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Studies on the DNA Damage of Freshwater Catfish *Clarias Batrachus* Exposed to $Znso_4$ Using Micronuclei & Comet Assay

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ABSTRACT:-

The present study has been investigated to the micronuclei and comet assay carried out in the hematological parameters of the freshwater catfish *clarias batrachus* exposed to control and different concentration of treated zinc sulphate. The Sub-lethal concentration was 25%, 50%, and 100% the DNA was determined by the comet assay DNA comet tail. The control fish shows the normal structure whereas the zinc sulphate exposed to individual alterations in the DNA tail the length of the DNA tail maximum genetic changes was observed the 28 days.

Keywords: Zinc Sulphate, Comet Assay, micronuclei, *clarias batrachus.*

INTRODUCTION

The Contamination of freshwaters with a large number of toxins has arisen as a serious natural issue, overall [Vutukuru SS., 2005; N. Dirilgen, 2001] The quick improvement of industry and horticulture has brought about an expansion in oceanic contamination with poisonous synthetic substances, which has caused a critical natural risk in spineless creatures, fish and people [Uluturhan E, Kucuksezgin F. 2007]. Among sea-going contaminations, weighty metals being universal in nature, structure a significant pressure related poison in oceanic bodies due to their non-biodegradable nature, harmfulness, long diligence, capacity to bio-gather and biomagnify in sea-going fauna [Waqar A. 2006; GB Kamble, 2000; Dinodia GS, 2002]. These metal particulates enter the amphibian medium through effluents released from tanneries, materials, metal getting done with, mining, coloring and printing businesses, fired and drug ventures and so on [Azmat R, Talat R. 2006].

Studies have shown that an expanded lipid peroxidation in respiratory organs (gill, air-sacs) (Parihar and Dubey, 1995) and liver (Parihar et al., 1996) of Heteropneustes fossilis which would recommend evolving favorable to oxidant processes and a requirement for cell reinforcement reactions to safeguard against expanded ROS creation which brings about assimilation of oxidases on the outer layer of the films (Robinson, 1978). Such cells use a NADPH oxidase chemical framework to produce straightforwardly O2 as a feature of their ordnance (Babior, 1994).

The adequacy of the cell reinforcement safeguard framework corresponding to the oxidative harm is quite compelling on account of fish which show metabolic varieties connected with the vacillation of climate. To explain the connection between the Sub-deadly impact of zinc and DNA harm far reaching study was acted in the current examination. Concentrates in the micronuclei of *C. batrachus* presented to Sub-deadly groupings of zinc sulfate for 7, 14, 21 and 28 days.

Through their gills, which cover more than half of their bodies, fish stay in close contact with the water around them (Pratap HB et al.). al (1989). Increasingly more of our natural surroundings are being disintegrated step by step because of expanded ecological contamination through different anthropogenic exercises. Heavy metals, pesticides, and other chemicals can be found in industrial effluents that are discharged into water bodies. Any poison which is released into water will change the surface pressure, warm properties, conductivity, thickness and pH esteem alongside biodegradable (proteins, fats, pesticides, fungicides and so on), and non biodegradable toxins. As a mark of climate reasonableness, fish is by all accounts vital since it is impacted by the residing living space (to be specific water), which is today dirtied by effluents from enterprises, pesticides cleaned out from agrarian terrains and cleansers from family channels and so forth. This paper is pointed toward deciding the gills, liver, of the *Clarias batrachus* to sub deadly centralization of Zinc sulphate.

Weighty metal pollution affects the biological equilibrium of beneficiary climate and variety of oceanic biota [Farombi EO, Adelowo OA, Ajimoko YR. 2007; Patnaik BB, Howrelia JH, Mathews T, 2011]. Heavy metals are any metal or metalloid with a relative atomic density greater than 5 g/cm3 [Järup L. 2003]. They fall under the category of potentially hazardous materials (arsenic, cadmium, lead, mercury, etc.), semi-fundamental (nickel, vanadium, cobalt) and fundamental (copper, zinc, iron, manganese). When taken in large quantities over a long period of time, toxic metals can cause harm even at very low concentrations; however, essential metals can cause harm when taken in large quantities. Among vertebrate oceanic fauna, fish

are viewed as quite possibly of the most characteristic component, in freshwater biological systems, for the assessment of weighty metal contamination [Rashed MN. 2010]. Fish are at a higher trophic level in the food web and can bioaccumulate a lot of certain metals especially in liver, gills and kidney, upto focuses a few times higher than in the encompassing water. Weighty metals are taken up through various organs of the fish in light of the fondness between them [Yousafzai AM, et al 2010. One of the extraordinary benefits of involving histopathological biomarkers in ecological observing is that this class of biomarkers permits analyzing these particular objective organs [Vutukuru SS. 2005].

