Biotechnol Res 2016; Vol 2(2):77-80
eISSN 2395-6763

ORIGINAL RESEARCH

Determination of genotoxic effect of azo dye C.I. RR 120 on fish *Catla catla*

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• Received: 22 February 2016 • Revised: 28 March 2016 • Accepted: 02 April 2016 • Published: 05 April 2016 •

ABSTRACT
Micronuclei (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water pollutants. The aim of this study was to determine the genotoxic potential of environmentally relevant concentrations of C.I. Reactive Red 120 (RR 120) azo dye on *Catla catla*, important edible freshwater fingerlings, using DNA damage in gill cells and haemocytes as sensitive biomarkers. It was also about to determine the dose and time dependent induction of frequencies of micronuclei in different tissues examined. For this, fingerlings were exposed to various concentrations of C.I. RR 120 azo dye (10, 20, 30 and 40 ppm). Samples (gills and peripheral blood) were collected and analyzed to check the effect of textile azo dye C.I. RR 120 result suggest that frequency of micronuclei at exposed fingerlings was higher compared with control group. A comparison between micronuclei frequencies in gills cells and haemocytes showed that the highest micronucleus frequency detect in gills cells followed by haemocytes cells. Induction of micronuclei was dose dependent and also time dependent.Results from this study recommend the use of the micronucleus test in fish tissues as a sensitive genotoxic monitor for aquatic pollution. It also enlightens the genotoxic effects of azo dye on various targeted samples by MN test.

KEY WORDS: *azo dye, Catla catla, Genotoxicity, Micronuclei test, RR 120*

Introduction

Water is the most vital environmental factor on which every living creature is directly or indirectly depends for their day to day survival. Like, all living organisms use water in their daily routine, the people uses water for agricultural purpose and some living organisms use water as a habitat. Nowadays, the urbanization and industrialization developed more rapidly in our country and are also water dependent. Some of them are textile industries, paper & pulp industries, leather & tanning industries, fertilizer & pesticide industries, etc. Maximum water is consumed and wasted by these industries, when they discharged their effluent in near water body. Some common water pollutants are dyestuff, bleaching agents, heavy metals, surfactants, acids and alkaline, various salt fertilizers & pesticides and other solid wastages present in their effluents (Soatar *et al*. 2005, Vanhulle *et al*. 2008, Florina *et al*. 2015).

The Textile industry in India is concentrated in the state of Gujarat, especially around the city of Surat, which hosts more number of plant and accounts for India's exports. Surat is the famous as textile hub in our country. The textile industry is the largest consumer of azo dyes (Jagvani *et al*. 2013). Azo dyes are the largest and most versatile class of dyes because of their pros like low cost, good fastness, ease of application, wide ranges of colors (Puvanesvari *et al.*2006). But these azo dye have some anesthetic cons also, visible at low concentration, non-biodegradable, carcinogenic and mutagenic. Approximately 10-15% dyes are released into the environment during dyeing process, making the effluent highly coloured and aesthetically unpleasant (Weber

& Adams, 1995, Ratna & Padhi, 2012). The effluent from textile industries thus carries a large number of dyes and other additives (Wang et. al., 2002). Water pollution adversely affects the water quality and it disturbs the balance of aquatic ecosystems. Moreover it poses toxicity i.e. lethal effect, genotoxicity, mutagenicity and carcinogenicity to aquatic organisms as well as animals (Gunasekaran *et al*, 2006, Jagvani *et al*. 2013).

Fishes provide a suitable model for monitoring aquatic genotoxicity and waste water quality because of their ability to metabolize xenobiotic and accumulate pollutants (Grisolia and Corderio, 2000, Nuzhat and shadab, 2011). Fish selected as an experimental model because they are large aquatic population and known model for the aquatic toxicology. Genotoxicity studies using cytogenetic analyses as micronuclei test detecting nuclear abnormalities are the most widely applied methods due to its proven suitability for fish species. Micronuclei assay detects both clastogenic and aneugenic effects and therefore can detect the genotoxicity of a wide range of compounds. Nuclear abnormalities, such as micronuclei, and other nuclear malformations as fragmented apoptotic cells binucleated cells and sticky adherent cells are considered as good indicators of cytotoxicity and genotoxicity, respectively (Shreedevi and chitra, 2014).

