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RESEARCH ARTICLE

GENOTOXICITY IN A FRESHWATER FISH, *CIRRHINUS MRIGALA* EXPOSED TO DYEING INDUSTRY EFFLUENT BY USING MICRONUCLEUS TEST

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ABSTRACT

The micronucleus test has been used to detect the genotoxic effect of mutagens in environment. Genotoxic effects of dyeing industry effluent have been evaluated by using piscine micronucleus test. In the present study, 96h LC_{50} of dyeing industry effluent against *Cirrhinus mrigala* came out to be 48.97%. Three sublethal concentrations (24.48%, 12.24% and 6.12%) were made and fishes were exposed to these concentrations. Blood from anterior kidney was used to study micronuclei in erythrocytes of fishes after 24h, 48h, 72h and 96h. There were significant differences ($p<0.05$) between the control and treated fishes. The results showed that at higher concentration (24.48%) and exposure time (96h), there was maximum number of micronuclei in erythrocytes as compared to other concentrations at various time durations as well as in control.

INTRODUCTION

Pollution of water resources is a serious and growing problem. Despite the existence of relevant legislation, the pollution of the aquatic environment by toxic pollutants continues to occur. Domestic and industrial effluents are the main sources responsible for the contamination of aquatic environments (Claxton *et al*., 1998). Toxic pollutants such as industrial effluents released from point and nonpoint sources have severe impact on health and ecological integrity of the aquatic ecosystem. Dyeing industry is one of the toxic effluent producing industry which poses severe damage to the aquatic environment as it produces large volume of wastewater containing complex compounds. This effluent contains genotoxic pollutants which may lead to the formation of micronuclei in aquatic organisms. At present very few studies are available regarding the genotoxicity induced by effluents in aquatic organisms.

Aquatic organisms such as fish accumulate and store pollutants directly from contaminated water or indirectly through the ingestion of contaminated aquatic organisms. Fish is a very sensitive bio-indicator of water quality as it can respond to mutagens at low concentrations and can highlight the potential danger of the chemicals introduced in the aquatic environment. It responds to toxicant in a manner similar to higher vertebrates. In addition, it is considered to be more sensitive for the induction of genetic damage as compared to mammalian cells. A variety of teleost fish, including larvae, have been used for the study of the mutagenic, clastogenic and teratogenic effects of environmental contaminants. Metcalfe used fishes *Umbra limi* and *Ictalurus nebulosus* as test organisms for the study of micronuclei and nuclear abnormalities in erythrocytes. The *in vivo* techniques such as the micronucleus test performed in fish have been shown to be efficient not only for assessing genotoxic potential but also for water quality monitoring (Al-Sabti and Metcalfe, 1995).The study of micronuclei is a rapid, reliable and easy to conduct technique for the assessment of genotoxicity. The micronuclei are chromosomal fragments formed due to the effect of clastogenic agents. These fragments of chromosomes or whole chromosome that lag behind during anaphase stage of cell division due to either loss of centromere or any abnormality in cytokinesis forms micronucleus. Several studies have shown a high incidence of micronuclei in fish peripheral erythrocytes after exposure to different pollutants under both field and laboratory conditions (Al-Sabti, 1986). This technique has also been employed in the determination of the genotoxic potential of contaminated water resources such as rivers and lakes. For the present study, *Cirrhinus mrigala*, commonly known as mrigal, has been selected as a test model as it is found abundantly in the rivers of Punjab, easily available and has great consumer preference amongst carps. Dyeing industry is one of the red industries causing severe damage to the aquatic environment. So, dyeing industry effluent has been selected for the study. Dyeing industry effluent contains mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease (EPR, 2010). These toxic substances have clastogenic effect on fish chromosomes leading to chromosomal loss. Genotoxic studies with native fish species represent an important effort in determining the potential effects of toxic agents. This study was carried out to evaluate genotoxicity caused by dyeing industry effluent in fish by using micronucleus test (MNT). The main objective of the present study was to assess genotoxicity of dyeing industry effluent by application of micronucleus test on fish, *Cirrhinus mrigala.*

MATERIAL AND METHODS

Specimens of *Cirrhinus mrigala* measuring 6-8 cm in length and 30 – 55 g in weight were collected from government fish seed farm, Patiala and brought to the laboratory in wide mouthed

