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STUDIES ON INDUCTION OF ERYTROCYTE ABNORMALITIES IN *CIRRHINUS MRIGALA* EXPOSED TO DYEING INDUSTRY EFFLUENT

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ABSTRACT

Erythrocyte abnormality test has been used to detect the genotoxic effect of dyeing industry effluent on the fish. The 96h LC_{50} of effluent against *Cirrhinus mrigala* was calculated by Finney method and three sublethal concentrations 24.48%, 12.24% and 6.12% were made. Fishes were kept in concentrations and blood was smeared after 24h, 48h, 72h and 96h. Eleven types of erythrocyte abnormalities were observed. Nuclear abnormalities included Nuclear Extrusion (NE), Blebbed (B), Binucleate (BN), Lobed (L), Notched (N) nuclei and cellular abnormalities included Enucleated (EnC), Vacuolated (VC), Deformed (DC), Echinocytic (EC), Spindle shaped (SC) and Apoptotic (AC) Cells. Data was statistically significant at ($p<0.05$) level of significance. 12.24% and 24.48% concentrations proved to be more toxic. In 6.12% concentration, abnormalities decreased at 96h. Erythrocyte abnormalities indicated that effluent induced clastogenic effects on the erythrocytes of *Cirrhinus mrigala* and may have similar effects on the human population located around the river and consume fishes.

KEY WORDS: Erythrocyte abnormality test, aquatic pollution, dyeing industry effluent, *Cirrhinus mrigala*.

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1. INTRODUCTION

Discharges from the industries, agriculture and municipal wastes are increasing water pollution to an alarming rate. Freshwater bodies have actually become the disposal sites for these wastes. In developing countries like India (Punjab) dyeing industries are in huge number, approximately 268 industries clustered around Ludhiana. Dyeing industry wastewater contain mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease[1].This industry discharges large volume of untreated wastewater to the surface water and although most of the contaminants are not treated adds significant amount of toxic substances in water bodies of Punjab. The freshwater organisms particularly fishes are very sensitive to various pollutants and play different roles in the trophic web. Fish can respond to mutagens at low concentrations and can highlight the potential danger of chemicals introduced in the aquatic environment. The potential damage to aquatic ecosystem by effluents had been advocated and demonstrated by various toxicity tests. Erythrocyte abnormality test is one of the most useful methods for evaluating genotoxicity in aquatic organisms and has been extensively applied on fish species[2]. The erythrocyte abnormalities have received considerable attention as these abnormalities are good indicator of genetic damage[3]. Fish erythrocytes are distinct because they possess a nucleus and their interpretation in the form of morphological changes became an important bioindicator of pollution. *Cirrhinus mrigala* commonly known as mrigal has been selected as the model as it has great consumer preference amongst carps. The main objectives of the present study are a) To determine 96h LC_{50} of dyeing industry effluent in order to select three sublethal concentrations b) To study erythrocyte abnormalities.

2. MATERIALS AND METHODS

2.1 Collection of specimen

Healthy live specimens of *Cirrhinus mrigala* measuring 6-8 cm in length and 30 – 55 gms in weight were collected from government fish seed farm, Patiala and brought to the laboratory in wide mouthed plastic bags containing fresh water. 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.

2.2 Effluent from dyeing industry

Effluent of dyeing industry was taken directly from the waste outlet of an industrial unit based in Ludhiana to conduct the genotoxicity test against the fish. Dyeing industry effluent contains mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease.

2.3 Determination of 96h LC_{50} and selection of concentrations

96h LC_{50} was determined by the method suggested by Finney[4] and it came out to be 48.97%. Three sublethal concentrations 6.12%, 12.24% and 24.48% (1/2, 1/4, 1/8 of 96h LC_{50} value) of the effluent were selected.

2.4 Experimental design

Acclimatized and apparently healthy, uninjured and uninfected fish specimens were taken. Fishes of control group were maintained in well aerated water. For treated group, fishes were exposed to three sublethal concentrations *viz*., 6.12%, 12.24% and 24.48% for 24h, 48h, 72h and 96h of the effluent. A of total 50 fishes were used for the experiment. Twelve fishes were used in each group (control and treated).

2.5 Measurement of erythrocyte abnormalities

Slides were prepared from blood of anterior kidney from the control and treated fishes after 24h, 48h, 72h and 96h of exposure. Five fishes were used for each concentration. From each fish, four slides were prepared. For each duration of exposure, 4000 cells (1000 cells from each slide) of control and treated groups were observed and photomicrographed.

2.6 Statistical analysis

Data of erythrocyte abnormality test was expressed as Mean(%)±S.E by applying ANOVA and Tukey test. Statistical analysis was performed by using computer software called Graphpad prism. $p<0.05$ was considered to be the level of significance. Statistical significance in the frequencies of erythrocyte abnormalities in the treated and control groups for each concentration and duration was evaluated.