The Indian major carp, *Catla catla* is highly sensitive, one of the popular edible fish, fastest growth rate and preferred to culture throughout a year. Hence, the present study was undertaken to evaluate toxicity of RR 120 on Indian major carp *Catla catla* using micronuclei (MN).

Materials and methods

Test Chemical

The dye used in the present study, Azo dye Reactive red 120 [$C_{44}H_{24}Cl_2N_{14}Na_6O_{20}S_6$] (C.I. 25810) was obtained from local source and directly used for experimental purpose without further purification. Stock solution was prepared by dissolving accurately weighed dye in distilled water to the concentration of 50 mg/ml and **s**tored at 4°C. Desired concentrations were obtained by diluting the dye stock solutions in accurate proportions to different initial concentrations.

Experimental design

Catla catla fingerlings were procured from local fish farm at Sivan village, Gujarat. Fingerlings were disinfected by 0.02% $KMnO_4$ and acclimatized in laboratory condition for 15 days in 100 L aquaria (APHA).

Acclimatized fingerlings were grouped into 5 groups (10 in each) and exposed to various concentrations (10, 20, 30 and 40 ppm) of C.I. RR120 azo dye for 4 days (96 hrs). One was kept as a control group. Samples (gills and peripheral blood) were collected at 4 days (96 hrs.) and were analyzed for MN. The changes were compared with those of the control.

Gills were dissected out and homogenized in hypotonic KCl and fixed with carnoy's fixative than centrifuged the material with three fixative washes. Prepared slide by air dry preparation. Peripheral blood samples were collected directly from caudal vein of each fingerling (control and exposed). Smears were prepared and air dried for 5 min. Both gill cells air dried smears and blood smears were fixed and stained by 10% Geimsa stain.

The number of cells having micronuclei out of the 1000 cells examined was counted for each specimen. Micronuclei frequency was calculated using the following formula:

FMN=MN/N

Where, FMN represents the frequency of the micronuclei and MN/N is the ratio between the number of micronuclei and the number of nuclei for each specimen examined.

Results

The frequency of micronuclei (per individuals) in peripheral erythrocyte and gill cells in fingerlings species *Catla catla* exposed for 4 days, are summarised in table 1.

Table 1: Frequency of micronuclei (MN) in 1000 Haemocytes of peripheral blood and Gill cells of Catla catla after 4 days of exposure to dye C.I. RR 120

Concentration of C.I. RR 120 (mg/L)	Frequency of micronuclei (MN) in haemocytes (%)	Frequency of micronuclei (MN) in Gill cells (%)
Control group	0.2	0.2
10ppm	0.9	1.2
20ppm	1.3	1.5
30ppm	1.7	2.1
40ppm	1.9	2.3

Result show that more number of micronuclei was seen in

the gill cells compared to the peripheral erythrocyte (Fig.1). Figure 2 shows that the frequency of micronuclei in peripheral erythrocyte is comparatively higher than controlled one. Whereas, gill cells showed significantly higher induction of micronuclei as compared to blood cells as well as control (Fig. 3). All these changes observed in dye exposed fingerlings were increase proportionally with concentration of dye.

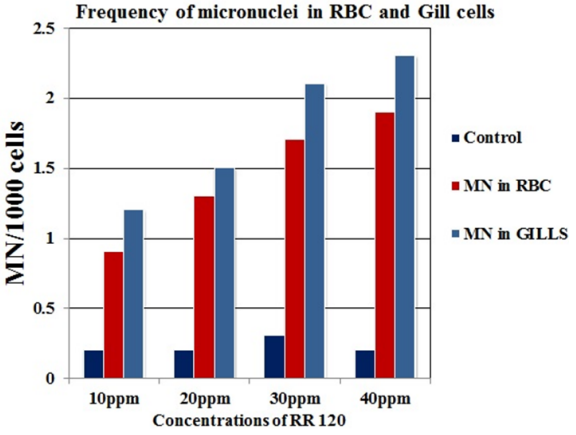

Figure 1: It represents the relative increase of MN in haemocytes and gill cells of fingerlings exposed to various concentrations of RR 120 for 4 days with respect to control.

