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GENOTOXIC EFFECTS OF DYEING INDUSTRY EFFLUENT ON A FRESHWATER FISH, CIRRHINUS MRIGALA BY CHROMOSOMAL ABERRATION TEST

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ABSTRACT

Pollution of water resources is a serious and growing problem, despite the existence of relevant legislations. Genotoxic studies on industrial pollutants are very important as they tend to accumulate in aquatic animals. Thus use of aquatic organisms to detect the genotoxicity is very useful in environment monitoring. For this chromosomal aberration test was employed to study genotoxicity caused by dyeing industry effluent on a freshwater fish, Cirrhinus mrigala. Kidney tissue was used for the present study. The 96h LC_{50} was calculated and three sublethal concentrations 24.48%, 12.24% and 6.12% were prepared. Chromosomal preparations of control and treated fishes were made after 24h, 48h, 72h and 96h of exposure period. Somatic metaphase plate was prepared from control fishes to study normal chromosome complement and compared with aberrated somatic metaphase plates of treated fishes. Chromosomal aberrations included Chromosomal fragmentations (Cf), Ring chromosomes (Rc), Terminal chromatid deletions (Tcd), Minutes (M), Centromeric gaps (Cg), Stickiness (Stk), Clumping (C), Pycnosis (Py), Stretching (Stch) and Pulverization (P). Results revealed that dyeing industry effluent to be potentially genotoxic ($p<0.05$). Centromeric gaps, Clumping and Ring chromosomes were predominant aberrations observed in all concentrations. Higher concentration (24.48%) and maximum exposure period (96h) induces more chromosomal aberrations. 6.12% and lower than this concentration however can act as safe disposal concentration of effluent to be dumped in rivers. This type of study could be used as criteria for determining genotoxicity caused by industrial effluents, which can be used to avoid its toxic effects on aquatic environment.

KEY WORDS

Chromosomal aberration test, aquatic pollution, dyeing industry effluent, Cirrhinus mrigala.

1. INTRODUCTION

Pollution of the aquatic ecosystem is recognized as a potential threat to all living organisms. It is produced by man himself; therefore pollution and its effects are considered man's greatest crimes against himself. Water quality is being unswervingly affected by man activities towards development. Maximum aquatic pollutants come from industrial effluents and agricultural run-off.

The river Satluj and its tributaries form the largest river system in Punjab. At Ludhiana, effluents from industries dealing with dyeing, hosiery, machine parts, paint, chemical, tannery and electroplating are added via a tributary, the Buddha Nallah while at Harike, industrial wastes from Jalandhar and Kapurthala enter Satluj via a tributary, the East-Bain. Industrial wastes are known to contain heavy metals and other poisonous substances. These pollutants may be

genotoxic and may lead to several human afflictions like cancer, atherosclerosis, cardiovascular diseases and premature ageing. The increasing degradation of the aquatic environment by anthropogenic contaminants has been the motivation behind increasing need and intensive efforts being made to determine critical concentrations of toxicants in rivers and other water bodies and to evaluate effects of pollutants in biological systems.

Bioassays of pollutants can be performed *in vitro* and *in vivo* to assess their effects. Measurement of chromosomal aberrations offers an acceptable parameter for monitoring mutagenic substances in water. Changes in DNA or chromosomal functions may be a step in the pathway to carcinogenesis. Chromosomal aberrations in animals thus could serve as useful indicators of the presence of clastogenic chemicals. Chromosomal aberration test (CAT) is a promising tool for assessing the genotoxicity in animals. Several reports are available on chromosomal aberrations in fishes exposed to polluted aquatic environment [1-5].

In river Satluj, several species of fishes belonging to the family Cyprinidae are found. The people of Punjab consume a large amount of fish. In Punjab, there are 268 dyeing industries in Ludhiana alone which discharge their wastes into Buddha Nallah that joins the river Satluj at Gorsian, Kedarbaksh in the north-western corner of Ludhiana district. Punjab Pollution Control Board [6] has identified 85 industries as red industries whose wastes are highly toxic to aquatic environment and dyeing industry is one of these red industries. The river Satluj is highly contaminated by dyeing industry effluent. Most fishes sold in market are captured from local water areas of the river Satluj. In the present study, *Cirrhinus mrigala* a popular edible fish of Punjab has been selected to observe genotoxic effects of dyeing industry effluent. The present study is aimed to investigate 1) To calculate 96h LC_{50} of the dyeing industry effluent 2) To determine three sublethal concentrations to observe chromosomal aberrations 3) To compare chromosomal aberrations with normal chromosomal complement 4) To determine the relative toxicity of three sublethal concentrations of the effluent.