3 RESULTS

The 96h LC_{50} value of dyeing industry effluent against *Cirrhinus mrigala* came out to be 48.97%. In *Cirrhinus mrigala,* a normal erythrocyte is elliptical in shape with centrally placed nucleus in the clear cytoplasm (Figs. 1). Such normal erythrocytes were observed in control group while in treated fishes both nuclear and cellular abnormalities were observed. Nuclear abnormalities included Nuclear Extrusion (NE, Fig. 2), Blebbed (B, Fig. 3), Binucleate (BN, Fig. 4), Lobed (L, Fig. 5) and Notched (N, Fig. 6) nuclei. Cellular abnormalities included Enucleated (EnC, Fig. 7), Vacuolated (VC, Fig. 8), Deformed (DC, Fig. 9), Echinocytic (EC, Fig. 10), Spindle shaped (SC, Fig. 11) and Apoptotic (AC, Fig. 13) Cells. Data of erythrocyte abnormalities induced by dyeing industry effluent is shown in the Table 1.

a) Nuclear abnormalities

Five types of nuclear abnormalities were observed in three sublethal concentrations. In 24.48% and 6.12% all nuclear abnormalities increased upto 96h whereas in 12.24% all nuclear abnormalities decreased at 96h except Nuclear Extrusion which increased from 24h to 96h.

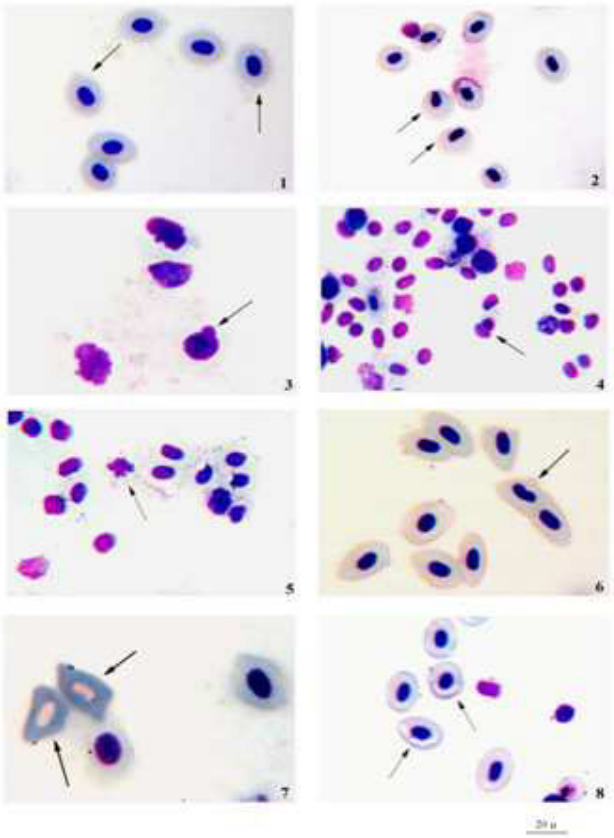
b) Cellular abnormalities

Six types of cellular abnormalities were observed in all the three sublethal concentrations. In 24.48% Echinocytic, Vacuolated and Deformed cells decreased from 24h to 96h whereas Enucleated, Spindle shaped and Apoptotic cells increased from 24h to 96h. In 12.24% Enucleated, Spindle shaped and Apoptotic Cells decreased from 24h to 96h whereas Echinocytic, Vacoulated and Deformed cells increased from 24h to 96h. In 6.12% all cellular abnormalities decreased from 24h to 96h. Mean percentage of erythrocyte abnormalities increased with increase in concentration and duration of dyeing industry effluent. At 72h of all the three concentrations, frequency of erythrocyte abnormalities rose steadily from 36.12 ± 2.73^{c} (6.12%) to 61.82 ± 0.66^{c} (12.24%) and 71.80 ± 2.73^{c} (24.48%). However, in extreme exposure (96h), the initial frequency of erythrocyte abnormalities increased from 29.50 ± 2.40^{d} (6.12%) to a maximum of 60.37 ± 1.76^{d} (12.24%) and 84.15 ± 2.00^{d} (24.48%) respectively as shown in Histogram. Thus, as the concentration and exposure duration increased, the cell alteration registered a quantitative increase with the increase of exposure time.

Table 1
Frequencies of erythrocyte abnormalities in Cirrhinus mrigala treated with dyeing industry effluent.

