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Research Paper

Induction of chromosomal aberrations and altered behavioural responses in fish *Labeo rohita* exposed to tannery industry effluent.

Zoology

KEYWORDS : *Labeo rohita*, chromosomal aberrations, behavioural responses, sublethal concentrations

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ABSTRACT

In present study, Labeo rohita was used to determine acute toxicity of the tannery industrial effluent for 120h. Two sublethal concentrations 7.74% and 1.93% were selected on basis of (1/2 and 1/8) of 96h Lc_{50} value. Various behavioural responses were observed along with chromosomal aberration to study genotoxic effect. Concentration 7.74% proved to be highly toxic than 1.93 % concentrations. Control fishes were also continuously monitored and compared with the changes caused by effluent in each concentration.

Introduction:

Water pollution has emerged as one of the most pressing problems of this century. Although over 70% of the planet is inundated with water less than 3% freshwater of that less than 1% is available to support life. Among various industrial effluent Leather/ tannery is a major industry in India which makes a significant contribution to the country's foreign exchange earnings. It is estimated that 30-35 L of water is used per kilogram of leather processed, generating about 680 X 106 L of effluent daily (Sounderraj *et al*., 2012). Heavy metals present in effluents discharged by the tannery industries constitute the most common group of toxic and non-degradable substances among other industries. On reaching the aquatic ecosystem, they pose a serious threat to the biotic components especially fish by altering the physiochemical characteristics of water and production of fish food organisms (Joir *et al*,. 1991). Behaviour of an organism represents a unique view which links its physiology with the environment (Little and Brewer, 2001). Altered fish behaviour is the first reflex response to any toxicants and is regarded as good biomarker (Kane *et al*., 2005). Ecogenotoxicology (genetic ecotoxicology) is an approach that applies the principles and techniques of genetic toxicology to assess the potential effects of environmental pollution in the form of genotoxic agents on the health of the ecosystem (Shugart and Theodorakis, 1998). Fish is an ideal model for toxicological research as it is highly sensitive to any physicochemical or biological change in the water quality.

Chromosomal aberration test is one of the promising test to detect genotoxic effect. The most common abnormalities are categorizes as chromosome/chromatid break, acentric fragments, chromatid and sub-chromatic exchanges, chromatid gaps at metaphase. Such aberrations are the result of unfinished repair or misrepair of DNA. The present study is attempt to study behavioural responses and genotoxic effect in terms of chromosomal aberration test in *Labeo rohita* exposed to tannery effluent.

Materials and methods:

Healthy live specimens of *Labeo rohita* about 6-8 cm in length and 32–58 gms in weight was collected from fish seed farm and were acclimatized in laboratory for 20 days. Tannery industrial effluent was taken directly from the waste outlet of industrial units based in Jalandhar. The effluent were collected three times were characterized from laboratory of Punjab Pollution Control Board (PPCB) for the determination of various constituents present in the effluents.

96h LC_{50} was determined by the method of Finney, 1971. Two sublethal concentrations 7.74% and 1.93% (1/2 and 1/8 of 96h of LC_{50}) were prepared for chromosomal aberration test and behavioural changes. Fishes were maintained in water (control) and in two sublethal concentrations for 24h, 48h, 72h, 96h and 120h as suggested by (Manna and Sadhukhan, 1986). Three sets of experiments were performed for each concentration. A total of 90 fishes were used for all the three experiments. Kidney tissue was used for the chromosomal preparations. Behavioural responses in two sublethal concentrations were recorded after 24h, 48h, 72h, 96h and 120h durations of exposure. Control group fishes were also monitored to assess the normal behaviour. Chromosomal aberrations data was 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.

Results:

Characterization of tannery effluent:

The physico-chemical analysis of the effluents was done by PPCB and its parameters observed were tabulated in table 1. The effluent was grey in colour with pungent smell. The effluent was acidic in nature with high amounts of TDS, TSS, turbidity and electrical conductivity. The effluent has high amount of BOD and COD. Various heavy metals observed were hexavalent chromium, total chromium, sulphide and boron. There was presence of high amount of chloride ions along with oil and grease.