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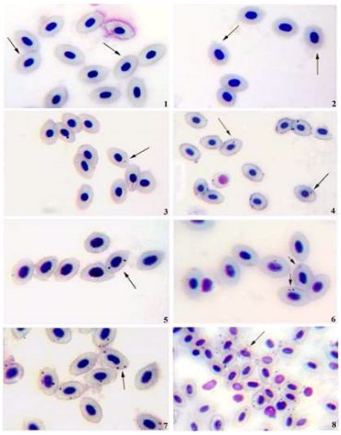
plastic bags containing fresh water and oxygen. Fishes were treated with 0.1% $KMnO_4$ solution for 30 minutes to remove any external infections and were acclimatized in laboratory for 20 days. Fishes were fed with pelted feed. Feeding was stopped 24h prior to commencement of genotoxicity tests and fishes were not fed during experimental periods.Effluent of dyeing industry was taken directly from the waste outlet of an industrial unit based in Ludhiana to conduct genotoxicity test against the fish. 96h LC_{50} was determined by the method suggested by Finney (1971). Acclimatized and apparently healthy, uninjured and uninfected fish specimens were taken. 10 fishes (per concentration) were exposed to 50L of normal water (control) and five concentrations (20%, 30%, 40%, 50% and 60%) of dyeing industry effluent. Mortality of fishes was recorded for 96h. The values of mortality were converted into Probit value and concentrations into log values to determine LC_{50} value. This experiment was performed in triplicate. The 96h LC_{50} value of dyeing industry effluent against *Cirrhinus mrigala* came out to be 48.97%.

For treatment, three sublethal concentrations of the dyeing industry effluent were tested which included 1/8, 1/4 and 1/2 of the 96h LC_{50} value (6.12%, 12.24% and 24.48% respectively). In one set of experiment, 40 fish specimens were used, out of which, 10 specimens maintained in well aerated water constituted the control group and 30 fish specimens exposed to 50L of 6.12%, 12.24% and 24.48% (10 in each concentration) of the effluent constituted the treated groups. This set of experiment was repeated thrice. Fishes were not fed during the experiment. Slides of blood smear were prepared from the control and treated fishes after exposure periods of 24h, 48h, 72h and 96h by using the method given by Ayllon *et al.* (2000). Slides were stained with Giemsa by using method given by Tijo and Whang (1965). For each concentration, 4000 cells were analysed after 24h, 48h, 72h and 96h of exposure each. Cells showing the presence of micronuclei were enumerated. Data of micronucleus test was expressed as Mean (%) ± S.E. To calculate S.E. (at $p<0.05$), ANOVA and post Tukey test were applied using computer software Graphpad prism.

RESULTS

In *Cirrhinus mrigala*, a normal erythrocyte is elliptical in shape with centrally placed nucleus in the clear cytoplasm. Such normal erythrocytes were observed in the blood taken from kidney of fishes in control (Figs. 1, 2). Presence of one (Figs. 3, 4), two (Figs. 5, 6) and three to five (Figs. 7, 8) micronuclei were observed in erythrocytes of treated fishes. The data pertaining to frequencies of micronuclei in erythrocytes is presented in the Table 1. In control fishes, out of 16000 studied cells, 137 cells showed MN. In treated groups, as the concentration increased, number of cells with MN also increased. In 6.12%, 501 cells were found having MN. Number of cells with one MN was high (491) while cells with two MN and three to five MN were observed only occasionally. As the concentration increased to 12.24%, total number of cells with MN increased to 643. Number of cells with one MN increased (637) while cells with two MN were very less (6). No cell with three to five MN was observed. At 24.48% concentration, total number of cells with MN further increased to 722. Number of cells with one MN decreased (400) but number of cells with two MN increased considerably (322).



Figs. 1 and 2: Normal erythrocytes
Figs. 3 and 4: Erythrocytes with One micronucleus
Figs. 5 and 6: Erythrocytes with two micronuclei
Figs.7 and 8: Erythrocytes with three to five micronuclei

However, cells with three to five MN were not observed. Significant differences between the control and treated groups were observed at different exposure periods. In control group, mean (%) ± SE recorded at 24h, 48h, 72h and 96h were 1.00±0.33, 0.87±0.33, 0.80±0.33 and 0.75±0.57 respectively. At 6.12% concentration, the Mean(%)±SE values of cells with micronuclei were 3.97 ± 0.57^{a}, 3.92 ± 0.66^{b}, 2.87 ± 0.33^{c} and 1.75 ± 0.33^{d} after exposure of 24h, 48h, 72h and 96h respectively. At 12.24% concentration, Mean(%)±SE values were 5.00 ± 0.66^{a}, 4.52 ± 0.66^{b}, 4.05 ± 0.33^{c} and 2.50 ± 0.33^{d} after 24h, 48h, 72h and 96h respectively. At 24.48% concentration, Mean (%)±SE values 3.27 ± 0.66^{a}, 4.62 ± 0.33^{b}, 4.87 ± 0.57^{c} and 5.27 ± 0.33^{d} after 24h, 48h, 72h and 96h respectively. The data indicates that with 6.12% and 12.24% concentrations, total number of cells with MN decreased from 24h to 96h. On the other hand, with 24.48%, total number of cells with MN increased from 24h to 96h.

