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IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|287-295

Research Article
Biological Sciences

ERYTHROCYTE ABNORMALITIES IN A FRESHWATER FISH, LABEO ROHITA EXPOSED TO TANNERY INDUSTRY EFFLUENT

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ABSTRACT

The erythrocyte abnormality test was analyzed in kidney cells of freshwater fish, Labeo rohita exposed to tannery industry effluent. The 96h LC_{50} value of tannery effluent against fish was calculated by Finney method and it comes out to be 7.07%. Three sublethal concentrations on basis of 96h LC_{50} value (3.53%, 1.76% and 0.88%) were selected. Fishes were exposed to these concentrations for 24h, 48h, 72h and 96h. Erythrocyte abnormalities (Nuclear and cellular) other than micronuclei have drawn major attention in recent years. Eleven types of abnormalities (5 nuclear and 6 cellular) were observed. Nuclear abnormalities included Nuclear Extrusion (NE), Blebbed (B), Binucleated (BN), Lobed (L) and Notched (N) nuclei, whereas cellular abnormalities included Enucleated (EC), Vacuolated (VC), Deformed (DC), Echinocytic (EC), Spindle shaped (SC) and Apoptotic (AC) cells. The importance of these erythrocyte abnormalities as biomarkers of exposures has not been previously exploited. In present study effort has been made to compare all erythrocytes abnormalities in fishes exposed to the sublethal concentrations of tannery industry effluent. 3.53% and 1.76% concentration of tannery industry effluent proved to be more toxic concentrations and induced abnormalities which damage erythrocytes completely. However, at 0.88% concentration erythrocyte abnormalities were seen but decreased at 96h as compared to other two concentrations. This concentration can act as safe concentration for disposal of effluent in aquatic ecosystem. The increase in erythrocyte abnormalities indicated that tannery industry effluent increased the clastogenic effects on peripheral erythrocytes of Labeo rohita and may have similar effects on the human population located around the river and consume fishes.

KEY WORDS

Erythrocyte abnormality test, aquatic pollution, tannery industry effluent, Labeo rohita.

1. INTRODUCTION

The aquatic environment plays a vital role in functioning of ecosystem. However, industrialization, urbanization and non-sustainable developments are posing serious threat to the aquatic system. Water bodies like rivers, lakes and ponds are polluted due to the discharge of industrial effluents, agricultural runoff and domestic wastes into them. In Punjab (India) due to industrialization the condition is more severe as River Satluj which is the largest and lifeline of the state is continuously being subjected to effluents from industries. Tannery industries located at Jalandhar contributes its major share of effluents in river Satluj. Further, tannery effluent is very toxic having foul smell and is damaging the aquatic flora and fauna. Fishes are excellent subject for the study of genotoxicity because they are in direct contact with the toxicants present in the water. They can also metabolize, concentrate and store water borne pollutants [1]. Fish erythrocytes are distinct because they possess a nucleus and their

interpretation in form of morphological changes became an important bioindicator of pollution. Erythrocyte abnormality test (change in nucleus and cell structure of erythrocytes) is one of the best diagnostic tools to judge the genotoxicity caused by the pollutants present in the aquatic ecosystem. Over recent years changes in erythrocyte nuclei have been increasingly used to evaluate genotoxic effects of various industrial effluents from textile mill, paper mill, peteroleum refinery and polycyclic aromatic. [2]. The formation of erythrocyte alterations especially nuclear alterations was first described by Carrasco *et al.* [3] and various other abnormalities like blebbed, Notched, Binucleate and Lobed nuclei have also been used as possible indicators of genotoxicity [4-6].

In the present study, genotoxic effect of tannery industry effluent on *Labeo rohita* has been done. Genotoxic evaluation in terms of erythrocyte abnormalities have not been previously studied in Punjab. Tannery industry is included in the list of red industries [7], and their effluents are highly toxic to the aquatic environment. There are about 30 tannery industries in Jalandhar which dump their effluents in Kala Sanghia drain which flows into chitti bein and then joins river Satluj. People living nearby have various skin diseases and are continuously exposed to this effluent. Family Cyprinidae is one of the largest families of freshwater fishes, having 2400 species belonging to 220 genera. These fishes are abundant in rivers of Punjab. Thus, they are ideal model to monitor genotoxic effect. *Labeo rohita* is selected because of its abundance, easy availability and high consumption by the people of Punjab. The main aims of the present study are 1) To determine LC_{50} for the preparation of three sublethal concentrations 2) To detect erythrocyte abnormalities (nuclear as well as cellular).