96h LC_{50} value:

96h LC_{50} value of effluent against *Labeo rohita* came out to be 15.48%. Two sublethal concentration selected were 7.74% and 1.93% (1/2 and 1/8 of 96h of LC_{50}).

Behavioural responses:

Control fishes: The control fishes were maintained in well aerated water showed active feeding, normal schooling behavior, well-synchronized body movements and attentive to slight disturbance near the tank. No mortality was recorded in the control group. The behaviour of the control groups did not show any change upto 120h and hence taken as standard for the entire experimentation.

Treated fishes: Fishes treated with two sublethal concentrations showed behavioural responses were tabulated in table 2. High concentration showed erratic swimming, gulping air at surface and hitting against the wall from 24h to 120h. Opercular movements became fast as fishes were introduced upto 48h and then slow from 72h to 120h. The fishes loose their equilibrium and were sluggish from 72h to 120h. Two fishes died at 120h. In low concentration the effect was less severe. The fishes showed erratic swimming, gulping of air at surface, slow opercular movement from 72h to 120. The fishes started hitting against wall from 96h to 120h. They fishes became sluggish after 120h.

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Chromosomal aberration test:
Control somatic metaphase plate revealed diploid number, 2n=50 (Fig.1). Chromosomal aberrations induced in fishes after 24h, 48h, 72h, 96h and 120h are summarized in the Table 3. Control fishes showed negligible chromosomal aberrations while exposed fishes possess five types of aberrations *viz.,* Chromosomal fragmentations (Cf, Fig.2), Ring chromosomes (Rc, Fig.3), Terminal chromatid deletions (Tcd, Fig.4), Minutes (M, Fig.5), Centromeric gaps (Cg, Fig.6). Chromosomal aberrations induced in fishes after 24h, 48h, 72h, 96h and 120h are summarized in the Table 3. All these aberration showed clastogenic effect. In higher concentration mean percentage of chromosomal aberrations increased from 24h to 120h whereas it decreased from 24h to 120h. Chromosomal aberrations followed a increasing trend in order Rc> Cf> Cg> M> Tcd in higher concentration whereas Cf> Tcd> Cg> Rc> M in lower concentration. The results showed concentration and time dependent response.

Discussion:
Aquatic ecosystem is used as storage house for many chemicals discharged by industries, domestic, sewage and agriculture wastes. The continuous release of these chemicals disturbs the water quality and aquatic organisms because of their persistence, bioaccumulation, toxicity and biomagnifications in the food chain. Aquatic organisms including fishes accumulate toxicants directly from contaminated water or indirectly through the food chain and causes for various pathological alterations in the tissues (Tilak *et al.*, 2004). The first response of an aquatic organism exposed to toxicant is change in its behavior and external morphology. Altered swimming behaviour as well as mucus secretion and gulping of air were indicative of the underlying morphological, biochemical or physiological alterations and hence may reflect a series of toxic effects and compensatory responses (Campbell *et al.*, 2005). Restlessness, erratic and jerky swimming, loss of equilibrium, hitting against the wall and hyperactivity in fishes might occur due to the inactivation of acetylcholinesterase (AChE), leading to accumulation of acetylcholine at synaptic junctions (Fulton and Key, 2001).

In the present study, genotoxic effect of tannery industry effluent in fish shows time and concentration dependent response. Higher concentration (7.74%) proved to be highly toxic because chromosomal aberrations are increasing with the increase of exposure while in low concentration (1.93%), aberrations are decreasing from 24h to 120h. Tannery industry effluent mainly contains chromium and heavy metals. Chromium reduces to chromium (IV) in the body which generates reactive free radicals (Tsalev and Zaprianov, 1983). These free radicals caused single strand DNA breaks and DNA cross-links. Thus, presence of chromium and heavy metals are responsible for the genotoxic damage in fishes.