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF FISH

The fresh water fish *Clarias batrachus* (17 ± 2 cm length and 38 ± 2 g weight) were collected locally from Panampattu village Villupuram, district, Tamilnadu, India, were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate day. Water in the tank was renewed, three or four times in a week and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank.

Toxicity Studies

Acute toxicity tests are conducted to measure the impact of toxicant on aquatic animals within a short period of four days. During acute toxicity test, organisms are transferred to freshly prepared, desired toxicant medium at periodic intervals usually 24 hours. In the present investigation the LC_{50} value is estimated and it is concerned with the determination of the median lethal response or median tolerance limit, which is no more than a condition which produces a 50 per cent mortality or permits a 50 per cent survival of the test organisms under the experimental conditions during a specific time interval (Sprague, 1973). following the completion of each experiment, the test containers were cleaned and dried to remove the pesticide content from adhering to the wall of the trough. The trough was covered with nylon net to prevent the escape of fish.

GENOTOXIC ASSAY

Micronuclei Assay:

After the expiry of 7, 14, 21 and 28 d of exposure, blood was collected from caudal vein from fish specimens using heparinised syringe and thin smear on pre-cleaned slides was made. Slides were fixed by dipping in absolute methanol for 5-10 min then air dried for at least 1 hour and stained in Giemsa stain for 10 min. Slides were air dried overnight and mounted with DPX and observed under microscope using 40/100X objective lenses and the micro nucleated cells were scored.

Single cell Gel Electrophoresis (Comet Assay):

Comet assay was performed according to the Tice Xitalics et al., (2000) with some modification. Blood samples were collected from caudal vein of fish. About 15 µL of the blood was mixed with 145 µL of low-melting point agarose (LMP 0.5%). A 40 µL of the mixture was layered on the microscopic slide which was precoated with normal melting agarose (NMA 1%). The slides were kept in refrigerator for 10 min. After solidification, the slides were immersed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mMTris-base, pH 10, with 10% DMSO and 1% Triton X-100 added fresh) inside refrigerator at 4°C for 1 hour. The slides were placed side by side in electrophoresis chamber containing fresh and cold buffer (300 mMNaOH, 1 mM EDTA, pH 13) for 45 min in refrigerator (4ºC). The electrophoresis was done at 25 V and 300 mA (25-30 min at 4ºC). Observation of slides were done with the CETI fluorescent microscope (Model: 3100.5000- Triton II) that were equipped with Sony camera (Model: No.DSC-H9). Two slides per specimen were prepared and 50 cells per slide (300 cells per concentration) were scored randomly. The DNA damage was measured by visual classification of cells into five type “comets” according to the tail length (Cavas, 2011; Lee and steinert, 2003); 0: no damage, 1: low damage, 2: moderate damage, 3: high damage and 4: complete damage. A genetic damage index (GDI), arbitrary units, was employed as below (Grisolia et al., 2009; Silva et al., 2000). GDI = (n1+2n2+3n3+4n4) / (Σ / 100) GDI: Genetic Damage Index, n1: Minimum damage, n4: Maximum damage, Σ: Total number of the cells.

