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A STUDY ON THE EFFECT OF ELECTROPLATING EFFLUENT ON THE HAEMATOLOGICAL AND GENOTOXICOLOGICAL ANALYSIS OF THE FRESHWATER FISH, *OREOCHROMIS MOSSAMBICUS*

Anju. K. Raj, Binu Kumari. S*, Mohan Kumar. M, Sujila. T, Drishya. M. K and Sruthi Gopinathan

PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore-641029, Tamilnadu, India.

***Corresponding Author Email: sbinukumari@gmail.com**

ABSTRACT

The study was carried out to investigate the acute and sublethal toxicity of electroplating effluent on haematological variables and genotoxicological changes of a fresh water fish, Oreochromis mossambicus under laboratory conditions. The 96 hour LC_{50} value of electroplating effluent to the fish, O.mossambicus was estimated by probit analysis method and was found to be 7.9 mg/l. (with 95% confidence limits). For sublethal studies a non-lethal dose of $1/10^{th}$ of 96-hour LC_{50} value (0.79mg/l) was taken. During acute and chronic exposure periods, Haematological variables like RBC, Hb, PCV, MCV, MCH, MCHC were significantly decreased in fish exposed to electroplating effluent. However, a significant increase in WBC value was observed in the exposed fish during above exposure periods when compared to that of the control groups. Comet assay damage occurred with water from electroplating industry showed increase in the DNA length. The DNA damage was determined by the length of comet tail. Increased tail length parallel by a reduction head size decreasing with treating time during 21 days exposure period.

KEY WORDS

Haematology, Genotoxic, Electroplating Effluent, Oreochromis mossambicus

I. INTRODUCTION

Water is the most precious of all resources in the lifeline of all living organisms on earth. Rivers are an important part of earth's life cycle. They play an efficient and prominent role in sculpting earth's topography by carrying huge quantities of water from land to sea. Increasing numbers and amount of industrial, agricultural and commercial chemicals discharged into aquatic environment have led to various deleterious effects on aquatic organisms including fish, accumulates pollutants directly from contaminated water and indirectly via the food chain.

Electroplating is considered as the major polluting industry because it discharges toxic materials and heavy metals through water, air emissions and solid waste in environment that is various processing industries have reported to contain high amounts of heavy metals such as nickel, iron, lead, zinc, chromium, cadmium and copper. The presence of heavy metals in industrial water

is letting out into different water sources and makes both environment and aquatic life stress.

Haematology is the science of studying the anatomical, physiological and pathological aspects of blood. The blood parameters in fishes are influenced by many factors. Quality of water, temperature, food availability and physiological status on fish either directly or indirectly. According to sex, season and age of fishes are directly reflected on blood parameters [1]. Haematology can be useful tool for monitoring health status, detecting illness, and following the progress of disease and response to therapy. Despite advances in fish medicine in recent years, interpretation of fish haematology often is hampered by a lack of meaningful reference values and the bewildering diversity of fish species. A multitude of intrinsic and extrinsic factors cause normal and abnormal variation in haematologic data. The article provides overview of some of the haematological abnormalities in fish induced by infectious agents and environmental, husbandry and nutritional issues.

The value of haematological parameters depend on season and slow or active movements fishes, reported that haematological parameters are influenced by microbial infection of fish toxicants. The recent technology that relaxed with haematology is comet assay. The comet assay and application in the field of ecotoxicology is a mature tool that continues to expand its perspectives.

Since Singh and colleagues in 1988 launched to the scientific community the alkaline Single Cell Gel Electrophoresis (SCGE) protocol, or comet assay, its uses and applications has been increasing [2]. The thematic areas of its current employment in the evaluation of genetic toxicity are vast, either in vitro or in vivo, both in the laboratory and in the environment, terrestrial and in the environment, terrestrial or aquatic.