2. MATERIAL AND METHODS

2.1 Collection of specimen

Freshwater fish, *Labeo rohita* of about 6-8 cm in length and 32–58 gms in weight were collected from government fish seed farm Patiala in wide mouthed plastic bags containing freshwater and oxygen. They were acclimatized in laboratory for 20 days and were treated with 0.1% of $KMnO_4$ solution for 30 minutes to remove any external infections. They were fed with pelted feed and feeding was stopped 24h prior to commencement of genotoxicity tests. They were not fed during experimental periods.

2.2 Effluent from tannery industry

Effluent of tannery industry was taken directly from the waste outlet of an industry unit based at Jalandhar. Tannery industry effluent contains several complex organic and inorganic components like sulphides (sodium sulphide, sodium hydrosulphite and calcium hydrosulphide), sodium chloride, cyanides, dimethyl amines, chromium sulphate salts, oil, grease, alum salts and suspended solids [8].

2.3 Determination of LC_{50} and selection of concentrations

The 96h LC_{50} of tannery industry effluent was calculated by following the method given by Finney [9]. Three sublethal concentrations 3.53%, 1.76% and 0.88% (1/2, 1/4, 1/8 of 96h of LC_{50} value) were selected. Apparently healthy, uninjured and uninfected fish specimens were used.

2.4 Experimental design

Fishes were released in tubs containing water (control) and three sublethal concentrations (3.53%, 1.76% and 0.88%) for 24h, 48h, 72h and 96h by using the method given by Ayllon *et al.* [4]. A of total 50 fishes were used for the experiment.

2.5 Measurement of erythrocytes abnormalities

Kidney Blood was used to make smear on a clean slide. Erythrocytes abnormalities were classified according to Claxton *et al*. [10]. Five fishes were

used for each concentration. From each fish, four slides were prepared. For each hour 4000 cells (1000 cells from each slide) of control and treated groups were observed and photomicrographed.

2.6 Statistical analysis

Data of erythrocyte abnormalities 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 erythrocyte abnormalities for control and treated groups of each concentrations and durations was also evaluated. Frequencies of erythrocytes abnormalities were expressed as Mean (%) ± S.E.

3. RESULTS

The 96h LC_{50} value of tannery industry effluent against *Labeo rohita* came out to be 7.07%. In *Labeo rohita,* a normal erythrocyte is elliptical in shape with centrally placed nucleus in the clear cytoplasm (**Fig. 1**). Such normal erythrocytes were observed in control fishes. Erythrocyte abnormalities induced by effluent is summarized in the **Table**.

Nuclear abnormalities are considered as precursors of micronuclei whereas cellular abnormalities results into cell death. Five types of nuclear abnormalities *viz*., Nuclear Extrusion (NE, **Fig. 2**), Blebbed (B, **Fig. 3**), Binucleated (BN, **Fig. 4**), Lobed (L, Fig. 5) and Notched (N, **Fig. 6**) nuclei were observed. In 3.53% and 1.76% concentrations, nuclear abnormalities increased from 24h to 96h whereas in 0.88% concentration, these abnormalities increased from 24h to 48h then decreased upto 96h except in Lobed nuclei which decreased from 24h to 96h and Notched nuclei which increased from 24h to 72h then decreased sharply at 96h. Six types of cellular Abnormalities *viz*., Enucleated (EC, **Fig. 7**), Vacuolated (VC, **Fig. 8**), Deformed (DC, **Fig. 9**), Echinocytic (EC, **Fig. 10**), Spindle shaped (SC, **Fig. 11**) and Apoptotic (AC, **Fig. 12**) cells were observed. In 3.53% and 1.76% concentrations, abnormalities increased from 24h to 96h whereas in 0.88% Vacuolated, Deformed, Echinocytic and Apoptotic cells increased from 24h to 48h then decreased up to 96h, while Enucleated and Spindle shaped cells decreased from 24h to 96h.

