RESEARCH PAPER | Zoology | Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

Evaluation of genotoxic potential of tannery industry effluent in a freshwater fish, *Labeo rohita* via chromosomal aberration test

KEYWORDS: *Labeo rohita*, tannery industry effluent, chromosomal aberrations, genotoxicity

Gurinder Kaur Walia
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002 (Punjab) India

Diana Handa
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002 (Punjab) India

Harbhajan Kaur
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002 (Punjab) India

Rohit Kalotra
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002 (Punjab) India

ABSTRACT *Chromosomal aberration test is one of the most exclusively used biomarker to detect effects of DNA damage. In the present study, this test has been used to elucidate the genotoxic impact of tannery industry effluent on kidney tissue of Labeo rohita. 96h LC_{50} against fish was calculated and three sub-lethal concentrations (3.53%, 1.76% and 0.88%) were prepared. Fishes were exposed to sublethal concentrations for 24h, 48h, 72h and 96h durations. Ten types of chromosomal aberrations were observed. Out of these, Chromosome fragments, Ring chromosomes, Centromeric gaps and Minutes were predominant while Pulverization and Stretching were lowest in all the concentrations. Results indicated that higher concentration (3.53%) and exposure (96h) induced maximum chromosomal aberrations. These were statistically significant ($p<0.05$) as compared to control.*

Introduction:
Water pollution is a global issue. Industrialization in developing countries affects the faunal diversity of water because waste water from these industries is directly dumped into the water bodies. Punjab is one of the leading states of India in agriculture and industrialization. The waste water generated from urban areas, industries and commercial activities is directly discharged into rivers, streams and lakes which is the main cause of water pollution. The situation becomes very serious during past few years. In Punjab, there are two main rivers, Satluj and Beas besides Ghaggar and Ravi. They get heavily polluted by untreated or inadequately treated industrial effluents, domestic sewage and agricultural run-off. PPCB (1999) enlisted 85 industries as red category industries and their wastes are highly toxic to aquatic environment. Tannery industry is one of them. There are numbers of tannery industries in Jalandhar which throw their untreated effluent in Kala Sanghia drain which finally reach to Satluj river via a tributary, chitti bein.

Aquatic organisms especially fishes are excellent subject for the study of mutagens, carcinogens and toxicants as they can metabolize, concentrate and store water borne pollutants and also respond to toxicants in a similar way as higher vertebrates (Al-Sabti 1991; Al-Sabti and Metcalfe 1995). Pollution of water bodies forces them to acclimatize to environmental changes which are imposing considerable stress on their lives. Genotoxicity deals with the study of deleterious effects of toxic agents present in the environment on the organisms. Chromosomal aberrations test (CAT) is a sensitive biomarker in monitoring the toxicity.

Present work was planned to study genotoxicity in a fish, *Labeo rohita* of family Cyprinidae after *in vivo* exposure to tannery industry effluent. The main objectives were **a)** to calculate 96h LC_{50} value **b)** to study chromosomal aberrations **c)** to evaluate highly toxic sublethal concentration.

Material and methods:
Fishes of about 6-8 cm in length and 30–60 gms in weight were collected from government fish seed farm, Patiala and were acclimatized in laboratory for 20 days. Tannery industry effluent was taken directly from the waste outlet of an industrial unit based in Jalandhar. 96h LC_{50} was determined by the method of Finney, 1971. Three sublethal concentrations 3.53%, 1.76% and 0.88% (1/2, 1/4 and 1/8 of 96h of LC_{50}) were prepared for genotoxicity tests. Fishes were maintained in water (control) and in three sublethal concentrations for 24h, 48h, 72h and 96h as suggested by Manna and Sadhukhan, 1986. Three sets of experiments were performed for each concentration. A total of 72 fishes were used for the experiment. Kidney tissue was used for the chromosomal preparations.

