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DNA damage responses to oxidative stress in tilapia (*Oreochromis mossambicus*) exposed to polluted water

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ABSTRACT

The aim of the present investigation was to assess the biological damage and oxidative stress caused by exposure of the test organism (Oreochromis mossambicus) to various mutagenic agents present in the polluted waters of the Chrompet Lake, located in Chennai, Tamil Nadu, India. This urban lake receives domestic, industrial waste discharge and storm water runoff. The evaluation of the quality of the water of Chrompet Lake is important and necessary, since it is utilized for domestic and recreational purpose. In addition, the aim was to collect data with respect to the validation of the comet assay as a biomarker in fish and on the utilization of O. mossambicus as a bio-indicator of genotoxicity in environmental impact assessment of continental waters. For this purpose, we performed a single cell gel electrophoresis (the Comet assay), testing DNA migration in an electrophoretic field using erythrocytes of O. mossambicus, both from the Chrompet Lake and the fish reared in the laboratory condition as negative control. The results of the experimental fish revealed a significantly greater number of comets and suggested a genotoxicity of the aquatic environment at Chrompet Lake. The comet assay in O. mossambicus provides an adequate sensitivity to be utilized as a tool in the monitoring of water pollution and environmental risk assessment.

Keywords*:* DNA damage, *Oreochromis mossambicus*, Environmental monitoring, Environmental risk assessment, Comet.

INTRODUCTION

Pollution of environmental waters is a serious and growing problem all over the world. Although there is legislation dealing with this problem in various countries, water pollution from toxic chemicals still occurs [1, 2]. Aquatic organisms, such as fishes and mollusks, accumulate pollutants directly from contaminated water and indirectly through the ingestion of contaminated organisms [10, 12]. Genotoxic pollutants contaminate not only aquatic organisms but also the whole ecosystem and in the end, humans through contamination of our food [16, 17].

In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the following suite of fish biomarkers may be examined: biotransformation enzymes (phase I and II), oxidative stress parameters, biotransformation products, stress proteins, metallothioneins, MXR (multixenobiotic resistance) proteins, hematological parameters, immunological parameters, reproductive and endocrine parameters, genotoxic parameters, neuromuscular parameters, physiological, histological and morphological parameters [15].

In this study, the water of Chrompet Lake was submitted to environmental testing using the comet assay in fish (*Oreochromis mossambicus*). This urban lake receives domestic waste discharge, storm water and industrial runoff. The study of monitoring the quality of the water of this lake was important and necessary, since it is utilized as a recreational area. In addition, the aim was to collect data with respect to the validation of the comet assay as a biomarker in fish and on the utilization of *O. mossambicus* as a bio-indicator of genotoxicity in environmental monitoring of continental waters.

MATERIALS AND METHODS

Animal and blood cells collection:
Oreochromis mossambicus was collected from polluted Chrompet Lake in Chennai, India. Individual's animals weighing 100±20 grams. In order to assess the effects of pollution in the tilapia and their oxidative stress profiles are analyzed. The survival rates of the tilapia were monitored in each set of site. Sets of tilapia were harvested for oxidative stress profiling at different sites. The blood cells were collected from four tilapia at each site (site 1, 2, 3 and 4) after commencement of exposure to pollution. The blood samples (approximately 2mL per individual) were collected using a syringe from the tail muscle. The samples from each tilapia were immediately centrifuged at 800×g at 4°C for 10 min to collect the blood cells. Three replicates were examined at each sampling time.

