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Research Article

Detection on Electroplating Effluent-Induced Cytopathological Alterations and DNA Damage in the Blood Samples of *Cirrhinus mrigala* (Hamilton, 1822)

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Abstract

In the present study, fresh waterfish *Cirrhinus mrigala* has been used as a model organism to understand the mechanistic link between electroplating industrial effluent (EIE) and oxidative stress in aquatic ecosystems. To this end, the fishes were exposed to industrial effluent, in the tank water for 10, 20 and 30 days under controlled laboratory conditions. Various hematological parameters (Total RBC and WBC, Hemoglobin, MCV, MCH, PCV), biochemical metabolites (Glucose, protein, Cholesterol) and markers of genotoxicity (comet assay and micronuclear assay) were assessed. Fishes exposed for 30 days showed marked increase ($p < 0.001$) in RBC with concomitant decrease in total WBC. There is significant ($p < 0.001$) decline in indices of blood cells such as Hb, MCV, MCH and PCV. Results from comet assay showed significantly ($p < 0.05$) increased frequency genotoxicity in erythrocytes of as evident from observed increase in tail length in fishes exposed to EIE for 30 days. Overall, the results of this study, clearly demonstrated concentration dependent response of electroplating industrial effluent on freshwater fish *Cirrhinus mrigala* on haematological, biochemical and genotoxic markers. The results obtained herein suggests EIE as potential xenobiotics contributing towards ecotoxic and genotoxic effect in fishes.

Keywords: Electroplating industrial effluent (EIE); *Cirrhinus mrigala*; Hematological; oxidative stress; Genotoxicity

Introduction

Fish serves as an excellent model organism in understanding aquatic toxicology involving diverge nature of toxicants [1,2]. Electroplating Industrial Effluent (EIE) has redox potential to suppress immune system and induce oxidative stress in fishes [3,4]. Genotoxic potential of electroplating effluenthas been reported in humans, rodents and fish cell lines [5-8]. In the current scenario, the assessment of the genotoxicity of electroplating effluentin terrestrial and aquatic ecosystems has emerged as major thrust area of research and there is increased trend to develop methods for detection of genotoxic effects for ecotoxicological applications [9]. Though, many methods like micronucleus test, chromosomal aberrations and DNA damage assays have been used for assessing genotoxicity of various chemicals in different animals, the DNA damage (comet assay) protocol is known to be simple, sensitive, more reliable and cost effective, and has been used to investigate the genotoxic potential of toxicants in the environment [10]. Influx of unwanted substances like toxic metals, into water bodies causes physical, chemical and biological changes and ecological imbalance. Heavy metal, released by both natural and anthropogenic process induces oxidative stress. This heavy metal induced oxidative stress, is not only prevalent in aquatic ecosystems worldwide, but has been reported in many fresh water species even within Indian subcontinent [11-13]. Apart from oxidative stress, hematological tests are important diagnostic tools and valuable indicators of disease or stress due to pollutants and environmental fluctuations. Since Electroplating Industrial Effluent (EIE) is involved in creating heavy metal pollution by direct discharge of effluent into water bodies, piscine haematology can be very useful in assessing the health status and changing environmental conditions. As such, hematological alterations in fishes have taken shape as an important tool in studying both general physiological states of fishes as well as environmental quality [14,15]. Fish erythrocyte is distinct from mammalian erythrocytes because they possess a nucleus and their interpretation in the morphological changes is an important bioindicator of pollution. Various abnormalities like bilobed, notched, binucleated and lobed nuclei also serves as an indicators of genotoxicity [16]. According to Matsumoto et al. [17,18] the comet assay is sensitive to be used for monitoring quality of the contaminated water with effluents containing heavy metals. In view of the background information wherein hematological parameters including oxidative stress has a significant role in fishes exposed to EIE and scarce scientific data on the genotoxic potential of electroplating effluent in aquatic animals, the present inventory was taken up with the following objectives: (1) To investigate the effects of sub lethal concentration of electroplating effluent on oxidative stress, hematological, serum biochemical and genotoxicity of the fresh water fish, *Cirrhinus mrigala* on short and long term exposure. (2) To unravel the relationship, if any between oxidative stress and DNA damage.

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Materials and Methods

Experimental animals

C. mirgala, commonly known as Indian mrigal carp, was selected as an experimental animal model. The fish (average length 7.4 ± 0.54 cm average weight 9.2 ± 0.85gm) were procured from Aliyar fish farm, Tamil Nadu, India, and acclimatized to the laboratory conditions for 15 days. The fish were placed in glass aquaria with tap water (pH 7.2 ± 0.6, temperature 28.2 ± 0.5°C, dissolved oxygen 3.65 mg/L, conductivity 222 µs/cm, Total Suspended Solids (TSS) 105.6 mg/L and salinity 0%). No mortality was observed during the acclimatization. The fish were fed with aquarium flake food twice a day and 12 hr light and 12 hr dark photo period was maintained during the acclimatization.