The comet fish technique is a useful tool to detect overall and region-specific DNA damage and repair in individual cells. [3] It combines two well established methods, the comet assay and the technique of fluorescence in situ hybridization (FISH). Whereas the comet assay allows separating fragmented from non-fragmented DNA, FISH helps to detect specifically labeled DNA sequences of interest.

II. MATERIALS AND METHODS

Acclimatization of the fish

Fishes are maintained in a large tank and acclimatized to laboratory conditions for 21 days. Water was changed daily to maintain the oxygen content and to remove the excreta of fishes. Fishes were maintained at room temperature and fed with rice bran and oil cake daily, at least one hour prior to the replacement of tank water. Feeding was stopped one day prior to the experiment in order to keep the animal more or less in the same state of metabolic requirement.

Evaluation of median lethal concentration (LC_{50})

The concentration of pollutant at which 50% of the test animals die during a specific period or the concentration lethal to one half of the test population is referred as median lethal concentration (LC_{50}) or median tolerance limit [4]. Batches of 10 healthy fishes were exposed to different concentrations of electroplating effluent to calculate the LC_{50} value. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 10-50 ml of the electroplating effluent were choose and the number if dead or affected fish in each set up was counted at regular intervals up to 24 hours.

Appropriate narrow range of concentration 1-5 ml was used to find the median lethal concentration, using a minimum of 10 fishes for each concentration, using a minimum of 10 fishes for each concentration and mortality was recorded for every 24 hours up to 96 hours. It was found as 7.9 mg/l for 96 hours. For this stock solution various sub lethal concentration was prepared for bioassay study. Four groups of fishes were exposed to 0.79 mg/l (sublethal concentration of 96 hours LC_{50} value) concentration of electroplating effluent for 7, 14 and 21 days respectively. Another group was maintained as control at the end of each exposure period, the blood was collected from gills using syringe and anticoagulants were added the haematological parameters were analyzed. The RBC and WBC were counted by haemocytometer, Hb was

estimated by acid haematin method [5] and PCV was calculated employing standard method and formulate [6]. The mean corpuscular volume was calculated by using values of PCV% and the red blood cell counts expressed in µm-3 [7]. The mean corpuscular haemoglobin content was calculated by using the value of haemoglobin content and the red blood cell counts and expressed in pg. The alkaline comet assay was performed according to the method of [8].

III. RESULT AND DISCUSSION

In the present investigation the effect of electroplating effluent on haematological nature of RBC, WBC, HB, MCV, MCH, MCHC, PCV in the freshwater fish, *Oreochromis mossambicus.* The amount of RBC in the blood of the fish exposed to 7.9 mg/L electroplating effluent for 24, 48, 72 and 96 hours are 1.97, 1.51, 1.43 and 1.32 and for 7, 14 and 21 days are 1.21, 1.00, 0.27×10^6 /mm^3 and mean control was found to be 2.41×10^6 /mm^3 respectively. Decrease in the RBC may be due to the disruptive action of the effluent on the peripheral cell due to which viability of the cells was affected. The general reduction in blood parameter is an indication of anemia. The RBC count coupled with low haemoglobin content may be due to destructive action of pollutants on erythrocytes. The anaemic condition in fish may be detected using haematocrit [9].

The WBC values are found to be increased from the control. The values were 2300, 2900, 3000, 3100, 4000, 4500, 4700, 5200×10^6 mm^3 in the control of 24, 48, 72 and 96 hours and 7, 14, 21 days respectively. The increase in WBC in present study has been attributed to several factors like increase in thrombocytes, lymphocytes or squeezing of WBC's in peripheral blood. Increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of fishes exposed to toxicants. High WBC count indicates damage due to infection of body tissues, severe physical stress as well as leukemia. Similar increase was reported by [10] in *Channa punctatus* due to copper sulphate and potassium dichromate induces toxicity [11] [12]. The significant increase in the total leucocyte count might be due to immunological reaction to produce more antibodies to cope with the stress induced by the toxicant.