2. MATERIALS AND METHODS

2.1 Collection of specimen

Specimens of *Cirrhinus mrigala* measuring 6-8 cm in length and 30 – 55gms in weight, were collected from government fish seed farm, Patiala. 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.

2.2 Collection of dyeing industry effluent

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. The dyeing industry effluent contain mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease [7].

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

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

Experimental design

Fishes were released in tubs containing water (control) and three sublethal concentrations (24.48%, 12.24% and 6.12%) for 24h, 48h, 72h and 96h by using the method given by Manna and Sadhukhan [9]. Three sets of experiments

were performed for each concentration. A total of 72 fishes were used for the experiment.

2.4 Measurement of chromosomal aberrations

Kidney tissue was used to make metaphasic plates. Slides were stained by the method given by Tijo and Whang [10]. Twenty four fishes (6 per hour) were used for each concentration. For each duration, thirty slides were prepared. For each hour 300 plates (100 plates from ten slides) of control and treated groups were observed and photomicrographed.

2.5 Statistical analysis

Data of chromosomal aberrations were subjected to ANOVA and Tukey test. Statistical analysis was done by using computer software 'Graph pad prism'. $p<0.05\%$ was considered to be the level of significance. Statistical significance of chromosomal aberrations for control and treated groups of each concentrations and durations was also evaluated. Frequencies of chromosomal aberrations were expressed as Mean (%) ± S.E.

3. RESULTS

The 96h LC_{50} value of dyeing industry effluent against *Cirrhinus mrigala* using Finney method (1971) came out to be 48.97%. Somatic metaphase plates obtained from kidney tissue revealed diploid number of 50 chromosomes (**Fig. 1**). Observations pertaining to aberrations induced in chromosomes of fishes were recorded after 24h, 48h, 72h and 96h of exposure is summarized in **Table**.

Control fishes showed almost negligible chromosomal aberrations when compared to those exposed to dyeing industry effluent. Ten types of aberrations were determined from three sublethal concentrations and these were Chromosomal fragmentations (Cf, **Fig.3**), Ring chromosomes (**Rc, Fig.4**), Terminal chromatid deletions (Tcd, **Fig.5**), Minutes (M, **Fig.6**), Centromeric gaps (Cg, **Fig.7**), Stickiness (Stk, **Fig.8**), Clumping (C, **Fig.9**), Pycnosis (Py, **Fig.10**), Stretching (Stch, **Fig.11**) and Pulverization (P, **Fig.12**).

At 24.48% concentration, Chromosomal fragments, Ring chromosomes, Terminal chromatid deletions, Minutes, Centromeric gaps and Pycnosis increased from 24h to 96h. Clumping and Stretching increased up to 72h then decreased and Stickiness and Pulverization decreased from 24h to 96h. Centromeric gaps were predominant and Stretching was lowest. At 12.24% concentration there was increase in chromosomal aberrations from 24 h to 96 h duration of time. Centromeric gaps and Ring chromosomes showed progressive increase with increase in duration of time. Clumping was the highest and Stickiness was the lowest among various aberrations. At 6.12% concentration, there was increase in chromosomal aberrations with increase in duration of exposure up to 72h but afterwards, there was a decrease. Centromeric gaps were observed to be the highest and Stickiness to be the lowest.

Mean percentage of chromosomal aberrations increased with increase in concentration and exposure duration of dyeing industry effluent. At extreme exposure 96h, of all the three concentrations, frequency of chromosomal aberration rose steadily from 48.66 ± 2.96^{d} (6.12%) to 59.33 ± 2.33^{d} (12.24%) and 83.00 ± 1.73^{d} (24.48%) as shown in Histogram. Thus, as the concentration increased, the chromosomal aberrations registered a quantitative increase with the increase of exposure time. Overall percent frequencies of each chromosomal aberration in each concentration as well as control are shown in Pie Charts (a-d).