Comet assay
DNA strand breaks and FPG-sensitive sites were detected in erythrocytes by single cell gel electrophoresis, the comet assay [14]. Clear microscope slides were pre-coated with 1% normal melting agarose. For each slide, 100µl of cell suspension (approximately 10,000 cells) was mixed with 200 µl of 0.5% low melting point agarose, spotted as first layer onto the pre-coated slide and covered with a coverslip. After agarose solidification the coverslip was gently removed; a second layer of 200µl of normal melting agarose (NMA) was added over the first layer, covered with a coverslip and allowed to solidify. Cover slips were removed and slides were placed in chilled lysis buffer (2.5MNaCl, 100mM EDTA, 10mM Tris–HCl; pH 10, 1% Triton X-100 and 10% DMSO added just before use) at 4 ◦C for 1 h. After lysis, the slides were placed on the platform in an electrophoresis tank that contains the pre-chilled (4°C for at least 1 hr) electrophoresis solution (300mM NaOH, 1mM EDTA, pH 13). The buffer should just barely cover the slides and was incubated for 30 min at 4 ◦C before beginning electrophoresis. The electrophoresis was subsequently conducted at 25V constant voltage and 300mA for 30 min. Then slides were removed from electrophoresis apparatus and washed with three changes of neutralization buffer in staining jar for 5 min each at 4°C. Each slide was stained with 75 µl of ethidium bromide (20mg/ml) and covered with a cover slip. The slides were examined under a fluorescent microscope and analyzed within 3–4 h. Slides were scored using an image analysis system (Comet Imager 1.2.13) attached to a fluorescent microscope (Carl-Zeiss, Germany) equipped with appropriate filter. The microscope was connected to a computer through a charge coupled device (CCD) camera to transport images to software for analysis. The final magnification was 400×. Analysis of mean % DNA in the tail, one of the reliable indicators of DNA damage was done using image analysis software. Images from 50 cells (25 from each duplicate slide) were analyzed. To show the reproducibility of our method, we measured DNA damage in lymphocytes from Chrompet Lake *Oreochromis mossambicus* subjects on five different sites. For this, blood samples were taken twice from the same subject on different sites; the respective samples were used for the comet assay and checked for and significance ($p < 0.05$).

Statistical analysis
Comparison between groups were performed using one-way ANOVA with $p<0.05$ as the criterion for significance. All analysis was done using windows based SPSS statistical package (version 12.0, Chicago, IL).

RESULTS AND DISCUSSION

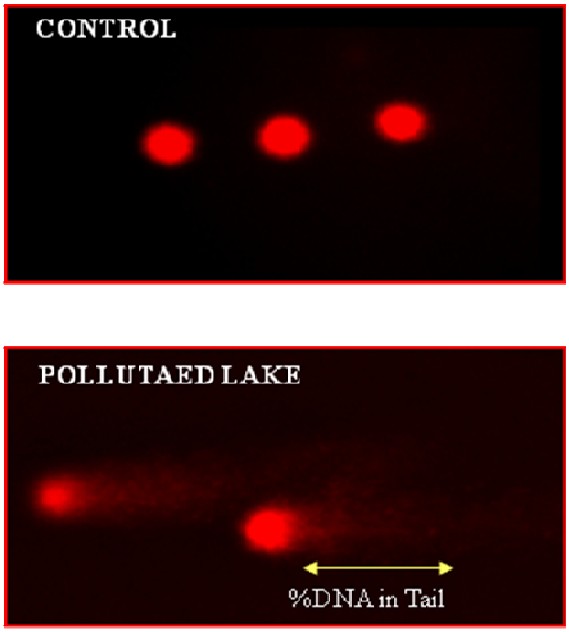
The level of DNA damage in *O. mossambicus* erythrocyte was shown as the tail moment, tail length and as the percentage of migrated DNA. An endpoint "tail moment" is defined as the product of the tail length and the fraction of DNA in the tail. The results are shown in Fig.1 and 2, and Table 1. DNA damage in blood cells demonstrated that there was a significant difference ($P < 0.05$) between the fish from Chrompet Lake and the animal kept in the laboratory (control), in which there was approximately double the number of cells with DNA damage, indicating genotoxicity in the environment.

In the present study, the comet assay was utilized as a biomarker of the genotoxic potential in the waters of Chrompet Lake, which was found to be effective in showing double the DNA damage in the group of *Oreochromis mossambicus* from the lake when compared to that found in the control. Based on these results, we can suggest that *O. mossambicus* is a good bio-indicator of genotoxicity. The comet assay demonstrates the capacity to detect DNA damage (genotoxicity), in agreement with the induction of DNA damage detected in other tests for genotoxicity (chromosomal aberrations and genetic *hgprt* mutation, MN) [9].

Table 1: Comet tail length (mean ± S.D.)