Experimental design

After acclimatization for 2 weeks the fish were divided into four groups (n = 15). One group was served as control and the other three are exposed groups for three different time durations of 10, 20 and 30 days. Fishes were exposed to a mixture of electroplating effluent on fresh water containing 100% solution. The experiment was planned in such a way that the fish from all the groups were sacrificed on the same day. Level of Lipid Peroxidation (LPO), Reduced Glutathione (GSH) and activities of enzymic-antioxidants Superoxide Dismutase (SOD, Catalase (CAT) and Glutathione Peroxidase (GPx). The activities of lipid peroxidation (Abcam), reduced glutathione (Thermofisher), and superoxide dismutase, catalase and glutathione peroxidase (Sigma Aldrich) were assessed as per manufacturer's instructions.

Haematological analysis

Hemoglobin was estimated by acid haematin method [19]. Red Blood Cells (RBC) and White Blood Cells (WBC) were counted using the improved Neubaurhaemocytometer [20]. Hence blood was diluted (1:200) with Hayem's fluid [21]. Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 106 mm^3 (Wintrobe, 1967). White Blood Cells (WBC) were counted using animproved Neubaurhaemocytometer [20,22]. Blood was diluted (1:20) with Turk's diluting fluid and placed in haemocytometer, 4 large (1 sq.mm) corner squares of the haemocytometer were counted under the microscope (Olympus Microsystems). The total number of WBC was calculated in mm^3 x 10^3 [23]. The Mean Corpuscular Volume was calculated by using values of PCV% and Red Blood Cell counts and expressed in µm [24]. Blood cell was sucked into heparinized haematocrit capillary tube (7.5 cm length, 0.1 cm width). After sealing both the sides of the tube it was centrifuged in the microhaematocrit centrifuge at 6000 rpm for 2 min. From the volume of blood taken and cell volume after centrifugation, the PCV percentage was calculated employing standard method and formula [25]. Mean Corpuscular Haemoglobin (MCH in pg), was calculated as (Hb x 10)/ RBC.

Biochemical analysis

The blood samples were collected from caudal vein of the fish, *C.mrigala*. The blood samples (without EDTA) was centrifuged for 10 minutes at 4000 rpm, supernatant serum was decanted and stored at -2°C. Protein, glucose and cholesterol concentration was measured according to the Lowry et al., [26]; glucose test kit (AGAPPE Diagnostics, India) using GOD-PAP methodology [27] and serum cholesterol was analyzed using cholesterol test kit (AGAPPE Diagnostics, India) based on CHOD-PAP methodology [28] respectively.

Genotoxicity analysis

Blood was smeared on two clean glass slides, air dried for 24 h, then fixed in absolute methanol for 10 min. After fixing, the same slides were stained in 4% Giemsa for 10 min, air-dried and then prepared for permanent use. The Giemsa solution was centrifuge and filtered before staining to reduce precipitation that could interfere with the analyses. Micronuclei were identified and scored with an optical microscope (1000x). Two thousand erythrocytes were scored for each specimen (1000/slide) to determine the frequency of micronucleated erythrocytes. Micronuclei has to be smaller than one-third of the main nuclei, clearly separated from the main nuclei, and had to be no-refractive small nuclei (> 1/3 of the main nucleus) with intact cytoplasm [29]. The presence of other nuclear abnormalities in erythrocytes was also analyzed. The frequency of Micronuclei (MN) was calculated as (number of cells containing micronuclei X 1000) total number of cells counted.

Comet assay

Comet assay was performed according to the protocol that had been previously described by Singh et al., 1988 and modified by Tice et al., [30,31] Blood samples were diluted with 1 ml of PBS. 60 µl of the diluted and mixed with 200 µl of 0.65% Low Melting-Point (LMP), agarose 75 µlof the mixture were then layered on the slides precoatedwith 0.5% Normal- Melting Point (NMP) agarose and immediately covered with cover slip and then kept for 10 min in a refrigerator to solidify. After gently removing the cover slips, the slides were covered with a third layer of 90 µlow-melting-point agarose and covered with cover slips again. After solidification of the gel, cover slips were removed and slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mMTris, pH 10 with 10% DMSO and 1% Triton X-100 added fresh) and refrigerated at 4°C for 2 h. After lysis, the slides were placed on a horizontal electrophoresis box side by side. The mixture was incubated for 5min, and thecell suspension was transferred into another tube by avoiding debris. The number ofcells in the cell suspension were counted using hemocytometer and pelleted at 4°C. The pellet was suspended in 1ml of ice cold PBS at 1×105 cells/ml. Molten agarosewas prepared in a boiling water bath, cooled down to 37°C and mixed with isolatedcells in a 1:10 ratio in a eppendorf tube. Seventy-fiveµl of the mixture of agarose andcells were taken on comet slides. The comet slides were placed in dark for 10 min at 4°C to solidify the gel. After 10 min, the slides were placed in lysis solution containingdimethyl sulfoxide, for 30 min at 4°C. Then the excess solution was removed andthe slides were placed in alkaline solution to denature the DNA for 40 min at roomtemperature. After 40 min, the slides were subjected to electrophoresis in Tris Borateelectrophoresis buffer (TBE) with 1 volt/cm current between the two electrodes for10 min. After 10 min of electrophoresis, the slides were fixed with 70% ethanol for 5min. The slides were stained with syber green and air-dried. Control comet slides were prepared along with the exposed cells comet slides. The whole process was done under yellow light in order to minimize the UV light damage. The processed slides were examined for DNA damage using an epifluorescent microscope (OlympusBX51 TRF, USA). Blood samples of fishes were analyzed per treatment. In each of

the fishes, a minimum of 75 individual cells per sample were screened, and a total of 225 individual cells (triplicate) were examined. The data were analyzed using a DELL computer equipped with a DNA damage analysis software(Loats Associates Inc., USA).