Conclusion:
This study has demonstrated that exposure of *Labeo rohita* to tannery effluent caused behaviour and genotoxic effect in fishes. These changes reflect the continuous discharge of effluent in rivers without treatment causing hazardous effect to aquatic biota, domestic animals and human beings as well. It is therefore, suggested that both small scale as well as large scale industries to treat their waste water in treatment plant before dumping. Healthy aquatic environment should be maintained to save fish fauna and human health. Legal actions should be taken to maintain the aquatic ecosystem as well as the fish diversity.

Table 1. Physicochemical parameters of tannery industry effluent:

Sr.No.	Parameters	Regulatory standards for tannery industry (CPCB –industry specific standards Sr.No-16)			1 Sample collected on 20/5/2013	2 Sample collected on 18/6/2013	3 Sample collected on 16/7/2013
		A-Inland surface body	B- Public Sewer	C- Onland for irrigation			
1.	Temperature (^{0}C)	Shall not exceed 5^{0}C above ambient air temperature of water in the receiving body	Shall not exceed 5^{0}C above ambient air temperature of water in the receiving body-	Shall not exceed 5^{0}C above ambient air temperature of water in the receiving body-	26	24	28
2.	Colour	-	-	-	Grey	Grey	Grey
3.	Odour	-	-	-	Pungent	Pungent	Pungent
4.	Turbidity	NS	-	-	Present	Present	Present
5.	pH	6.0-9.0	-	-	4.5	5.7	3.9
6.	Electrical conductivity	NS	-	-	High	High	High
7.	TDS, mg/l	2100	-	-	2400	2872	2547
8.	TSS, mg/l	100	00	200	648	740	786
9.	BOD,(3 days at 27 ^{0}C), mg/l	30	350	100	520	524	528
10.	COD, mg/l	250	-	-	295	284	298
11.	Chloride, mg/l	1000	1000	200	1524	1421	1540
12.	Hexavalent Chromium as Cr^{-6} mg/l	0.1	0.2	0.1	0.3	0.5	0.7
13.	Total Chromium as Cr, mg/l	2.0	5.0	-	4.1	4.8	4.5
14.	Sulphide as S, mg/l	2.0	-	-	6.4	6.8	7.4
15.	Boron as B, mg/l	2.0	2.0	2.0	0.2	0.2	0.4
16.	Oil and Grease, mg/l	10	20	10	25	27	24

Table: 2 Behavioural responses and Morphological changes in *Labeo rohita* exposed to tannery industry effluent.

	EXPOSURE PERIOD AND CONCENTRATION														
A) BEHAVIOURAL RESPONSES	24h			48h			72h			96h			120h		
	C	1.93%	7.74%	C	1.93%	7.74%	C	1.93%	7.74%	C	1.93%	7.74%	C	1.93%	7.74%
1. Erratic swimming	A	L	M	A	L	M	A	L	M	A	M	M	A	M	M
2. Gulping air at surface	A	L	M	A	L	M	A	L	M	A	M	M	A	M	M
3. Opercular movement	N	N	F	A	N	F	N	F	S	A	S	S	A	S	S
4. Loss of equilibrium	A	A	A	A	A	A	A	A	P	A	P	P	A	P	P
5. Hitting against the wall	A	A	P	A	A	P	A	A	P	A	P	P	A	P	P
6. Restlessness	A	A	P	A	A	P	A	A	A	A	A	A	A	A	A
7. Sluggishness	A	A	A	A	A	A	A	A	P	A	A	P	A	P	P
8. Fish lied at water surface before death	A	A	A	A	A	A	A	A	A	A	A	P	A	A	P

Less= L, More= M, Fast= F, Normal= N, Slow= S, Present= P, Absent= A.