Experimental groups	Duration of exposure (h)	T	Number of aberrant cells											t	Mean ±S.E
			Nuclear aberrations					Cellular aberrations							
			NE	B	BN	L	N	EnC	VC	DC	EC	SC	AC		
Control	24	4000	1	1	0	0	0	0	0	1	0	0	0	3	0.07±0.00
	48	4000	0	0	0	0	0	0	0	0	0	0	0	0	0.00±0.00
	72	4000	0	0	0	2	0	0	0	1	0	0	0	3	0.07±0.00
	96	4000	0	0	0	0	0	0	0	0	0	0	0	0	0.00±0.00
Total			1	1	0	2	0	0	0	2	0	0	0		
Treated															
6.12%	24	4000	301	4	14	6	3	18	315	1065	88	578	332	2724	68.10±0.88[a]
	48	4000	299	4	16	14	9	16	285	816	89	666	335	2549	63.72±0.88[b]
	72	4000	105	11	22	30	22	10	230	384	44	270	317	1445	36.12±2.73[c]
	96	4000	229	28	24	63	20	2	201	130	34	210	239	1180	29.50±2.40[d]
Total			961	45	79	355	78	49	886	2770	962	3020	1229		
12.24%	24	4000	330	4	16	6	5	10	296	1119	107	648	371	2912	72.80±0.88[a]
	48	4000	172	16	24	196	35	5	99	375	420	1029	317	2688	67.20±1.33[d]
	72	4000	189	21	25	139	31	10	185	514	365	747	245	2473	61.82±0.66[c]
	96	4000	482	11	18	31	12	15	247	980	54	344	211	2415	60.37±1.76[b]
Total			1146	54	80	130	59	37	974	2613	239	1472	1138		
24.48%	24	4000	145	58	11	141	18	3	28	837	81	717	185	2224	55.60±1.86[a]
	48	4000	231	71	12	217	21	3	140	757	247	662	173	2534	63.35±1.00[b]
	72	4000	268	81	20	316	28	1	102	695	342	893	126	2872	71.80±2.73[c]
	96	4000	330	106	27	555	51	0	73	425	442	901	456	3366	84.15±2.00[d]
Total			974	316	70	1229	118	7	343	2714	1112	3173	940		

a, b, c and d: significant differences at 24 h, 48 h, 72 h and 96 h respectively from the control at p<0.05.
T= total number of cells, t= total number of abnormal cells.
NE= nuclear extrusion, B= blebbed, L= lobed, N= notched, EnC= enucleated cell, VC= vacuolated cell, DC= deformed cell, EC= echinocytic cell, SC= spindle shaped cell, AC= apoptotic cell



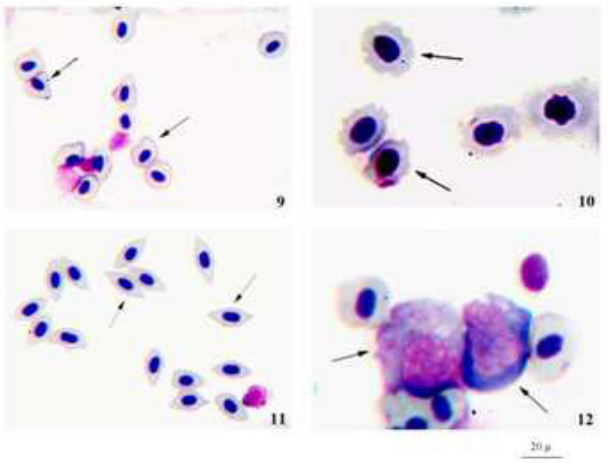
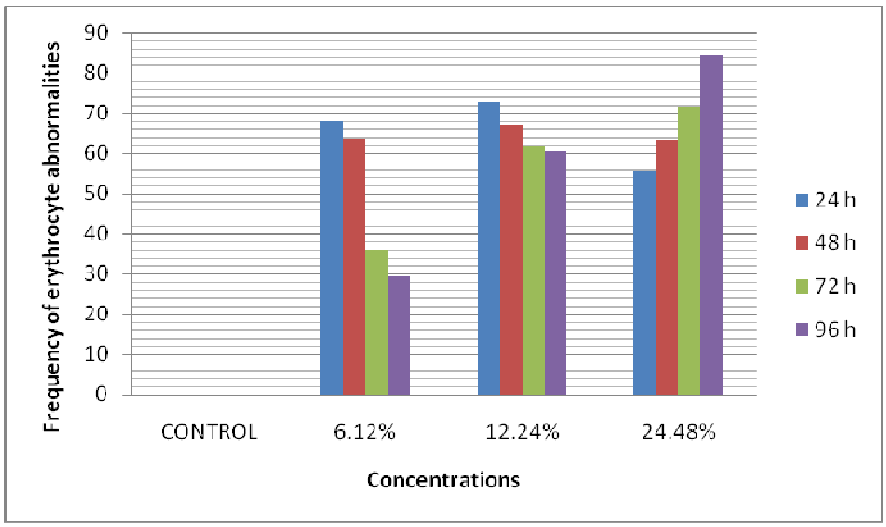