4. DISCUSSION

In view of ever increasing levels of pollution caused by a wide variety of toxic substances in various water bodies, testing for potential

genotoxic effect on aquatic organisms has assumed a considerable significance. Chromosomal aberration test is one of the best tests for assessing genotoxicity of a pollutant in fishes with relative large chromosome number. It is not widely done due to its laborious work of studying metaphasic plates. *Cirrhinus mrigala* a popular edible fish found abundantly in rivers of Punjab was selected.

There was a quantitative relationship between the occurrence of chromosomal aberrations and dyeing industrial effluent. This toxic effluent can alter the genetic material of fish following bioaccumulation in fish organs.

Ten types of chromosomal aberrations were found in the present study. Increase in frequency of chromosome aberrations exhibited time and concentration dependent response. Dyeing industry effluent mainly contains heavy metals which affect replication, translation and repair of genetic materials. Similar work has been reported by other authors and suggested heavy metals disrupt DNA duplications during S phase, interfere with nucleotide synthesis and mis-replicate damaged DNA leading to malformation of DNA molecules [11-13]. It has further been suggested that toxins may have strong oxidative effect on membrane phospholipid proteins and nucleic acids [14]. Heavy metals also generate reactive free radicals [15]. According to Natarajan and Obe [16], OH^- and O_2^- radicals are most relevant oxygen species which react with DNA causing DNA breaks that ultimately lead to chromosomal aberrations.

Earlier workers also examined the effect of polluted water from industrial, agricultural and sewage runoff on fishes. Al-Sabti and Kurelec [1] recorded chromosomal aberrations (breaks and fragments) in *Mytilus galloprovincialis* collected from ten locations of Rovinj area. Kumari and Ramkumaran [2] observed that 65% of *Channa punctatus* collected from polluted Hussainsagar Lake showed chromosomal aberrations. Hafez [3] observed higher frequency of Stickiness, fragments, gaps and deletions in *Mugil cephalus* at the most polluted site of Abu-qir bay. Mahmoud *et al.* [4] observed more Chromatid gaps, Chromatid deletions and Chromosome fragments in two fishes, *Oreochromis niloticus* and *Tilapia zillii* at highly polluted sites receiving sewage and other discharges. Similar observations were also recorded by Rose *et al.* [5] in freshwater fish, *Hypophthalmus molitrix* present in polluted sites of the river Coovum. Direct effect of dyeing industrial effluent was not studied before on fishes in Punjab.

The present study revealed chromosomal aberrations increased with increase in concentration and time. Concentration 24.48% was more toxic than 12.24%. 6.12% and lower than this concentration, however can act as safe disposal concentration of effluent to be dumped in rivers.

5. CONCLUSION

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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Table: Frequencies of chromosomal aberrations in kidney cells of *Cirrhinus mrigala* after treatment with dyeing industry effluent

Experimental groups	Duration of exposure (h)	T	Chromosomal aberrations (Number of plates)										t	Mean(%)±S.E
			Cf	Rc	Tcd	M	Cg	Stk	C	Py	Stch	P		
Control	24	300	2	3	0	0	0	0	1	0	1	0	7	2.33±0.66
	48	300	0	0	1	0	0	1	1	0	0	0	3	1.00±0.57
	72	300	1	1	0	0	0	0	0	1	0	0	3	1.00±0.57
	96	300	0	2	0	0	0	0	0	0	0	0	2	0.66±0.33
Total			3	6	1	0	0	1	2	1	1	0		
Treated														
6.12%	24	300	3	26	8	19	20	0	19	4	4	11	114	38.00±2.89[a]
	48	300	28	24	30	19	34	2	34	3	6	6	186	62.00±1.15[b]
	72	300	23	35	35	17	50	7	17	4	7	4	199	66.33±1.86[c]
	96	300	10	22	17	10	23	12	34	6	10	2	146	48.66±2.96[d]
Total			64	107	90	65	127	16	104	17	27	23		
12.24%	24	300	1	16	2	13	13	0	20	7	2	10	84	28.00±0.33[a]
	48	300	18	19	12	24	18	4	30	21	4	3	153	51.00±1.86[b]
	72	300	19	24	29	23	24	4	21	5	7	6	162	54.00±1.86[c]
	96	300	9	40	21	8	41	3	29	20	4	3	178	59.33±2.33[d]
Total			47	99	64	68	96	11	100	53	17	22		
24.48%	24	300	11	10	3	12	22	17	17	2	6	17	117	39.00±1.00[a]
	48	300	17	17	8	15	17	3	23	16	3	6	125	41.66±1.33[b]
	72	300	22	19	26	23	29	3	45	7	8	5	187	62.00±2.19[c]
	96	300	34	48	33	22	56	15	16	21	1	3	249	83.00±1.73[d]
Total			84	94	70	72	124	38	101	46	18	31		