Slides were stained by the method of Tijo and Whang, 1965. 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:
96h LC_{50} value of effluent against *Labeo rohita* came out to be 7.07%. Somatic metaphase plate revealed diploid number, 2n=50 (Fig.1). Chromosomal aberrations induced in fishes after 24h, 48h, 72h and 96h are summarized in the Table.

Control fishes showed negligible chromosomal aberrations while exposed fishes possess ten 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), Stickiness (Stk, Fig.7), Clumping (C, Fig.8), Pycnosis (Py, Fig.9), Stretching (Stch, Fig.10) and Pulverization (P, Fig.11).

3.53% concentration proved to be highly toxic and induced maximum chromosomal aberrations. Chromosome fragmentations, Terminal chromatid deletion, Minutes, Stickiness and Pulverization increased from 24h to 96h. Ring chromosomes, Centromeric gaps and Clumping increased upto 72h then decreased. Pycnosis and Stretching decreased from 24h to 96h. During all the time intervals, Centromeric gaps were predominant (67.00%) and Pulverization was lowest (1.33%). In 1.76% concentration, Terminal chromatid deletion, Minutes and Centromeric gaps increased from 24h to 96h. Chromosome fragmentations, Ring chromosomes, Stickiness and Clumping increased upto 72h then decreased. Pycnosis, Pul-

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

verization and Stretching decreased from 24h to 96h. During all durations, Centromeric gaps were predominant (54.66%) and Stretching was lowest (2.00%). Concentration 0.88% caused all types of aberrations but their frequency was less. Terminal chromatid deletion, Minutes, Centromeric gaps, Clumping and Pulverization increased from 24h to 96h but the increase was not significant. Chromosome fragmentations, Ring chromosomes, Pycnosis, Stretching and Stickiness increased upto 72h then decreased. During all time intervals, Centromeric gaps occurred predominantly (55.66%) while Pulverization (2.00%) showed lowest value.

Mean percentage of chromosomal aberrations increased with the concentration and duration of exposure (Histogram). At 72h, frequency of aberrations rose from 75.33 ± 2.33^{c} (0.88%) to 83.00 ± 1.73^{c} (1.76%) and 91.00 ± 2.31^{c} (3.53%). At 96h, frequency of aberration increased significantly from 68.00 ± 2.03^{d} (0.88%) to 84.33 ± 1.45^{d} (1.76%) and 92.33 ± 1.73^{d} (3.53%). Results depicted that chromosomal aberrations induced by tannery industry effluent in fishes were time and concentration dependent.

Discussion:

Aquatic environment plays a vital role in functioning of an ecosystem. Pollution of the aquatic ecosystem is recognized as potential threat to all living organisms. This not only reduces the fitness in fish population but also pose risk to human health via food chain. Thus, chromosomal aberrations test is a diagnostic tool to judge the genotoxicity caused by the pollutants.

Genotoxic effects have been reported in fishes by Al-Sabti and Kurelec (1985) on *Mytilus galloprovincialis* from polluted site of rovinj area of Northern Adriatic sea; Kumari and Ramkumaran (2006) in *Channa punctatus* from polluted Hussainsagar Lake, Hyderabad; Hafez (2009) in *Mugil cephalus* from the most polluted site of Abu-qir bay; Obiakor *et al*. (2010) in *Clarias gariepinus* from polluted river water; Mahmoud *et al*. (2010) in *Oreochromis niloticus* and *Tilapia zillii* from drainage canal receiving sewage and other discharges and Rose *et al*. (2010) in *Hypophthalmicthys molitrix* from polluted water of the river Coovum.

In the present study, genotoxic effect of tannery industry effluent in fish shows time and concentration dependent response. Concentration 3.53% is proved to be highly toxic because chromosomal aberrations are increasing with the increase of exposure while in 1.76% and 0.88% concentrations, aberrations are decreasing after 72h. Tannery industry effluent mainly contains chromium and heavy metals. Chromium gets reduced from chromium (VI) to chromium (III) which generates highly reactive free radicals responsible for single strand DNA breaks and DNA cross-links. Thus, presence of chromium and heavy metals are responsible for the genotoxic damage in fishes.