Mean percentage of erythrocyte abnormalities increased with increase in concentration and duration of tannery industry effluent. At 72h of all the three concentrations, frequency of erythrocyte abnormalities rose steadily from 47.52 ± 0.66^{c} (0.88%) to 58.22 ± 1.15^{c} (1.76%) and 74.27 ± 0.88^{c} (3.53%). However, in extreme exposure (96h), the initial frequency of erythrocyte abnormalities increased from 41.82 ± 2.03^{d} to a maximum of 71.05 ± 0.66^{d} and 80.55 ± 2.52^{d} in 0.88%, 1.76% and 3.53% respectively as shown in Histogram. Thus, as the concentration increased, the cell alteration registered a quantitative increase with the increase of exposure time. Overall percent frequencies of cells with erythrocyte abnormalities in each concentration as well as control are shown in Pie Charts (a-d).

Table. Frequencies of erythrocyte abnormalities in kidney cells of *Labeo rohita* after treatment with tannery industry effluent.

Experimental groups	Duration of exposure (h)	T	Number of abnormal cells											t	Mean (%) ±S.E
			Nuclear abnormalities					Cellular abnormalities							
			NE	B	BN	L	N	EnC	VC	DC	EC	SC	AC		
Control	24	4000	0	0	12	0	0	0	0	12	0	15	13	52	1.30±0.33
	48	4000	0	12	11	0	0	0	0	13	0	16	15	67	1.67±0.88
	72	4000	0	0	0	11	0	12	0	18	0	15	0	56	1.40±0.66
	96	4000	11	12	0	19	0	0	0	15	0	13	13	83	2.07±0.88
Total			11	24	23	30	0	12	0	58	0	59	41	258	
Treated															
0.88%	24	4000	70	85	15	225	10	24	34	796	90	667	195	2271	56.77±1.45 [a]
	48	4000	137	115	35	155	54	15	134	507	138	423	273	2086	51.15±1.76 [b]
	72	4000	67	90	25	26	63	18	48	651	110	542	261	1901	47.52±0.66 [c]
	96	4000	116	99	28	141	44	7	114	425	122	323	254	1673	41.82±2.03 [d]
Total			390	389	103	547	171	64	330	2379	460	1955	983	7931	
1.76%	24	4000	86	74	11	60	8	10	18	347	79	606	73	1372	34.30±0.66 [a]
	48	4000	124	84	17	153	24	30	114	487	87	780	112	2012	50.30±0.33 [b]
	72	4000	136	96	21	183	37	61	127	533	96	864	155	2329	58.22±1.15 [c]
	96	4000	232	103	37	242	56	99	154	553	171	955	240	2842	71.05±0.66 [d]
Total			578	357	86	638	125	200	413	1920	433	3205	580	8555	
3.53%	24	4000	148	85	127	94	92	353	243	560	125	343	93	2263	56.67±1.20 [a]
	48	4000	159	110	172	115	154	461	288	610	139	391	135	2734	68.35±1.20 [b]
	72	4000	162	146	188	129	166	471	272	680	198	412	147	2971	74.27±0.88 [c]
	96	4000	188	162	196	142	178	479	302	711	255	450	159	3222	80.55±2.52 [d]
Total			657	503	683	480	590	1764	1105	2561	717	1596	534	11190	

a, b, c and d: significant differences at 24h, 48h, 72h and 96h 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.