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 metaphase plates, t= total number of metaphase plates with chromosomal aberrations.
Cf= chromosome fragmentation, Rc= ring chromosome, Tcd= terminal chromatid deletion, M= minutes, Cg= centromeric gaps, Stk= stickiness, C= clumping, Py= pycnosis, Stch= stretching, P= pulverization.

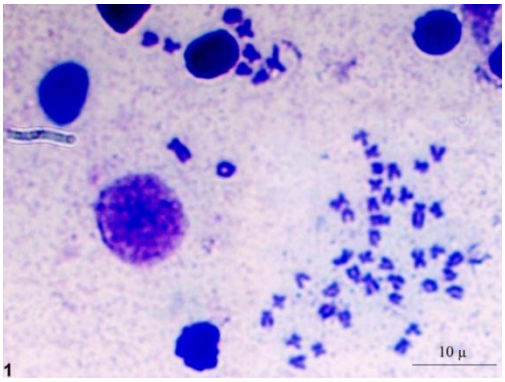
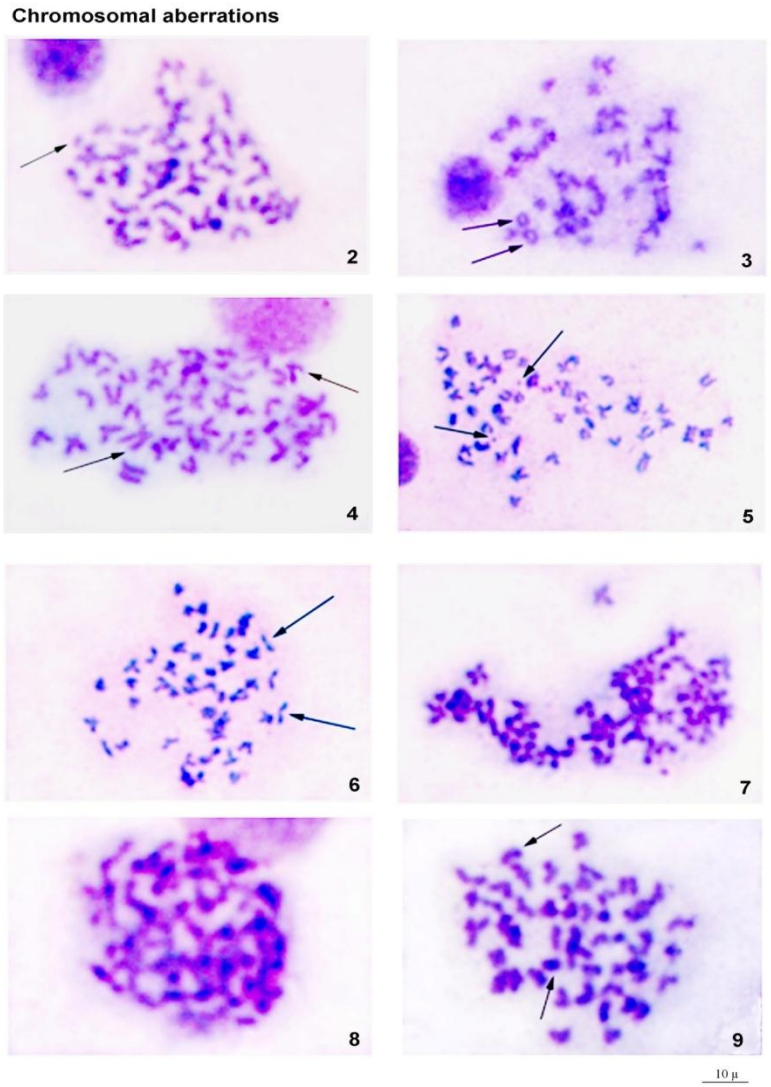
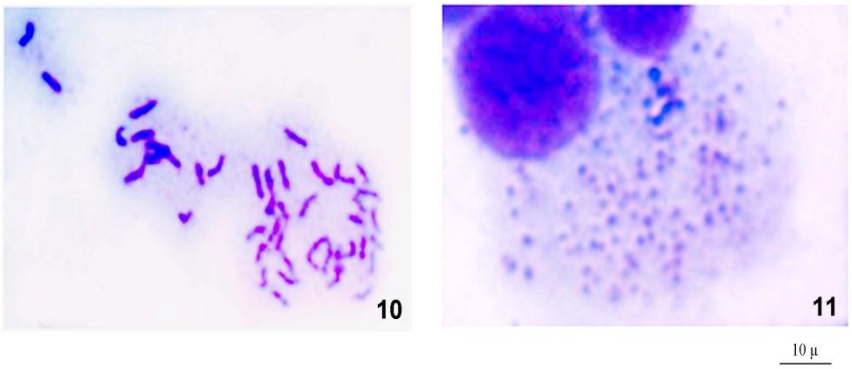