Table 3: Frequencies of chromosomal aberrations in kidney cells of *Labeo rohita* after treatment with tannery industry effluent

Experimental Group	Durations	TCO	NDC	Mitotic index±S.E.	T	Chromosomal aberrations					t	Mean(%)±S.E.
						Cf	Rc	Tcd	M	Cg		
Control	24	3600	575	15.90±0.33	300	0	1	0	0	0	1	0.33±0.33
	48	3600	562	15.60±0.66	300	1	0	0	0	0	1	0.33±0.33
	72	3600	559	15.55±0.66	300	0	0	0	0	0	0	0.00±0.00
	96	3600	549	15.20±0.57	300	0	1	0	0	0	1	0.33±0.33
	120	3600	549	15.20±0.57	300	1	1	0	0	0	2	0.66±0.33
Total						2	3	0	0	0		
Treated												
1.93%	24	3600	489	13.58±0.57	300	20	17	19	15	18	89	29.66±1.20[a]
	48	3600	499	13.86±0.33	300	18	15	16	13	16	78	26.00±0.57[b]
	72	3600	525	14.58±0.57	300	15	13	14	12	14	68	22.66±0.66[c]
	96	3600	530	14.72±0.66	300	12	11	12	10	12	57	19.00±1.52[d]
	120	3600	535	14.86±0.66	300	10	9	10	9	9	47	15.66±0.33[e]
Total						75	65	71	59	69		
7.74%	24	3600	445	12.36±0.88	300	25	28	10	14	13	90	30.00±1.52[a]
	48	3600	439	12.19±0.88	300	29	33	16	18	18	114	38.00±1.15[b]
	72	3600	415	11.52±1.45	300	34	37	19	21	25	136	45.33±1.45[c]
	96	3600	402	11.16±1.00	300	39	42	26	27	29	163	54.33±0.88[d]
	120	3600	399	11.08±0.57	300	41	44	28	29	31	173	57.66±0.88[e]
Total						168	184	99	109	116		

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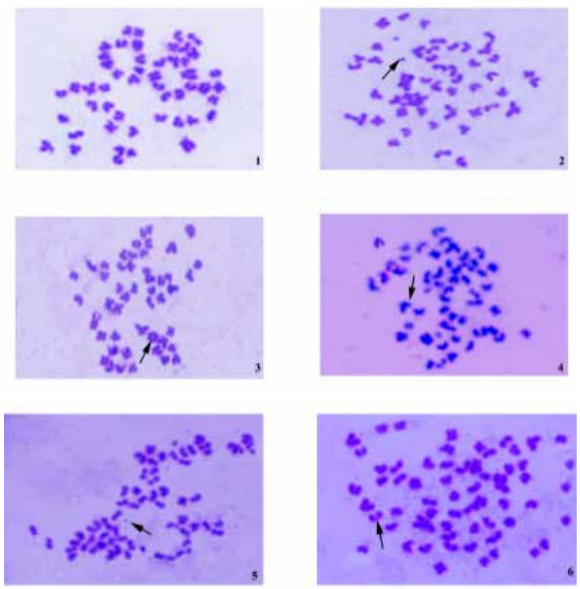
A, B, C, D AND E: SIGNIFICANT DIFFERENCES AT 24H, 48H, 72H, 96H AND 120H RESPECTIVELY FROM THE CONTROL AT P<0.05. TCO=TOTAL NUMBER OF CELL OBSERVED, NDC=NUMBER OF DIVIDING CELLS, T= TOTAL NUMBER METAPHASE PLATES, T= TOTAL NUMBER OF METAPHASE PLATES WITH CHROMOSOMAL ABERRATIONS. CF= CHROMOSOME FRAGMENTATION, RC= RING CHROMOSOME, TCD= TERMINAL CHROMATID DELETION, M= MINUTES, CG=CENTROMERIC GAPS.

Plate-1



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