The level of Haemoglobin in the fish, *Oreochromis mossambicus* exposed to 21, 48, 72 and 96 hours are follows 2.7, 2.3, 2.1, 1.3 and for 7, 14, 21 days are 0.8, 0.7, 0.4 g % respectively and mean control was found to be 3.8g %. The reduction of Hb might be attributed to the blood coagulation. The decrease in haemoglobin concentration indicates the fish inability to provide sufficient oxygen to the tissues [13]. A specific toxic effect on fish blood and tissue occurs due to various chemical heavy metals and toxins which enter to the aquatic environment. Prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be described as pathological condition in fishes exposed to toxicants.

The amount of PCV in the blood of fishes exposed to 7.9 mg/L effluent for 24, 48, 72 and 96 hours are 8.9, 7.6, 7.4, 6.5 % and for 7,14, 21 days are 5.4, 4.0, 3.2 % and mean control was found to be 9.5 %. The values of MCV in fishes for 24, 48, 72 and 96 hours are 18, 16.9, 13.1, 12.6 μm^3 and for 7, 14, 21 days are 11.0, 10.2, 0.7 μm^3 and mean control was found to be 25.1 μm^3. The amount of MCH in the blood of the fishes exposed to 7.9 mg/L effluent was recorded 24, 48, 72 and 96 hours are 15.1, 13.4, 12.1 and 10.3 Pg and for 7, 14, 21 days are 9.2, 7.4, 6.1 Pg and the control was found to be 16.4 Pg.

The amount of MCHC recorded for 24, 48, 72 and 96 hours are 16.1. 14.3, 13.1, 12.0 and for 7, 14, 21 days are 10.1, 9.4, 7.3 respectively and for control, it was 18.0 g/ dL. The decrease in MCH & MCHC in the present study clearly indicates that the concentration of haemoglobin in RBC is reduced. MCH is a good indicator of RBC swelling [14]. The significant decrease in the MCHC values in the present study may be due to swelling of RBC or decrease in haemoglobin synthesis. The decreased MCV & MCH clearly indicate the hypochronic microlytic anaemia.

Effect of electroplating effluent on genotoxicological changes in the blood of the freshwater fish, *Oreochromis mossambicus* under long term exposure periods. The fishes treated with electroplating effluent showed an

increase in the DNA length. The DNA damage was determined by the length of the comet tail was increased with increasing the concentration of the electroplating effluent under damage DNA documented by the commet array test. Increased tail length parallel by a reduction in head size increasing with treating time during 21 days exposure period [15].

The comet assay and its application in the field of ecotoxicology is a mature tool that continues to expand its perspectives. Comet assay, its uses and application has been increasing. The thematic areas of its current employment in the evaluation of genetic toxicity are vast, either in vitro or in vivo, both in the laboratory and environment, terrestrial or aquatic. [16] It has been applied to a wide range of experimental models such as bacteria, fungi, cell culture, arthropods, reptiles, mammals and humans. This document is intended to be comprehensive review of what has been published. The genetic blood toxicity is maintained by this method. Effects of electroplating effluence on Genotoxicological changes in the blood of the fresh water fish, *Oreochromis mossambicus* under long term exposure periods.

The fishes treated with electroplating effluent showed an increase in the DNA length. The DNA damage was determined by the length of comet tail was increased with increasing the concentration of the electroplating effluent under damage DNA documented by the comet array test. Increased tail length parallel by a reduction in head size increasing with treating time during 21 days exposure period [17].

IV. CONCLUSION

From the above investigation it can be inferred that the aquatic animals are affected by the industrial electroplating effluent. Bioassay studies should be conducted for all possible pollutants on aquatic organisms to reach safe concentration. Since these are one of the great utilities in regulating and controlling the limits of pollutants to be discharged into water bodies. So we should create awareness among the people not to discharge the electroplating effluent directly to the waterbodies without treatment.