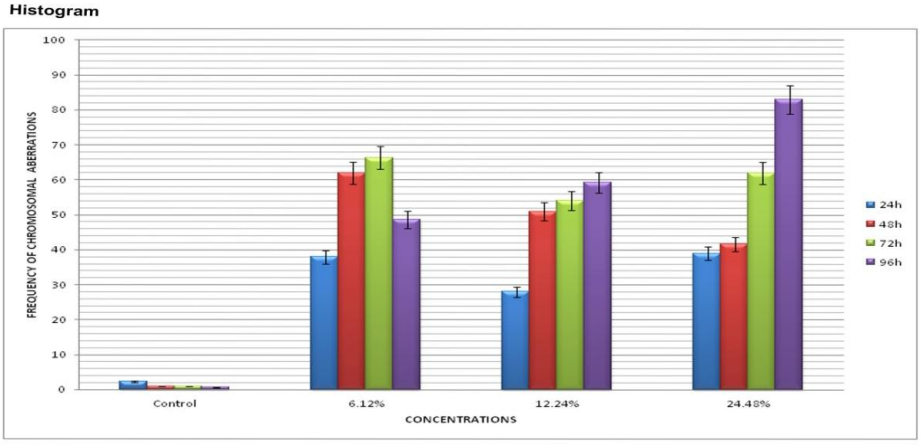
Fig.1 Normal metaphase complement



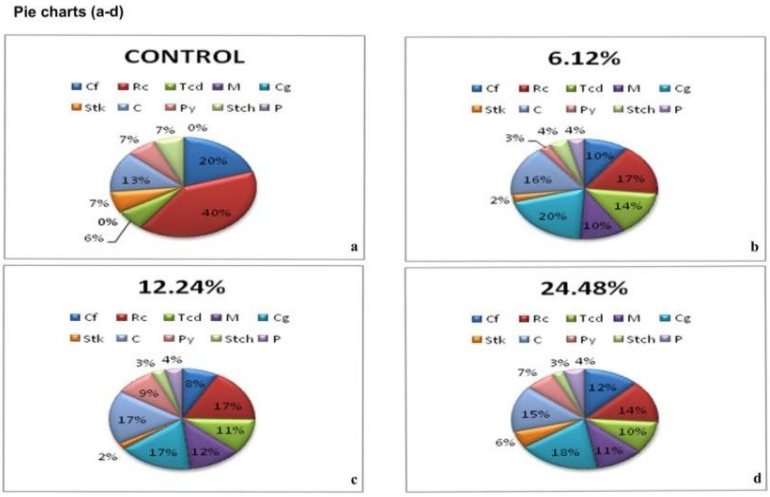
Figs. (2-9): Chromosomal aberrations: Cf= Chromosome fragmentation (Fig. 2), Rc= Ring chromosome (Fig. 3), Tcd= Terminal chromatid deletion (Fig. 4), M= Minutes (Fig. 5), Cg= Centromeric gaps (Fig. 6), Stk= Stickiness (Fig. 7), C= Clumping (Fig. 8), Py= Pycnosis (Fig. 9)



Figs. (10-11): Chromosomal aberrations: Stch= Stretching (Fig. 10), P= Pulverization (Fig. 11).



Frequency of chromosomal aberrations in kidney cells of *Cirrhinus mrigala* after treatment with dyeing industry effluent.



Percent frequency of chromosomal aberrations in kidney cells of *Cirrhinus mrigala* after treatment with dyeing industry effluent.

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