Statistical analysis

The data was analyzed using SPSS/PC+ Statistical package (version 11.5). Significant difference between control and experimental groups were determined using Duncan's test were performed to determine if there were significant differences among and between treatment groups. Significant differences were considered at $p < 0.05$.

Results

Level of lipid peroxidation, glutathione and Enzymic-antioxidants

Table 1 and 2 shows level of lipid peroxidation, glutathione content and alterations of antioxidant enzymes in erythrocytes and gills of *C. mrigala* exposed to Electroplating Industrial Effluent (EIE) for 10, 20 and 30 days. There is duration dependent increase in lipid peroxidation and depletion in glutathione with most pronounced effect seen on 30 day exposure. With regard to enzymic antioxidants, 10 day exposure showed significant ($p<0.001$) increase in the activities of SOD, CAT and GPx whereas both 20 and 30 day exposure showed decline consistently in both blood as well as gills. Oxidative stress was maximum in fishes exposed to EIE for 30 days.

Haematological analysis

The Red Blood Corpuscles (RBC)and White Blood Corpuscles (WBC) levels showed highest percentage decrease and increased in blood (-88.24%; 100.78%) during long term exposure for 30 days was a significant increase ($p < 0.005$) compared to control which are presented in (Figure 1). The Haemoglobin (Hb) content is demonstrated in (Figure 2a). The Hb level were showed highest percentage decrease in blood (100.78%) during long term exposure at 30 days of treatment groupswhich was a significant increase ($p<0.005$) compared to control. The Mean Corpuscular Volume (MCV) in content were observed in the electroplating effluent in treated with long term exposure period of fish showed significant decrease ($P<0.05$). MCV were decrease count of fish treated of the electroplating effluents compare to the control (Fig 2b). The Mean Corpuscular Haemoglobin (MCH) content were observed in the electroplating effluent in treated fish at different exposure period showed significant decrease ($P<0.05$). MCH were decrease count of fish treated with long term exposure of the electroplating effluents compare to the control (Figure 3a). Similarly PCV level was observed significant decrease ($p < 0.005$) at 30 days compared to control (Figure 3b).

Table 1: Effect of electroplating effluent (EIE) on the level of lipid peroxidation, glutathione content and activities of the antioxidant enzymes in erythrocytes of *Cirrhinus mrigala* exposed to electroplating effluent during for 10, 20 30 days.

Biochemical parameters	Control	Exposure Period		
		10 days	20 days	30 days
LPO	2.4 ± 0.17	2.83 ± 0.18[a]	3.33 ± 0.17[a]	3.77 ± 0.32[a]
GSH	3.24 ± 0.22	2.68 ± 0.25[a]	2.26 ± 0.14[a]	1.61 ± 0.12[a]
SOD	6.60 ± 0.42	7.34 ± 0.43[a]	4.25 ± 0.22[a]	3.08 ± 0.25[a]
CAT	1.80 ± 0.10	2.07 ± 0.13[a]	1.04 ± 0.08[a]	0.79 ± 0.06[a]
GPx	2.40 ± 0.10	2.61 ± 0.14[a]	1.51 ± 0.10[a]	0.88 ± 0.04[a]

Mean ± SD of three observations. The values that do not share the same superscript letter (a, b, c) are significant different from control group at ([c]p<0.05, [b]p<0.01 and [a]p<0.001). Units: GSH (mg/gm Hb), LPO (nM of MDA formed / g Hb/ Min), SOD (IU / gm Hb where one unit is equal to the amount of enzyme required to inhibit auto oxidation of pyrogallol by 50%, %), CAT (k/gm Hb / s), GPx (mmoles of GSH utilized/ g / Hb/ s).

Table 2: Effect of electroplating effluent (EIE) on the level of lipid peroxidation, glutathione content and activities of the antioxidant enzymes in gills of *Cirrhinus mrigala* exposed to electroplating effluent during for 10, 20, 30 days.