Discussion

Fish and aquatic invertebrates have been considered to be efficient and cost effective model systems to study the toxic, mutagenic, and carcinogenic potential of pollutants (Braunbeck *et al.* 2005) due to their ability to metabolize, concentrate, and store water-borne pollutants (Osman *et al.* 2007).

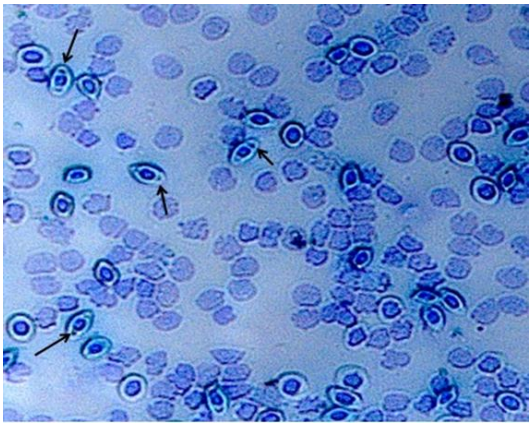

Figure 2: Arrow represents the relative increase of MN in erythrocytes of fingerlings exposed to highest concentration of RR 120 with respect to control.

All test concentrations of azo dye C.I. RR 120 used in the present study induced a significantly higher number of MN compared to the control. Both concentration and time-dependent increase in MN induction have been reported due to chemical exposure in fish (Bahari *et al.* 1994). Further, the MN induction has also been reported earlier due to dye exposure in *prussian carp* (Al-sabti. 2000). Although, the MN test has been found to be a sensitive assay to evaluate genotoxic compounds in fish under controlled conditions as an index of cumulative exposure (Bolognesi *et al.* 2006), it might suffer variations according to clastogen, test organism, and the life cycle of the cells (Grisolia and Cordeiro 2000). Further, as the pre-existing mature (and non-dividing) erythrocytes would predominate in the blood, the detection of induced MN in mature blood cells will be at a low frequency in the lower concentration.

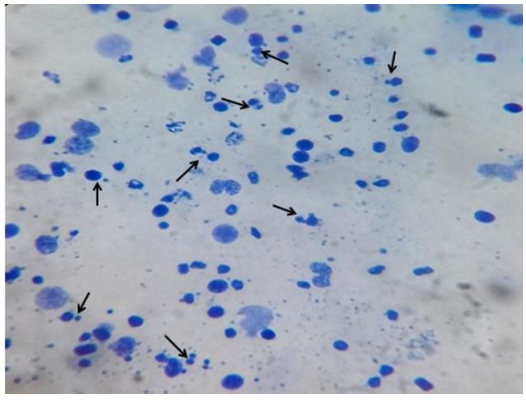

Figure 3: Arrow represents the relative increase of MN in gill cells of fingerlings exposed to highest concentrations of RR 120 with respect to control.

Micronuclei are results from acentric chromosome fragments or chromosomes that delay, in relation to others in their migration to the poles of the cell in anaphase. Micronuclei can also be formed by apoptosis, inactivation of the spindle formation and chromosome damage beyond action of physical agents (Heddle *et al.* 1991 and Al-Sabti and Metcalfe 1995). Studies by Ayllon and Garcia-Vasquez (2000) and Kirschbaum *et al.* (2009) showed this correlation indicating that nuclear abnormalities could be primary responses, i.e., prior the formation of micronuclei.

The observation that C.I. RR 120 caused greater increase in micronuclei frequencies in gills cells than peripheral erythrocyte is most likely due to the fact that the gills were in direct contact with the test chemical in solution. This work

has been also supported by our previous study (Barot, 2015). The present investigation gets confirmation from the results obtained by Dubey and Tripathi (Dubey and Tripathi, 2014) when they exposed fish *channa punctatus* to cadmium chloride.

Conclusion

Using the micronucleus test, the overall results of the present study shows that C.I. RR 120 textile azo dye is genotoxic because it induced cytotoxicity and micronuclei formation in erythrocytes and Gill cells of *catla catla.* Therefore, it should be always kept in mind that excess discharge of textile dyes into the water ecosystem proved to be very dangerous for the fish and other aquatic organism. These works suggest the researchers to develop new, effective and eco-friendly dye removal techniques.

Acknowledgment

Jagruti Barot thanks to University Grant Commission, India for financial support.

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