It is concluded that *O.mossambicus* exposed to electroplating effluent in different exposure period showed haematological changes and provoked DNA damage. Therefore, along term monitoring program of effluent discharge from industries into nearby aquatic water bodies would be valuable in the assessment of the potential biological risks to human health and the environment. It causes threat to fish survival and also affect the aquatic ecosystem.

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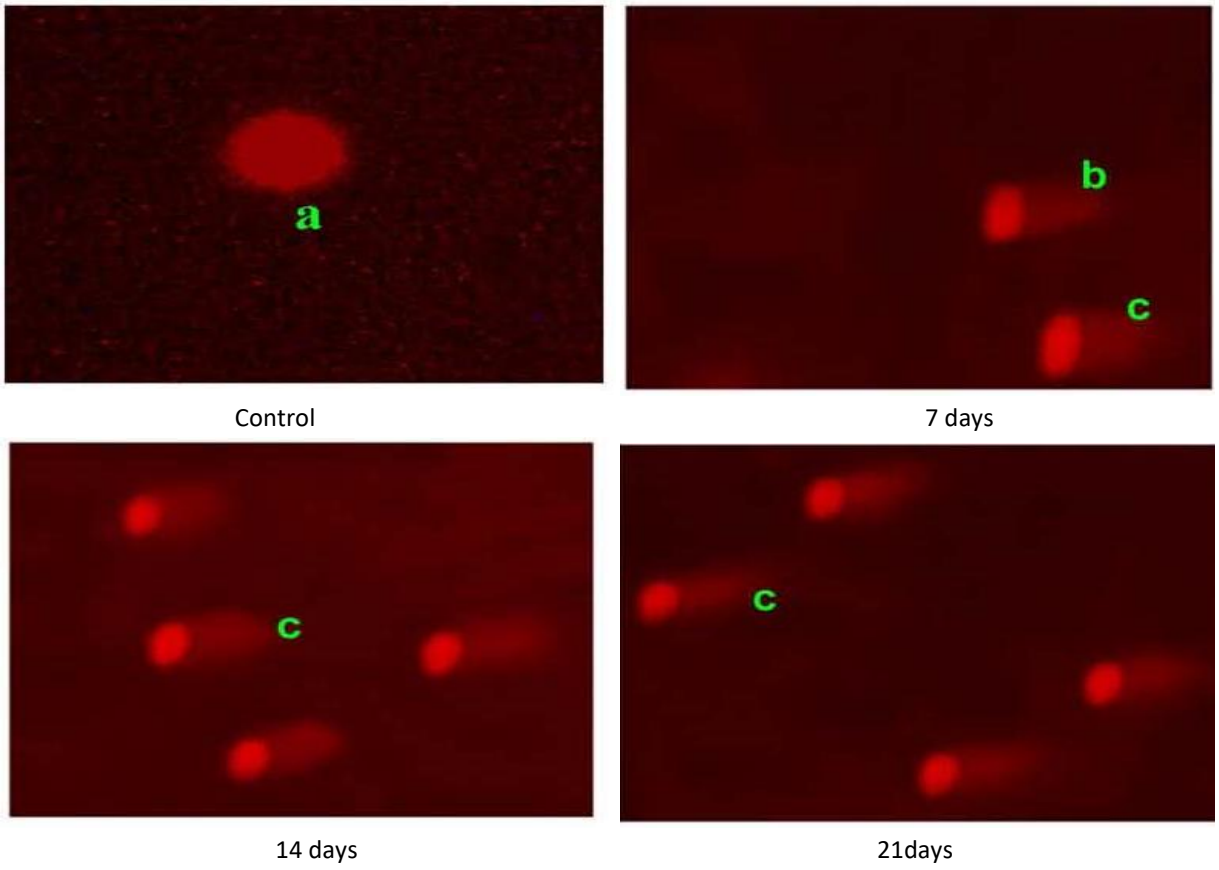
Table.1. Effect of electroplating effluent on haematological parameters in blood of the fresh water fish, *Oreochromis mossambicus*