STATISTICAL ANALYSIS

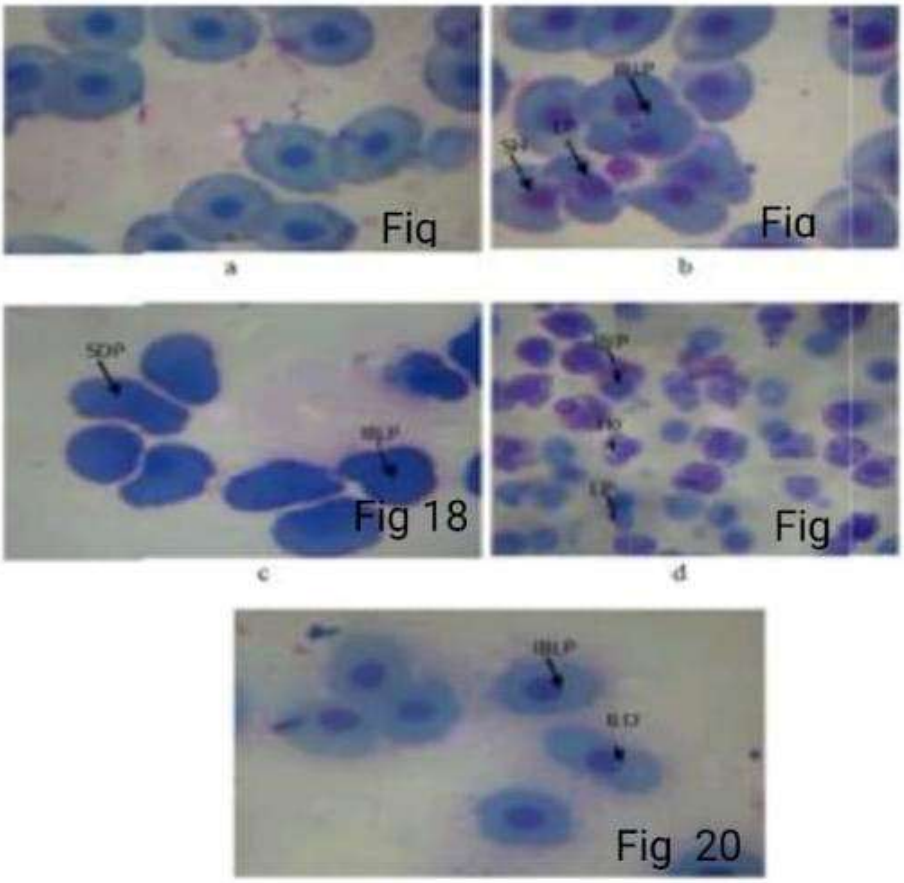
The data obtained in biochemical parameters, haematology and genotoxicity were subjected to standard statistical analysis each sampling time and their respective control groups in different groups. Duncan’s multiple range tests (Bruning and Kintz, 1968) was performed to determine whether the parameters altered significantly by exposure periods.

RESULTS

Micronuclei Assay

The morphology of the erythrocyte of *C batrachus* fish shows centrally located in the control group. Irregular boundary with more than one projection was seen in erythrocytes of exposed fish at all stages of exposure to sub lethal Zinc Sulphate solution. Micronuclei frequency in red blood cells of *C.*

batrachus exposed to zinc are shown in Figures 16 to 20. Although initially no significant differences were observed in Micronuclei frequencies between unexposed control fishes and those exposed, a statistically significant increase in red blood cells with altered nuclear morphology was noted and observed after exposure for 28 days.



Figures 16 to 20. Micronuclei induction in red blood cells of *C.batrachus* exposed to sub-lethal concentration of Zinc Sulphate.