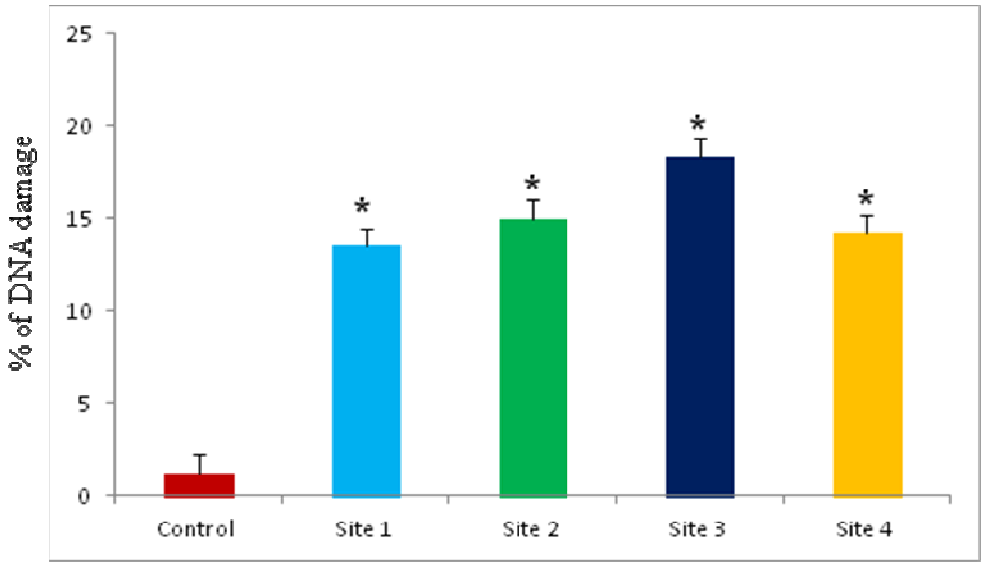
Subjects	Mean tail length (µm) ± S.D.
Control	2.7 ± 0.76
Site 1	16.13 ± 1.30
Site 2	16.54 ± 1.02
Site 3	8.21 ± 0.87
Site 4	17.3 ± 1.92

Fig 1*. DNA damage assessment *Oreochromis mossambicus



According to data this study and criteria established to [15], the Comet assay in *O. mossambicus* should be a good biomarker. The assay is reliable, relatively cheap and easy to perform. The DNA is sensitive to pollutant exposure and effects (DNA breakage) serve as an early warning parameter. Baseline data of the DNA damage in Comet assay is distinguished between natural variability and contaminant-induced stress, which are defined to each cell type and/or organism. The underlying mechanism of the relationships between DNA damage and pollutant exposure and this toxicological significance may be established, e.g. DNA damage. The Comet assay is non-invasive or non-destructive methodology, preserving the organism and ecosystem. The basic biology and physiology to the test organism are known.

Fig 2, represents the histogram showing significantly increased amount of DNA damage in the cells which were collected from different site of the polluted lake O*reochromis mossambicus* compared to control, here the % of DNA damage is the representation of the amount of DNA tail which has been formed.



[13] suggested that the determination of genotoxicity as a result of environmental contamination of water should be conducted with the water as a whole and not specifically for each (contaminating) component, and that the comet would be a good test for this type of monitoring. The data on genotoxicity in Chrompet Lake found in *O. mossambicus* demonstrated the poor quality of that environment, however, without determining the specific polluting components. According to [18], there is an elevated concentration of heavy metals such as lead and Zinc in the waters of Chrompet Lake, possibly the agents responsible for the DNA damage found in the comet assay in *O. mossambicus* from Chrompet Lake.

Various aquatic organisms have been utilized as bioindicators, for examples: fishes and mollusks [7]. Fish of the species *O. mossambicus* utilized in the present work, showed a good DNA damage pattern for analysis and environmental testing, thereby proving to be a bioindicator of genotoxicity for Chrompet Lake. Some studies utilizing different substances known to be mutagenic (e.g. cyclophosphamide, mitomycin C) in *O. mossambicus*, showed that this species was more sensitive when compared to *Oreochromis niloticus* and *Cyprinus carpio* in monitoring with the micronucleus assay [4, 5, 6].

CONCLUSION

This study suggests that *Oreochromis mossambicus* is good bio-indicator of environmental genotoxicity and that the comet assay constitutes a sensitive, rapid and economic method for the detection of DNA damage, showing that it is a biomarker for non specific genotoxicity in fish. It has been used successfully to reflect the variation in exposure in vivo in a particular species, and it also has the potential as a test to explore cell specific effects, inter-individual variation and the persistence of lesions. However, further work is needed, including the standardization of the methods and the measurements, before the comet assay can be used as a standard biomarker of aquatic environments.

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