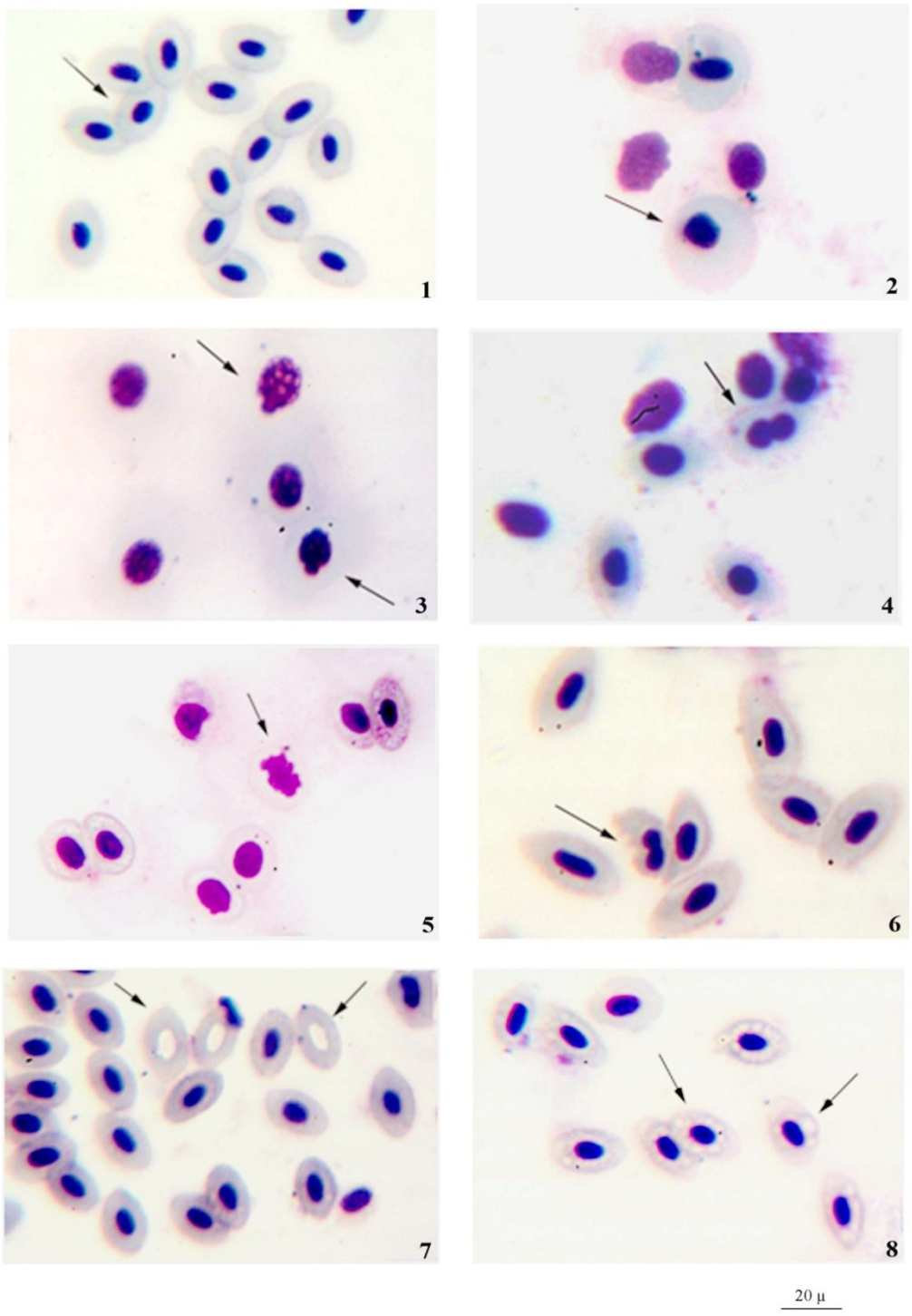


Fig. 1: Normal erythrocyte (Control), Erythrocyte abnormalities (Treated):

Nuclear abnormalities: Fig. 2: Nuclear extrusion, Fig. 3: Blebbed nuclei Fig. 4: Binucleate, Fig. 5: Lobed nuclei, Fig. 6: Notched nuclei