Sample (mg/g wet tissue)	EXPOSURE PERIODS							
	CONTROL	24HRS	48HRS	72HRS	96HRS	7 Days	14 Days	21 Days
RBC count	2.41 ± 0.80	1.97 ± 0.47	1.51 ± 0.96	1.43 ± 0.92	1.32 ± 0.91	1.21 ± 0.88	1.00 ± 0.85	0.07 ± 0.05
't' value		13.98**-	13.09**	11.23**	14.09**	14.22**	14.41**	15.34**
%		-18.26	-37.34	-40.23	-45.23	-49.79	-58.51	-97.1
WBC count	2300 ±12.24	2900 ± 15.57	3000 ± 22.43	3100 ± 28.84	4000 ± 32.59	4500 ± 39.67	4700 ± 42.97	5200 ± 45.31
't' value		96.45**	104.63**	108.42**	143.50**	187.31**	193.64**	210.63**
%		34.78	30.43	26.09	73.91	95.65	104.35	126.09
Hb (gm %)	3.8 ± 0.14	2.7 ± 0.11	2.3 ± 0.15	2.1 ± 0.12	1.3 ± 0.09	0.8 ± 0.10	0.7 ± 0.05	0.4 ± 0.03
't' value		12.45**	14.05**	15.07**	16.55**	17.49**	17.12**	18.75**
%		-28.25	-39.47	-44.74	-65.79	-78.95	-81.58	-89.47
PCV count (%)	9.5 ± 0.27	8.9 ± 0.28	7.6 ± 0.26	7.4 ± 0.25	6.5 ± 0.21	5.4 ± 0.19	4.0 ± 0.17	3.2 ± 0.19
't' value		9.12**	10.75**	10.97**	11.36**	12.15**	13.21**	14.82**
%		-6.32	-20	-22.11	-31.58	-43.16	-57.89	-66.32
MCV(μm^3)	25.1 ± 0.54	18.0 ± 0.48	16.9 ± 0.29	13.1 ± 0.19	12.0 ± 0.11	11.0 ± 0.10	10.2 ± 0.12	0.7 ± 0.09
't' value		29.93**	31.22**	35.74**	36.42**	37.14**	38.61**	43.57**
%		-28.29	-32.67	-47.81	-52.19	-56.18	-59.36	-97.21
MCH (pg)	16.4 ± 0.35	15.1 ± 0.31	13.4 ± 0.15	12.1 ±0.13	10.3 ± 0.10	9.2 ± 0.11	7.4 ± 0.08	6.1 ±0.08
't' value		14.81**	17.13**	17.92**	19.63**	20.06**	22.71**	22.89**
%		-7.93	-18.29	-26.22	-37.2	-43.9	-54.88	-62.8
MCHC(g/dL)	18.0 ± 0.41	16.1 ± 0.38	14.3 ± 0.28	13.1 ±0.17	12.0 ±0.15	10.1 ±0.12	9.4 ± 0.09	7.3 ± 0.05
't' value		13.39**	15.15**	15.92**	16.23**	18.78**	19.33**	21.57**
%		-10.56	-20.56	-27.22	-33.33	-43.89	-47.78	-59.44

Results are mean (±SD) of five observation

Values are mean ±SD, n=5 *- Significant at 5% (t<0.05) **- significant 1% (t<0.01) Ns- Non-significant

Fig 1: Appearances of comets in peripheral blood erythrocytes of *Oreochromis mossambicus* exposed to electroplating effluent at long term exposure periods



Control 7 days

14 days 21days

a. Undamaged (Control)
b. Slightly damaged
c. Nuclei from erythrocytes of fish consist of a head with DNA migration into the tail region

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***Corresponding Author:**
Binu Kumari. S
Email: sbinukumari@gmail.com