Biochemical parameters	Control	Exposure Period		
		10 days	20 days	30 days
LPO	30.40 ± 1.37	34.96 ± 2.10[a]	41.22 ± 1.98[a]	49.46 ± 2.77[a]
GSH	1.60 ± 0.08	1.42 ± 0.07[a]	0.96 ± 0.05[a]	0.68 ± 0.03[a]
SOD	7.70 ± 0.39	9.08 ± 0.62[a]	4.62 ± 0.24[a]	3.15 ± 0.15[a]
CAT	2.40 ± 0.11	2.72 ± 0.18[b]	1.79 ± 0.09[a]	1.28 ± 0.06[a]
GPx	0.80 ± 0.04	0.91 ± 0.06[a]	0.60 ± 0.03[a]	0.52 ± 0.03[a]

Mean ± SD of three observations. The values that do not share the same superscript letter (a, b, c) are significant different from control group at ([c]p<0.05, [b]p<0.01 and [a]p<0.001). Units: GSH (µg per mg tissue), LPO (nM of MDA formed per mg protein), SOD (units per mg protein where one unit is equal to the amount of enzyme required to inhibit auto oxidation of pyrogallol by 50%, %), CAT (µmoles of H_2O_2 consumed per min per mg protein), GPx (µg of reduced glutathione utilized /min/ mg protein.

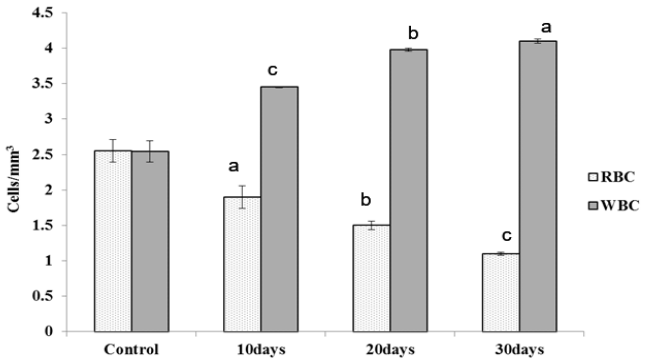

Figure 1: Changes in RBC and WBC level in the blood of *Cirrhinus mrigala* exposed to induced concentration of electroplating effluent for 10, 20 and 30 days. Each point represents a mean values and standard deviation of three replicates. Values that do not share the same superscript letter (a, b, c) are significant different from control group at ([c]p<0.05, [b]p<0.01 and [a]p<0.001).

Biochemical analysis

The total Glucose, Protein and Cholesterol content in blood of fish, *C. mrigala* were studied and control and treatment groupsare presented in (Table 4). The results were statistically analyzed and showed highest percentage decrease in blood (28.51%) during long term exposure 30 days were noted. Whereas, the amount of protein were statistically analyzed and showed highest percentage decrease in blood (83.44%) during long term exposure of 30 days. Similarly, the value of cholesterol was found to be significant in blood and maximum percentage decrease (62.76%) during long term exposure after 30 days.

Micornucleus assay

The frequencies of micronuclei in peripheral blood erythrocytes of fish, *C. mrigala* induced by the electroplating effluent are presented (Figure 4a). A total number of cells was scored per experimental fishes. The frequency of micronuclei of fishes treated with electroplating

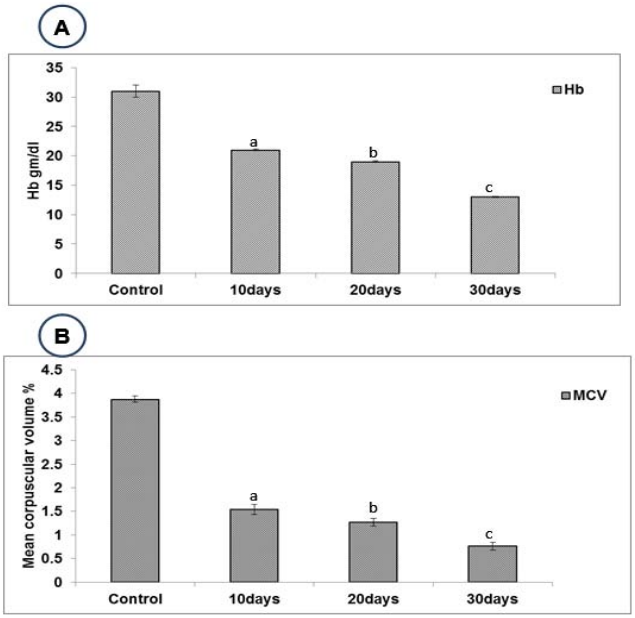

Figure 2: A and B. Changes in Hb and MCV level in the blood of *Cirrhinus mrigala* exposed to induced concentration of electroplating effluent for 10, 20 and 30 days. Each point represents a mean values and standard deviation of three replicates. Values that do not share the same superscript letter (a, b, c) are significant different from control group at (^{c}p<0.05, ^{b}p<0.01 and ^{a}p<0.001).