Conclusion:

Tannery industry effluent is proved to be genotoxic as it is responsible to cause chromosomal aberrations in fishes. Therefore, it is suggested that effluents should be passed through treatment plant before being discharged into the rivers. Only, safe concentration of effluents should be released into the rivers. Legal actions should be taken to maintain the aquatic ecosystem as well as the fish diversity.

Table. Frequencies of chromosomal aberrations in kidney cells of *Labeo rohita* after treatment with tannery 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	1	2	0	0	0	0	2	0	0	0	5	1.66±0.33
	48	300	0	0	0	0	0	0	1	0	1	0	2	0.66±0.33
	72	300	0	1	0	0	0	1	0	1	0	0	3	1.00±0.00
	96	300	1	0	0	0	0	0	0	0	1	0	2	0.66±0.33
Total			2	3	0	0	0	1	3	1	2	0	12	
Treated														
0.88%	24	300	23	23	10	20	39	25	13	10	1	1	165	55.00 ± 2.03^{a}
	48	300	8	31	15	12	44	22	15	5	1	1	154	51.33 ± 0.57^{b}
	72	300	3	39	25	49	49	31	22	1	2	5	226	75.33 ± 2.33^{c}
	96	300	12	44	16	34	35	32	19	7	2	3	204	68.00 ± 2.03^{d}
Total			46	137	66	115	167	110	69	23	6	10	749	
1.76%	24	300	15	40	11	13	36	13	22	13	2	6	171	57.00 ± 2.08^{a}
	48	300	13	31	7	25	30	27	23	9	2	2	169	56.33 ± 2.60^{b}
	72	300	19	44	16	48	54	44	19	5	0	0	249	83.00 ± 1.73^{c}
	96	300	7	42	18	55	44	32	33	18	2	2	253	84.33 ± 1.45^{d}
Total			54	157	52	141	164	116	97	45	6	10	842	
3.53%	24	300	18	36	17	14	51	27	26	4	4	1	198	66.00 ± 1.00^{a}
	48	300	9	40	6	24	31	29	18	12	2	1	172	57.33 ± 2.91^{b}
	72	300	19	47	15	58	62	46	24	4	0	0	273	91.00 ± 2.31^{c}
	96	300	24	33	26	60	57	48	21	3	3	2	277	92.33 ± 1.73^{d}
Total			70	156	64	156	201	150	89	23	9	4	920	

Volume : 3 | Issue : 12 | Dec 2013 | ISSN - 2249-555X

a, b, c and d: Significant difference at 24h, 48h, 72h and 96h 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.