Cellular abnormalities: Fig. 7: Enucleated cells, Fig. 8: Vacuolated cell

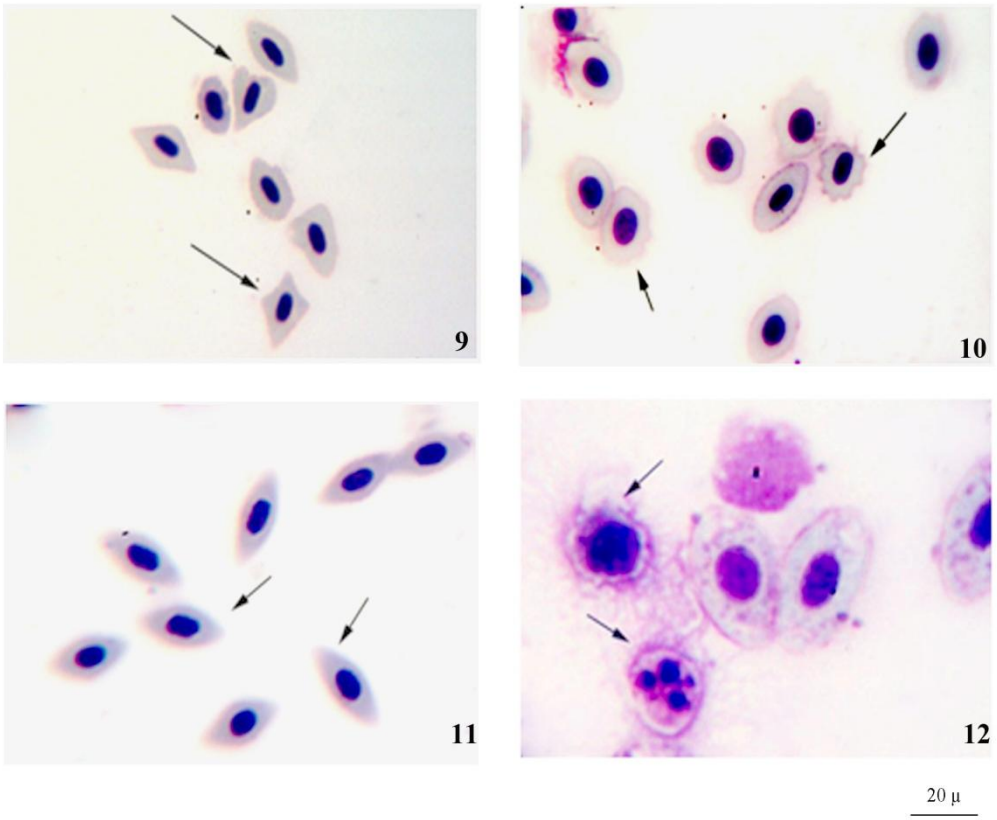
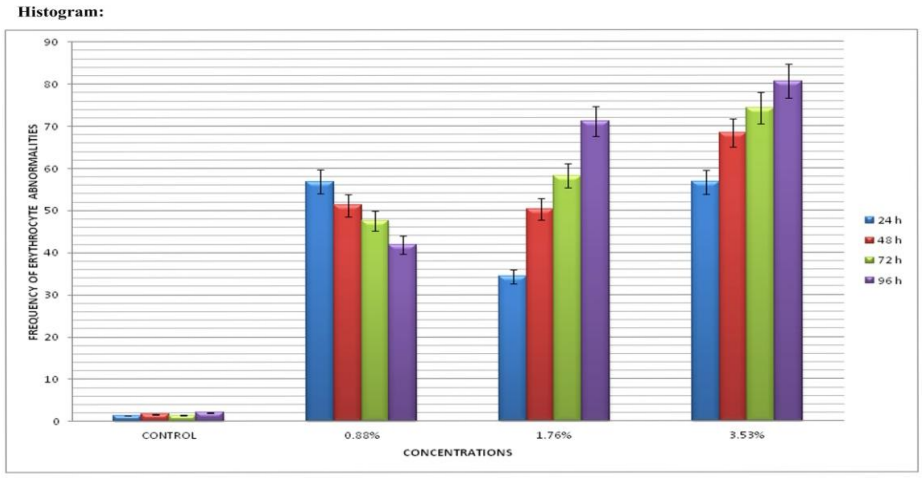
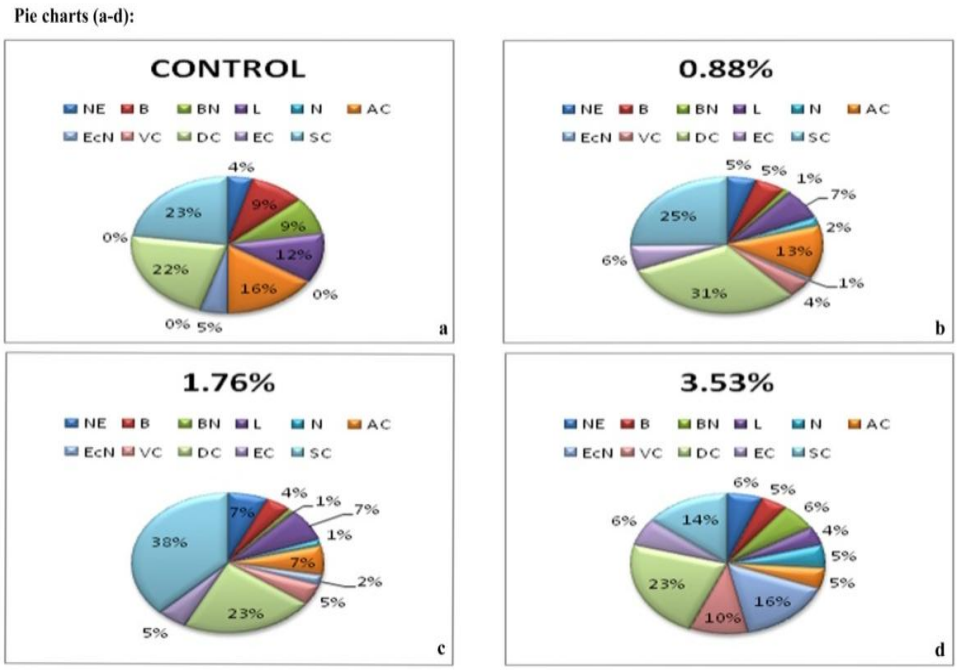