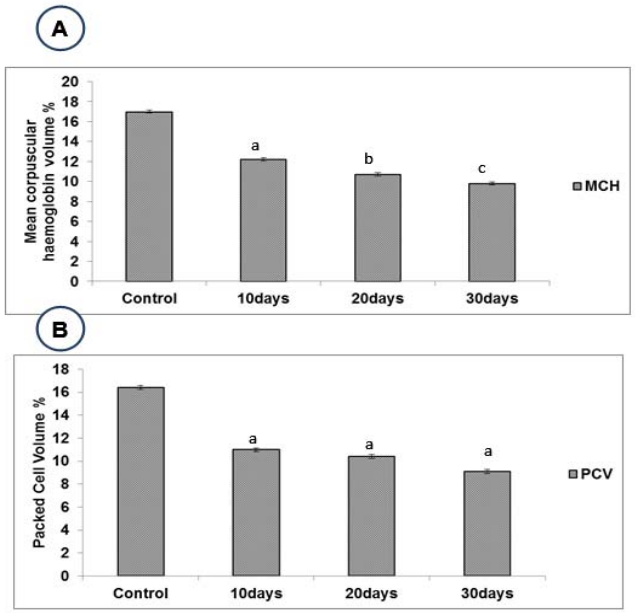

Figure 3: A and B. Changes in MCH and PCV level in the blood of *Cirrhinus mrigala* exposed to induced concentration of electroplating effluent for 10, 20 and 30 days. Each point represents a mean values and standard deviation of three replicates. Values that do not share the same superscript letter (a, b, c) are significant different from control group at (^{c}p<0.05, ^{b}p<0.01 and ^{a}p<0.001).

effluent during long term for 30 days showed significant increase as against control. The frequency of micronuclei ranged between 0.899 to 16.99 in the blood of fishes on exposure to electroplating effluent. Among the different exposure periods, the frequency of micronuclei was higher in 30 days exposure period compared to the control.

Table 3: Same physical and chemical parameters of the experimental water.

Water	
Illumination	16 h with florescent lamps (daylight 65/80W)
Temperature	21.2 ± 1 °C (YSI 550 A temperature meter)
Total hardness	340 ± 4.8 mg $CaCO_3$/L (EDTA titration method)
Total alkalinity	319 ± 0.5 mg $CaCO_3$/L (acidimetry method)
Turbidity	1.5 ± 1 (NTU-nephelometric turbidity units)
Dissolved oxygen	6.46 ± 0.6 mg/L (YSI 550 A oxygen meter)
Total dissolved solid content	660 ± 2.3 mg/L $CaCO_3$/L (EDTA titration method)
pH	7.6 ± 1 (WTW pH 330i meter)

Table 4: Biochemical changes in Blood sample of *Cirrhinus mrigala* exposed to electroplating effluent during for 10, 20 30 days.

Biochemical parameters	Control	Exposure Period		
		10 days	20 days	30 days
Glucose	52.60 ± 0.16	36.50 ± 0.16^{a}	29.70 ± 0.16^{b}	19.10 ± 0.16^{c}
Protein	6.10 ± 0.02	2.99 ± 0.02^{a}	1.69 ± 0.02^{b}	1.01 ± 0.02^{c}
Cholesterol	61.50 ± 0.16	35.40 ± 0.16^{a}	28.50 ± 0.16^{b}	22.90 ± 0.16^{c}

Mean ± SD of three observations. The values that do not share the same superscript letter (a, b, c) are significant different from control group at (^{c}p<0.05, ^{b}p<0.01 and ^{a}p<0.001).

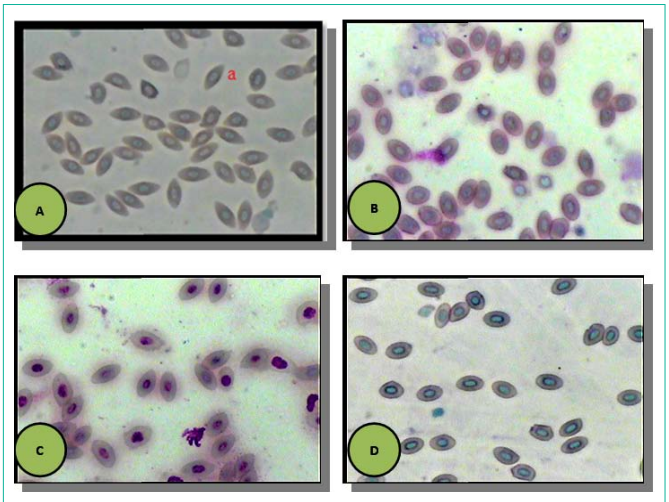

Figure 4: Photomicrography showing different micronuclei abnormalities of *Cirrhinus mrigala* in control (A) and experiment (B-D) groups. (A) General view of the blood sample. Note Normal shapes micronuclei. (B) 10 days exposure. Section of blood sample exposed to electroplating effluent for 10 days showing club shaped micronuclei. (C) 20 days exposure. It rupture of surface micronuclei and blood vessels damaged. 30 days exposure. Widespread blood tissue damage and loss of normal architecture (arrowhead) is remarkable (Scale bar = 50 µm).

Different types of micronuclei were observed (Figure 4b) and these were categorized into small micronuclei, large micronuclei, two micronuclei in a cell and notched nuclei.