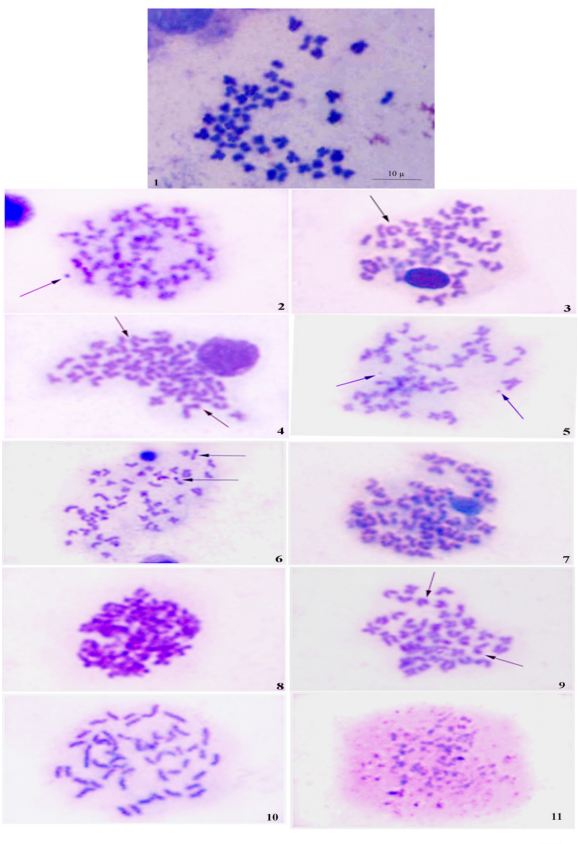
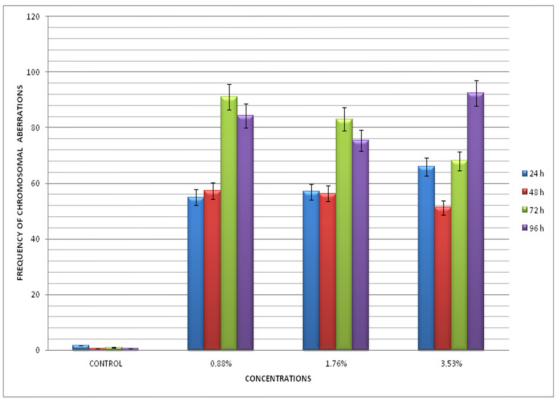


Fig.1 Normal metaphase complement, **Figs. (2-11) Chromosomal aberrations:** Cf= Chromosome fragmentation (Fig. 2), Rc= Ring chromosome (Fig. 3), Tcd= Terminal chromatid deletion (Fig. 4), M= Minutes (Fig. 5), Cg= Centromeric gaps (Fig. 6), Stk= Stickiness (Fig. 7), C= Clumping (Fig. 8), Py= Pycnosis (Fig. 9), Stch= Stretching (Fig. 10), P= Pulverization (Fig. 11).

Histogram:



Percent frequency of Chromosomal aberrations in *Labeo rohita* after treatment with tannery industry effluent.

REFERENCE

1. Al-Sabti, K. (1991) Handbook of genotoxic effects and fish chromosomes, edited by Kligerman A.D (Jozef Stefan Institute Press, Yugoslavia). | 2. Al-Sabti, K. and Kurelec, B. (1985) Induction of chromosomal aberrations in the mussel, Mytilus galloprovincialis. Bulletin of Environmental Contamination and Toxicolology, 35: 660-665. | 3. Al-Sabti, K. and Metcalfe, C.D. (1995) Fish micronuclei for assessing genotoxicity in water. Mutation Research, 343(2-3): 121-135. | 4. Finney, D. J. (1971) Probit analysis. Cambridge University Press, London. | 5. Hafez, A.M. (2009) Mugil cephalus Genome: A sensitive monitor for genotoxicity and cytotoxicity in aquatic environment. Australian Journal of Basic and Applied Sciences, 3(3): 2176-2187. | 6. Kumari, S.A. and Ramkumaran, S. (2006) Chromosomal aberrations in Channa punctatus from Hussainsagar Lake, Hyderabad (A. P). Indian Journal of fisheries, 53(3): 359-362. | 7. Mahmoud, A., Mohamed, Z., Yossif, G. and Sharafeldin, K. (2010) Cytogenetical studies on some River Nile species from polluted and nonpolluted aquatic habitats. Egyptian Academic Journal of Biology Sciences, 2(1): 1-8. | 8. Manna, G.K. and Sadhukhan, A. (1986) Use of cells of gills and kidney of Tilapia fish in micronucleus test. Current Science, 55: 498-501. | 9. Punjab Pollution Control Board. (1999) List of red category of industries, Punjab, India. | 10. Rose, M.H., Sudhakar, K. and Sudha, P.N. (2010) Effect of water pollutants on the freshwater fish, Hypophthalmicthys molitrix. The Ecoscan, 4(1): 31-35. | 11. Tijo, J.H. and Whang, J. (1965) Human Chromosome Methodology, Academic press, New York.