Fig. 9: Deformed cells, Fig. 10: Echinocytic cells, Fig. 11: Spindle shaped cells, Fig. 12: Apoptotic cells



Percent frequency of erythrocyte abnormalities in *Labeo rohita* after treatment with tannery industrial effluent.



Percent frequency of erythrocyte abnormalities in *Labeo rohita* after treatment with tannery industrial effluent.

4. DISCUSSION

The impact of industrial effluent on aquatic ecosystem is a growing problem and studies on industrial effluents indicated that genotoxicity tests have a viable role in environmental quality monitoring and control. Although there are large number of genotoxicity assays, only a relative number have been used for the evaluation of industrial effluents [10]. In Punjab there are no or minimal use of treatment plants for treating tannery industry effluents. Thus, untreated effluent is discharged into rivers which makes water turbid, emits foul smell, make oily layer and toxic. This toxic water from tannery industries of Jalander is carried by a rivulet (chittin bein) to River Satluj which ultimately damage aquatic flora and fauna.

Genotoxic effect of other industrial effluents have been reported by authors [5, 6, 11-14]. Cavas and Ergene-Gozukara [6] determined the increase in number of cells with nuclear abnormalities in fishes exposed to petroleum refinery and chromium processing factory effluent. Matsumoto *et al.* [15] found frequency of erythrocyte abnormalities to be more in *Oreochromis niloticus* exposed to water receiving Tannery industry effluent.

Eleven types of abnormalities were found in the present study. Increase in frequency of erythrocyte abnormalities exhibited time and concentration dependent response. Tannery industry effluent mainly contains chromium and heavy metals. Chromium undergoes intercellular reduction from chromium (VI) to chromium (III) and oxygen generates as highly reactive free radical which can react with DNA. Similar work has been reported by other authors and suggested that nuclear budding in interphase cause Blebbed and Lobed nuclei. The entire process represents the mechanism of elimination of amplified genes from the nuclei [16, 17]. Further, Von Sonntag and Steenken [18, 19] hypothesized that these abnormalities arise due to damage caused to the genetic material by

free radical produced under oxidative stress due to the toxicants. Aneuploidy is another abnormality that resulted due to tubulin failure and mitotic fuses caused by aneugenic actions of toxicants and resulted in formation of Binucleated cells and Notched Nuclei [20, 21]. Ateeq *et al.* [22] elaborated the sequence of cellular degradation under the impact of toxicants and also suggested that toxicants cause hypoxic conditions which result in depression of ATP that lead to abnormal shape of erythrocytes. Further, toxicants interrupted the lipid solubility of membranes of erythrocytes resulting in Vacuolated and Echinocytic cells and ultimately leading to apoptosis. Thus, presence of chromium and heavy metals induced such changes in fishes which are not reversed and cause cytotoxic damage resulting in death of fishes. Fishes are major protein source and reflect water quality. Their stock depletion reflects the water quality of major rivers.

5. CONCLUSION

The results obtained from this study Indicated that the tannery industry effluent being discharged into the river have genotoxic potential and is capable of causing significant ecological disruption in the receiving environment. The increase in the formation erythrocytes abnormalities (nuclear and cellular) in erythrocytes of *Labeo rohita* were identified as good genotoxic biomarkers for monitoring impact of industrial effluents in the environment. Since the assessement of cytotoxicity markers is quite helpful in terms of erythrocytes abnormalities. Examination of the biomarkers should be included into routine genotoxic survey to determine cytotoxicity at subcellular damage caused by environmental pollution. It is, therefore suggested that tannery industry effluent should be passed through treatment plants before being discharged into the aquatic ecosystem.

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