Genotoxicity

The result of the comet assay on peripheral blood erythrocytes of fish, *C.mrigala* at different duration periods are presented in (Figures 5a,b).The fishes treated with electroplating effluent showed an increase in the DNA length and percentage of DNA damaged cells compared to the control. The SCGE assay showed an increase in two

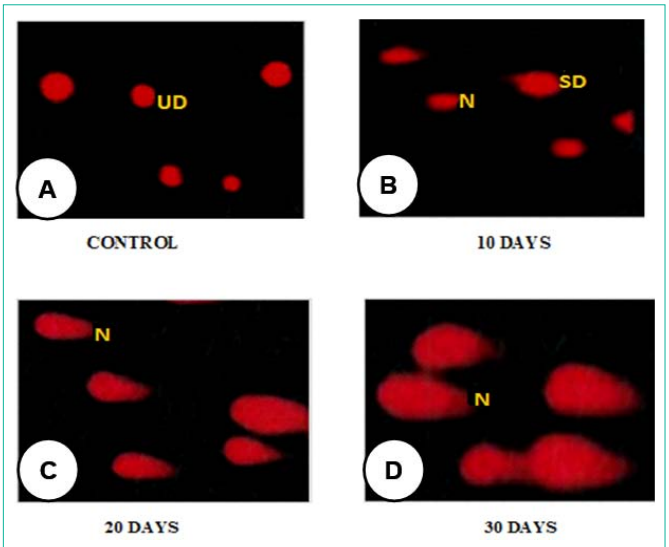

Figure 5: Light microscope appearance of comets in peripheral blood erythrocytes of *Cirrhinus mrigala* in control (A) and experiment (B-D) groups. (A) General view of the blood sample. Note Normal shapes nuclei from erythrocytes. (B) 10 days exposure. Section of blood sample exposed to electroplating effluent for 10 days showing rupture of nuclei. (C) 20 days exposure. It produced marked central nuclei damaged. 30 days exposure. Arrow indicates nuclei damage head with DNA migration into the tail region (Scale bar = 50 µm).

comet parameters in exposed fish where the genome damage was significantly different ($P < 0.05$) from control fish. The percentage of DNA damaged cells were ranged .The DNA damage was determined by the length of comet tail. The length of the comet tail was increased with increasing the concentration of the electroplating effluent. Under damage DNA documented by the comet assay test increased tail length parallel by a reduction in head size increased with treating time during 30 days exposure period.

Discussion

Electroplating Effluent (EIE) induced decrease in antioxidant defense, alterations in hematological parameters and marked genotoxicity exhibited by DNA damage and microscopic morphological changes in the blood samples of Indian mrigal carp *Cirrhinus mrigala*, were some of the hall mark features of this study. Increased level of lipid peroxidation and decreased glutathione was observed in both blood and gills of across all experimental groups irrespective of the treatment duration. However the level of antioxidant enzyme showed and altogether different trend. While 10 days of exposure increased the activities Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx), the enzymic antioxidants decreased in both 20 and 30 days of exposure to EIE. The decreased glutathione as observed in the present study may possibly due to its utilization in counteracting the deleterious effects of free radicals as documented by Akram et al., [4] in fresh water bighead carp (*Aristichthys nobils*) when exposed to bisphenol A. According to Halliwell and Gutteridge [32], Reactive Oxygen Species (ROS) mediated tissue oxidative damage and increased lipid peroxidation occurs if antioxidant defenses are overwhelmed due surge in free radicals. The results obtained in our study wherein duration dependent increase in lipid peroxidation was observed supports this contention. Increased lipid peroxidation and decreased glutathione were observed in erythrocytes and gills were observed in the fish *Rhabdosarga sarba* when exposed to red tide algal bloom and hydrogen peroxide [33]. Interestingly, our investigation exhibited increased activities of all the three antioxidant enzymes (SOD, CAT and GPx) when the fishes were exposed to EIE for 10 days. However later all the three enzymes decreased significantly when exposure duration increased to 20 and 30 days. This discrepancy can be explained based on adaptive response of antioxidant enzymes. In the present study, increased activity of SOD in erythrocytes and gills could be due to increased superoxide anion production, since SOD is the first line of defense to counteract the harmful effect of superoxide anion [34]. Later, when the cell is unable to cope up with continuous production of superoxide free radicals, the activity of the SOD together with catalase and glutathione peroxidase decreases when fishes are exposed to EIE for protracted duration of time upto 30 days. Similar to our long term exposure results, decreased SOD and CAT was documented in Nile tilapia (*Oreochromis niloticus*) when exposed to silver nano particles [35]. Glutathione peroxidase family of enzymes have evolved as a key antioxidant enzyme in fishes to counteract the deleterious effects of reactive oxygen species [36]. Hence modulation of GPx noted in our study suggests oxidative stress as the major mechanism involved in all toxic manifestation seen when fishes were exposed to EIE. Corroborating evidence exists from studies with other toxicants such as new flavonoid metal-insecticide, bisphenol- A, cadmium, chromium and copper on various fishes to support the findings that exposure to EIE altered antioxidant enzymes [4,37,38]. Taken together, oxidative stress seems to be the major physiological basis governing toxic exposure to electrochemical industrial effluent in the fish *Cirrhinusmrigala*. Measurements of hematological parameters are often important in diagnosing structural and functional status of the animal exposed to toxicant. Different blood parameters are often subjected to change depending upon stress condition and various other environmental factors. The decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies. In the present study exposure to EIE exhibited impaired erythropoiesis manifested by lower RBC count, Hb concentration, and hematocrit level. Alterations in erythropoiesis can be attributed to disturbance in the metabolism of the hematopoietic organs. Our results are in agreement with Nanda and Behera, [39] wherein the authors reported reduced red blood cell counts in Anabas *testudineous* and *Heteropheustesfossilis* were upon exposure to cadmium and nickel. The drop in hemoglobin level in our study is possibly associated with decreased RBC count indicative of anemia. decrease in the haemoglobin concentration in blood is usually caused by the effect of toxic metals on gills, as well as decrease in oxygen, which also suggests anaemia or confirms toxic impact of industrial effluent on the fish, *Arius nenga*. Anaemia is an ultimate manifestation of acute and chronic intoxication of heavy metals [40]. In the present study, MCV, MCH and PCV decreased in all exposure periods. The decrease of MCV and MCH clearly indicates hypochronic microcytic anemia. The reduction in packed cell volume may be caused by blood loss, haemodilution and osmoregulatory dysfunction. The decrease in MCH in the present study clearly indicates that the concentration of haemoglobin in RBC is reduced. The MCH is a good indicator of Red Blood Cell swelling [41]. According to Kumar, [42] endosmosis is the reason for increase in MCV. This results in haemodilution, further