Figure 1

Normal erythrocyte (Control), Erythrocyte abnormalities (Treated): a) Nuclear abnormalities: Fig. 2: Nuclear extrusion , Fig. 3: Blebbed nuclei, Fig. 4: Binucleate, Fig. 5: Lobed nuclei, Fig. 6: Notched nuclei b) Cellular abnormalities: Fig. 7: Enucleated cells, Fig. 8: Vacuolated cells, Fig. 9: Deformed cells, Fig. 10: Echinocytic cells, Fig. 11: Spindle shaped cells, Fig. 12: Apoptotic cells

Histogram



DISCUSSION

Water quality is unswervingly affected by anthropogenic activities towards development. In the developing countries industrialization, urbanization and green revolution are increasing water pollution to an alarming rate. Fish is an important indicator of water pollution as it remains in direct contact with water for food and oxygen and thus is highly sensitive to any change in aquatic environment. So, it can be used in bioassays to assess the effects of pollutants. Erythrocyte abnormality test is a promising tool for assessing genotoxicity of the pollutants. The present study is an attempt to assess the genotoxicity induced in a freshwater fish, *Cirrhinus mrigala* exposed to sublethal concentrations of dyeing industrial effluent. Dyeing industry effluent contains heavy metals, oil and grease. Pollution by heavy metals is an

important problem due to their stable and persistent existence in the environment. Heavy metals interferes the regular chromosome segregation during cell division mainly by inhibition of the mitotic spindle. Most of them have been known to form reactive oxygen species as well as electrophilic free-radical metabolites which interact with DNA and cause disruptive changes. It has been suggested that during the heavy metal exposure, electrophilic ions and radicals are produced which interactwith nucleophilic sites in DNA and lead to breaks and other related damage in the DNA. Cu and Zn act as aneugens, they induce aneuploidy resulting in erythrocyte abnormalities and result in chromosomal rearrangement. Also oil and grease makes water turbid and make layer on gills of fishes. This makes respiration difficult and induces stress in fishes.

Similarly, many other authors also believe nuclear budding in interphase causes the formation of Blebbed and Lobed nuclei. The entire process represents the mechanism of elimination of amplified genes from the nuclei[5]. Further, Von Sonntag and Steenken hypothesized that these abnormalities arise due to the damage caused to the genetic material by free radical produced under oxidative stress caused by toxicant[6]. Aneuploidy is due to failure of tubulin aggregation to form the spindle as well as cytokinesis under the aneugenic action of toxicants and results in formation of Binucleate cells and Notched nuclei[7]. Ateeq *et al.* while elaborating the sequence of cellular degradation under the impact of toxicants suggested that toxicants cause hypoxic conditions which result in depression of ATP that lead to abnormal shape of erythrocytes[8]. Further, toxicants interrupt the lipid solubility of membranes of erythrocytes resulting in Vacuolated and Echinocytic cells, and ultimately lead to apoptosis. Our results were concordant with findings of other authors. Nuclear abnormalities like Blebbed, Notched, Conical and Vacuolated in fishes were found in increasing order from Caning to Kakadip to Haldia[9]. With increase in concentration and exposure time, a significant rise in number of cells with nuclear abnormalities (Binucleates, Lobed, Blebbed and Notched nuclei) was observed in *Oreochromis niloticus* exposed to petroleum refinery and chromium processing plant effluents[10]. Matsumoto *et al.* found frequency of nuclear abnormalities (Blebbed, Notched and Lobed nuclei) to be more in *Oreochromis niloticus* exposed for 72h to water receiving tannery effluent[11]. A similar increase in frequency of nuclear abnormalities at polluted sites has been observed in *Clarias gariepinus, Mugil cephalus* and *Alburnus orontis*[12], *Mugil cephalus*[13], *Centropomus parallelus*[14] and *Cyprinus carpio*[15]. In the present study, an increase in the frequency of erythrocyte abnormalities was observed which was time and concentration dependent, at the highest concentration 24.48% and in 12.24% at (96h). At 6.12%, abnormalities decreased at 96 h and this concentration can act as safe disposal concentration in water after treatment. It is, therefore, suggested that dyeing industry effluent should be passed through treatment plant before being discharged into the aquatic ecosystem.

CONCLUSION

Dyeing Industrial effluents contain large amounts of heavy metals which are genotoxic. These when present in water get incorporated in fish through the food chain may enter the human body and affect the health. Thus, it is very important and urgent to study the genotoxic effects of dyeing industrial effluent on fish. From present study it is clear that dyeing industry effluent is highly genotoxic for fishes and should be passed through effluent treatment plant before being discharged into the rivers. Legal actions should be taken to avoid any further damage to the fish fauna.

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