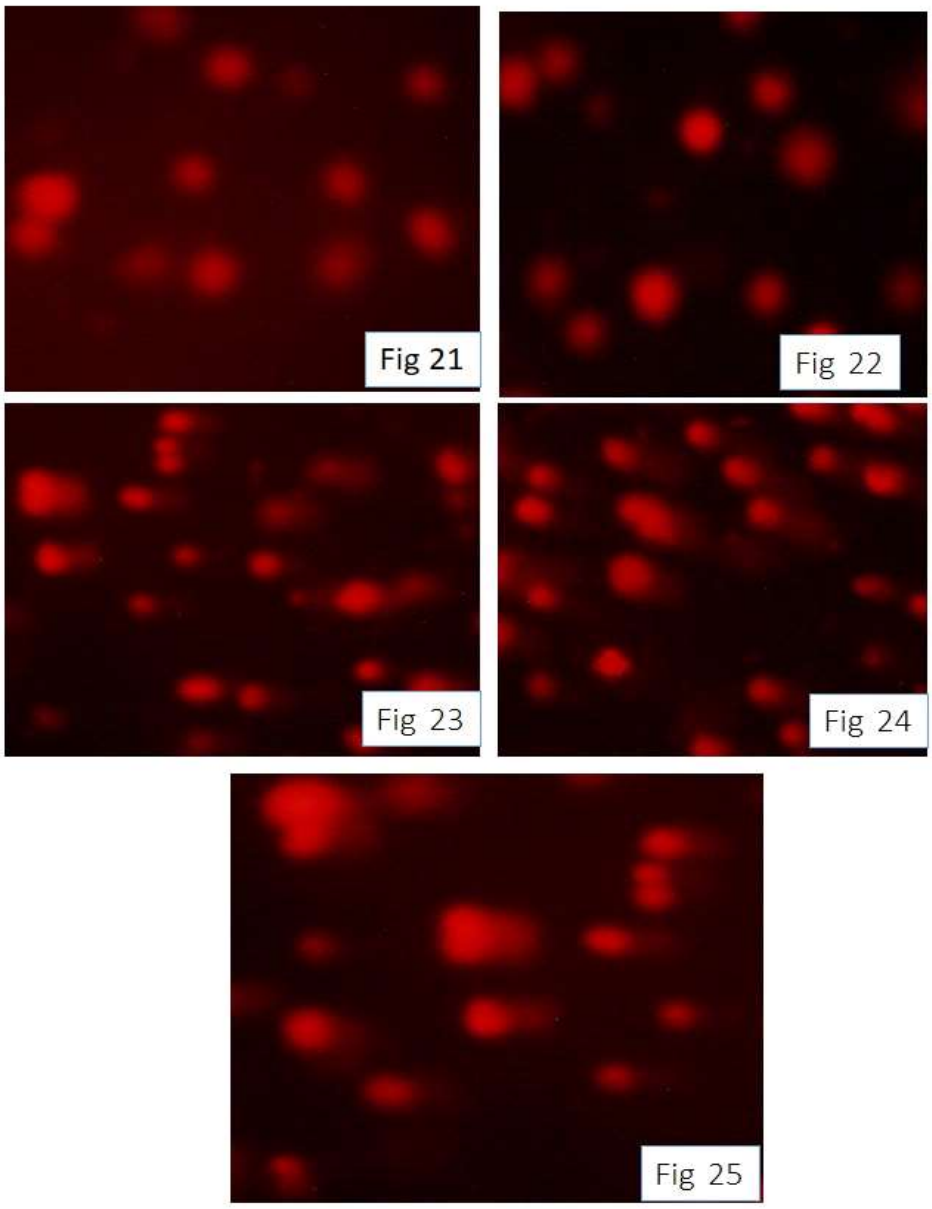
Single cell Gel Electrophoresis (Comet Assay)

Amount of DNA damage in the cells was estimated from visual classification as an extent of material genetically migrated and shown by Genetic Damage Index (GDI). The fish exposed to sub-lethal concentration of zinc exhibited significantly higher DNA damage in their blood cells than the control groups (Table 1, Fig. 21-25). Maximum GDI was noted after 28 days of exposure.

Table 1 Showing the Genetic Damage Index (GDI) in blood cells of *C. batrachus* in different experimental groups for 7, 14, 21 and 28 days

Control	**Duration (in days)**			
	7d	**14d**	**21d**	**28d**
0	1	2	3	4

0: undamage, 1: low damage, 2: moderate damage, 3: high damage and 4: complete damage.



Figures 21 – 25 DNA damage in the blood cells

DISCUSSION

Micronucleus test (MNT) is a widely used cytogenetic technique for MN the assessment of chromosomal damage induced by various genotoxity. Fish and aquatic invertebrates have been considered to be efficient and cost effective model systems for studying the toxic, mutagenic, and carcinogenic potential of pollutants (Braunbeck *et al.* 2005) due to their ability to metabolize, concentrate, and store water-borne pollutants (Osman *et al.* 2007). Sub-lethal concentration of zinc used in the present study induced a significantly higher number of MN compared to the control. Further, the MN induction increased significantly with the advancement of the exposure periods. Both concentration- and time-dependent increases in MN induction have also been reported due to chemical exposure in fish (Bahari *et al.* 1994). Although, the MN test has been found to be a sensitive assay to evaluate genotoxic compounds in fish under controlled conditions as an index of cumulative exposure (Bolognesi *et al.* 2006), it might suffer variations according to clastogen, test organism, and the life cycle of the cells (Grisolia and Cordeiro, 2000). Further, as the pre-existing mature (and non-dividing) erythrocytes would predominate in the blood, the detection of induced MN in mature blood cells is related to concentration of toxicant in the medium. Zinc sulphate when reacts with acidic substances present in the water medium produces zinc oxide which has the potential to produce chromosomal changes, including

chromosome aberrations and MN induction in test animals (Hoda and Sinha 1991). In the present study, the MN was significantly increased with the increase in duration of exposure. Present investigation on the genotoxic potential of zinc suggests a serious concern about its potential danger to aquatic organisms, especially to fish, and indirectly to human beings.