DISCUSSION

The present study is an attempt to assess the genotoxicity induced in fish exposed to sublethal concentrations of dyeing industry effluent. In control, a few cells with one MN were observed (mean % ranging from 0.75 to 1.00) but cells with two or more MN were altogether absent. The data on baseline MN frequencies shows a large inter-species variability, ranging from 0 to 13 per 1000 cells (% MN of 0 to 1.3) and the variability has been related to interspecies differences in metabolic competency and DNA repair mechanisms as well as cell proliferation in the target organ affecting the MN expression (Bolognesi and Hayashi, 2011). The % MN values in the present study are on the higher side but still lie within the range. In treated groups, cells with one, two and three MN were observed. In 6.12% and 12.24% concentrations, there was decrease in frequency of cells with MN from 24h to 96h, whereas in 12.24%, there was increase in frequency of cells with MN from 24h to 96h. Several toxicants have been seen to cause micronuclei formation in fish. Al-Sabti and Hardig (1990) observed micronuclei in perch found in Baltic Sea contaminated with pulp wastewater, while Poongothai *et al.* (1996) reported micronuclei in five different fish species collected from sewage polluted water. An increase in the frequencies of cells with MN was found in *Salmo trutta* in downstream region of River Trubia where heavy metal pollutants are added in high concentration from an old military factory (Ayllon *et al*, 2000).

Table 1 Frequencies of micronuclei in erythrocytes of *Cirrhinus mrigala.*

Experimental groups	Duration of exposure (h)	T	Number of cells with micronuclei			t	Mean (%) ± S.E
			1	2	3-5		
Control	24	4000	40	0	0	40	1.00±0.33
	48	4000	35	0	0	35	0.87±0.33
	72	4000	32	0	0	32	0.80±0.33
	96	4000	30	0	0	30	0.75±0.57
Total			137	0	0		
Treated							
6.12%	24	4000	157	2	0	159	3.97±0.57[a]
	48	4000	149	6	2	157	3.92±0.66[b]
	72	4000	115	0	0	115	2.87±0.33[c]
	96	4000	70	0	0	70	1.75±0.33[d]
Total			491	8	2	501	
12.24%	24	4000	195	5	0	200	5.00±0.66[a]
	48	4000	180	1	0	181	4.52±0.66[b]
	72	4000	162	0	0	162	4.05±0.33[c]
	96	4000	100	0	0	100	2.50±0.33[d]
Total			637	6	0	643	
24.48%	24	4000	24	107	0	131	3.27±0.66[a]
	48	4000	120	65	0	185	4.62±0.33[b]
	72	4000	140	55	0	195	4.87±0.57[c]
	96	4000	116	95	0	211	5.27±0.33[d]
Total			400	322	0	722	

a, b, c and d: Significant differences from the control at $p<0.05$ after 24h, 48h, 72h and 96h respectively. T= Total Number of cells, t= Total number of cells with micronuclei.

Similarly, an increase in frequency of MN was observed in *Lates calcalifer, Liza parsia, Liza tade, Rhinomugil corsula* and *Terapon jarbua* found in River Hooghly-Matlah at three sites, Haldia (petrochemicals and fertilizer industries wastes), Kakdip (industrial and sewage discharge) and Canning (few tanneries and sewage discharge) (Mallick and Khuda-Buksh, 2003). With increase in concentration and exposure time, a significant rise in number of cells with MN was observed in *Oreochromis niloticus* exposed to petroleum refinery and chromium processing plant effluents (Cavas and Gozukara, 2005). Matsumoto *et al.* (2006) found increase in frequency of MN in *Oreochromis niloticus* exposed for 72h to water receiving tannery effluent as compared to upstream. A similar increase in frequency of micronuclei and nuclear abnormalities at polluted sites as compared to unpolluted sites has been observed in *Clarias gariepinus, Mugil cephalus* and *Alburnus orontis* (Gozukara *et al.*, 2007). In the present study, an increase in the frequency of micronuclei was observed at the highest concentration (24.48%) and duration of exposure (96h). It has been suggested that toxic chemicals disrupt DNA duplications during S phase, interfere with nucleotide synthesis, mis-replicate damaged DNA and cause DNA breaks (Landolt and Kokan, 1983). Also, toxicants are known to produce OH and O_2 free radicals and have strong oxidative effect on membrane phospholipid proteins and nucleic acids (Natarajan and Obe, 1978). The entire process represents the mechanism of elimination of amplified genes from the nuclei (Shimizu *et al.*, 1998). The results of the present study clearly demonstrate that sublethal concentrations of dyeing industry effluent cause damage to the genetic material of *Cirrhinus mrigala* and thus are genotoxic. Genotoxicity has been observed to be maximum at the highest sublethal concentration (24.48%) which can prove to be toxic to the fish fauna. It is, therefore, suggested that dyeing industry effluent should be passed through treatment plant before being discharged into the river ecosystem. Immediate steps should be taken in this direction to avoid any risk on the fish fauna of Punjab.

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