increasing the MCV value. Sublethal concentrations of lead, copper and zinc has been shown to produce haemolyticanaemia due to lysis of erythrocytes with decrease in PCV% value [43]. Sachar and Raina, [44] have observed the decreased haematocrit value (PCV) in the fish, Aspidopariamorar on exposure to Lindane. A drastic decrease of PCV, MCV and MCH indicates anaemic conditions due to abnormal stress conditions, particularly seasonal, environmental and toxic substances in the EUS infected fish, *Channastriatus* of Dharmasagar, Hasanparthy and Bhandham lakes [45]. One possible mechanism involved in decreased hemoglobin is rapid oxidation of hemoglobin to methaemoglobin or release of O_2 radical due to oxidative stress induced by stressors present in EIE. Alternatively, similar decrease in RBC count has been also reported in fishes subjected to lethal exposure to cadmium based pollutant [46]. Apart from exposure to Electrochemical Industrial Effluent (EIE), observed in the present study, decreased RBC count due to inhibition of erythropoiesis has been reported in several organisms such *Clariasgariepinus*,*Cypri nuscarpio*, *Clariasgariepinus* and *Oreochromisniloticus* exposed to diverge nature of xenobiotic such as metal fishing company effluent, chloropyrifos and endosulfan [47,48]. In the present study, RBC was found to decrease in all exposure periods, an observation attributed in part due to stimulation of haemolyzing action by the toxic effluent [49]. Thus, the decrease in the RBC count, decreased hemoglobin and hematocrit observed in this study is possibly due to the inhibition of erythropoiesis, haemosynthesis or osmoregulatory dysfunction or due to an increased rate of erythrocyte destruction in the haemtopoietic organ corroborated by Vani et al. [50]. WBC plays an important role in defence mechanism of the body. An increase in WBC is termed as Leukocytosis. Across all experimental groups, leukocytosis was a common observation. According to Ray and Dubey, [51] the increased PCV, MCV may be considered as a combined effect of erythrocyte swelling and compensative mechanism of the fish to increase the oxygen carrying capacity of the blood. The results of the present study is in consonance with Gautamet al. [52] with observed decrease in RBC, Hb content and increase in WBC of blood in *Channastriatus* treated with endosulfan and diazinon.