The present study showed that the Comet assay can be applied successfully in fish, for assessment of genotoxicity potential of zinc. The comet assay under alkaline condition is able to identify DNA damage (e.g. single strand breakage, alkaline labile sites, DNA cross links, etc.) that was induced by pesticides (Kumar *et al.,* 2010; Tice *et al.*, 2000). This method has considerable advantages over the ordinary cytogenetic methods such as chromosomal aberration, sister chromatic exchange and micronucleus test used to detect DNA damage, because in this method the cells do not require to mitotic division and chromosomal properties (Ali and Kumar, 2008; Nwani *et al*., 2010; Pandrangi *et al.,* 1995). Moreover, the assay is an useful method for *in vivo* and *in vitro* genotoxicity on aquatic organisms (Cotelle and Ferard, 1999). According to several studies, the comet assay has been successfully applied in blood cells of many fish species. So, fish are suitable tools for monitoring of genotoxic pollutants in aquatic environment (Abdul-Farah *et al.,* 2003; Cavas and Ergene-G zükara, 2005). In the present study, the DNA damage was higher after 28 days of exposure period in the fish *Clarias batrachus.* Similar reports were observed with the finding of Kumar *et al.,* (2010) and Kushwaha *et al.,* (2000). Also, the results are in accordance with other researchers who worked on human. The DNA damage detected in this study could have originated from DNA single-strand breaks, DNA double-strand breaks, DNA adducts formation and DNA-DNA and DNA-protein cross links (Mitchelmore and Chipman, 1998), resulting from the interaction of zinc or its metabolites with genetic material (Garaj-Vrhovac and Zeljezic, 2000). The DNA damage, especially DNA single-strand breaks, can be used as biomarker for the identification of genotoxic pollutant in aquatic ecosystem. In general, heavy metals when undergoing different metabolic processes inside living organisms (such as fish) produces a lot of free radicals that very often can interfere with the biomolecules' structure like protein and nucleic acids (Mohanty *et al*., 2011).

The mechanism of DNA damage due to Sub-lethal zinc exposure is due to the formation of zinc oxide which induces DNA damage in different way such as phosphorylation process (Ali *et al.,* 2009; Blasiak *et al.,* 1999). In addition, it seems that oxidative stress has an important role to induce cytotoxic and genotoxic damage (Moore *et al.,* 2010). In conclusion, Comet assay is sensitive for the detection of DNA damage in aquatic organisms. Therefore, the assay can be applied for investigation of genotoxic pollutants.

SUMMARY AND CONCLUSION

Efforts have been made to investigate the effect of zinc sulphate on the histopathological, biochemical, haematological and genotoxic alterations of Sub-lethal concentrations of zinc sulphate (2. 9 ppm) on gill, skin and blood of the freshwater catfish *Clarias batrachus.* Tissue sampling was done at 7, 14, 21and 28 d of exposure periods.

- The morphology of the erythrocyte of *C. batrachus* fish shows centrally located nucleus in the control group. Irregular boundary with more than one projection was seen in erythrocytes of exposed fish at all stages of exposure to sub lethal zinc sulphate solution. Although initially no significant differences were observed in MN frequencies between unexposed control fish and those exposed, a statistically significant increase in erythrocytes with altered nuclear morphology was indeed observed after exposure for 28days.

- Amount of DNA damage in the cells was estimated from visual classification as extent of material genetically migration shown by Genetic Damage Index (GDI). The fish exposed to sub lethal concentration of zinc exhibited significantly higher DNA damage in their blood cells than the control groups. Maximum GDI have been noted after 28 days of exposure.

In the present study, various alterations in histopathological, biochemical, haematological and genotoxic analyses on *Clarias batrachus* after Sub-lethal zinc sulphate treatment clearly shows that a continuous low level metal stress have a gross biological impact on aquatic organisms.

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