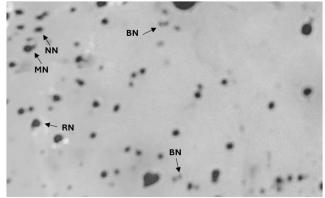


Fig 2: Pictorial representation of different nuclear abnormalities

#### 4.Discussion

It was an established fact that the frequency (%) induction value greater than 1 generally indicated a higher genotoxic risk, as it enhances an increase in chromosomal damage in the pollutant exposure group in comparison with the control group.

Moreover, the juveniles are affected by genotoxins compared to the adults, which is supported by earlier study. [19] But the frequency was less than 1, which means less genotoxic risk in the studied specimens.

#### 5. Conclusion

It is concluded that there was a tendency of genotoxic risk in the juveniles of L. bata compared to adults specimens but not alarming. The cause for genotoxicity is unexplored. It is suggested that regular monitoring of genotoxins in the waterbodies of fishponds.

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#### **Conflict of interest**

Authors declare no conflict of interest in the present study.

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