Several experiments postulate that blood glucose levels is linked to stress levels in fish and represent anomalies in respiration and nutrition [53,54]. Blood glucose is a sensitive and reliable indicator of pollutants causing environmental stress in fish. Stress, per se initiates high metabolic demand and the increase in blood glucose level is essentially an adaptive response of the body to meet with this increased energy demand [55]. Significant increase in blood glucose level was also reported in *Anguilla Anguilla* exposed to cocaine [56], in the catfish *Pangasianodon hypophthalmus* exposed to chromium (Majharul Islam et al., 2020). Cholesterol is an essential structural component of cell membranes, it is the outer layer of plasma lipoproteins and the precursor of all steroid hormones [57]. Electrochemical Industrial Effluent (EIE) contains diverge variety of chemicals including heavy metals such as lead, cadmium and chromium. This heavy metal is known to cause hepatoxicity and this in turn causes impairment in cholesterol biosynthesis leading to decline in serum cholesterol. Alternatively heavy metal induced oxidative stress possibly leads to lesser absorption of dietary cholesterol in fishes attributing to its decline. Under conditions of oxidative stress, both these mechanisms leading to decreased serum cholesterol have been well documented [58]. The decrease in cholesterol in present investigation may be possibly due to the low availability of acetyl CoA for cholesterol synthesis in the absence of glucose utilization and dependence of body on alternate energy sources. Kour and Kour, [59] reported that cholesterol content of blood serum showed decline in *Channapunctatus* during prespawning phase when exposed to nickel-chrome electroplating effluent. Sadhna Sharma Tripathi et al. [60] investigated that cholesterol showed continuous decline with increase in sublethal concentration of copper. Taken together, the results of our study and on the basis of the background discussion on the significance of glucose and cholesterol in overall physiological state of fishes, it is reasonable to assume that the increased glucose and decreased cholesterol can be attributed to impaired carbohydrate metabolism resulting from oxidative damage to the pancreas or liver. Discharge of electroplating industrial effluent into water bodies raises serious concerns regarding genotoxicity. In terms of eco-genotoxicology, both comet assay and micronucleus assay in fish erythrocytes has been established as important *in vitro* biomarker of freshwater pollution [61]. Hence sensitive markers can act as indispensible tools to screen genotoxicity of aquatic organisms. Due to ease of performance and low cost, micronuclei test is often preferred over comet assay [62]. In the present study, we found highest frequency of micronuclei in erythrocytes of fishes exposed to EIE for 30 days. The following pattern of micronuclei was obtained with increase in duration of treatment: club shaped on day 10, rapture of micronuclei on 20 days exposure and wide spread damage to normal histoarchitecture of erythrocytes on 30 day exposure. Under *in vitro* conditions, micronuclei induction has been observed in fish cell lines exposed to diverge nature of industrial effluents from thirty-eight industries such as leather, electroplating, ore processing, metal manufacturing, and paper pulp manufacturing indicative of MN test as a important indicator of genotoxicity. Interestingly, both Micronucleus Test (MN) and comet assay under controlled laboratory condition similar to our study have been investigated in several genotoxic and putative mutagenic agents such as Benxene (BZN), glyphosate formulation [63-65]. MN formation is a short term response to a noxious genotoxicants and it depends on the intensity of exposure to contaminants and probably independent of the duration of such exposure. Yet another hallmark feature of our study stems from our results from comet assay on peripheral blood erythrocytes of fish, *C.mrigala* wherein fishes treated with electroplating effluent which depicted increased DNA length and percentage of DNA damaged cells compared to control. Ping Nan et al., [66] studied the three parameters of genotoxicity tail moment, tail length and tail intensity have been widely used by researchers for evaluating DNA damage. As the amount of damage increases in a cell, more DNA migrates into the tail region and is quantified in terms of an increased amount of determined fluorescence in the tail region, as well as by tail length. The ratio of the DNA in the tail region (tail intensity) is commonly used for quantifying DNA strand breakage and represents the most reliable parameter [67]. Formation of comet tails and micronuclei have been reported with several other toxicants such as hexavalaent chromium in *Ctenopharyngodon idellus* and *Labeo rohita* [68,69], Naphthalene-2-sulfonate induced toxicity in blood cells of freshwater fish *Channa punctatus* [70]; amoxicillin residue on zebra fish (*Danio rerio*) embryos (Chowdhury et al., 2020); heavy metals on Astyanax *lacustris* [71], sub lethal concentrations of cadmium chloride exposure in Mozambique tilapia *Oreochromis*

mossambicus [72], oil-containing drill cuttings in marine benthic goby *Mugilogobius chulae* [73]. Corroborative evidence from all these studies highlight the importance of comet assay as an ideal genotoxicity marker in fishes and other marine organisms. Some of the potential toxic agents mentioned in these studies are similar to those reported In Electroplating Industrial Effluent (EIE) that have been found in our study. In the present study, the DNA damage in erythrocytes of *Cirrhinusmrigala* exposed to electroplating effluent in different durations was investigated wherein the highest damage was observed at 30 days of exposure period. Thus, percentage of DNA damage increased with the increase in exposure to electroplating effluent. DNA damage can initiate a cascade of biological consequences at cellular, organic, individual and finally at population and community levels. The results of our study on Micronucleus (MN) induction in fish model and increased DNA damage from comet assay can have serious implication, since the mechanism of toxicity in fishes can be easily correlated with higher vertebrates especially with regard to several toxic agents that are present in EIE that have the potential for teratogenicity and carcinogenicity in humans [74].

Conclusion

From the above investigations it can be inferred that *Cirrhinu smrigala* exposed to Electrochemical Industrial Effluent (EIE) at varying duration resulted in oxidative stress, hematological alterations, biochemical changes in glucose, protein and cholesterol levels together observed genotoxicity. Since fish is an affordable source of protein, it's very important to preserve the overall health of fishes. Any alteration in the health of fish would eventually lead to biomagnifications in the succeeding food chain. The results of our study marks Electrochemical Industrial Effluent (EIE) as a major pollutant for fresh water fishes. Our results suggest that fish blood can be considered as potential bio-indicator in assessing the physiological status of fish. The genotoxic effects of EIE is of special significance to subjects consuming fresh water fishes due to associated health risks in human subjects. Our findings open windows for further research as it would be pertinent to study level of oxidative stress and susceptibility to cancer in subjects consuming fishes that are naturally reared in water bodies contaminated with EIE. Based on the findings of our study, we suggest, creating awareness among the people not to discharge the electroplating effluent directly to the water bodies without treatment.

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