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RESEARCH ARTICLE

TANNERY EFFLUENT INDUCED DNA DAMAGE IN LIVER AND TESTICULAR TISSUES OF FRESHWATER FISH *CHANNA STRIATUS*

[1*]Sivachandran, R., [2]Mazher Sultana, [3]Saravanan, R. and [4]Malathy, S.

[1]Department of Advanced Zoology and Biotechnology, Presidency College, Chennai – 600 005
[2]Department of Advanced Zoology and Biotechnology, Ramakrishna Krishna Mission Vivekananda College, Mylapore, Chennai -600 004
[3]Department of Zoology, Dr Ambedkar Government Arts College, Vyasarpadi, Chennai-600 039
[4]Department of Botany, Government Arts College for Men, Nandanam, Chennai-600 035

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ABSTRACT

Various tests in organisms have been utilized for the detection and identification of toxic substances in air, water and soil. The development of comet assay for aquatic organisms is of particular relevance in testing for environmental pollutants. In the present study, the comet assay was applied on fish, *Channa striatus*. DNA damage was assessed in the metabolic tissue, liver and reproductive tissue, testis. The highest degree of DNA damage and comet % scores were seen in experimental fishes when compared to control. Results of DNA damage in both liver and testis were significant ($P<0.05$). Comet parameters (comet % and tail length) in the experimental tissue samples were significant ($P<0.05$). The results suggests a genotoxic nature of the tannery effluent. Comet assay in fish provides adequate sensitivity to be utilized as a tool in the monitoring of water pollution and environmental risk assessment.

INTRODUCTION

Heavy metal pollution affects not only aquatic organisms, but also public health as a result of bioaccumulation in food chain. Contamination of freshwater with heavy metals causes devastating effects on ecological balance of the aquatic environments (Goel, 2000). The accumulation of metals in an aquatic environment has direct consequences to man and to the ecosystem also (Ballschmiter, 2002). Chromium is an environmentally significant metal used in various industrial processes. Chromium compounds enter natural waters mainly through effluents from electroplating and tanning industries, from dyeing, from sanitary landfill leaching and from water - cooling towers can also enter drinking water distribution system (Goel 2000; Kaiser, 2001). Nearly 90% of all leather produced is tanned using chromium and its determination in environmental samples is of great importance due to its toxicity. The metal pollutants are present in water bodies in a mixture of two or more major metals, often forming complexes which are more toxic than individual toxicant (Abdul Jameel, 2000; Palanisamy *et al.*, 2006).

Corresponding author: Sivachandran R.
Department of Zoology, Ramakrisha Mission Vivekananda College, Mylapore, Chennai-600 004

Heavy metals may produce damage to organs as a result of significant alterations in various metabolic activities (Kamalakannan *et al*., 2007). Tannery in the recent period due to urbanization, rapid industrialization has increased natural waters with metals due to dumping of untreated wastes in the aquatic habitats, causing deleterious effects to fish (Radhakrishnan Nair, 2006). The pollution of aquatic environment by tannery effluent adversely affects the survival of aquatic organisms including the commercially important fish species which form the dominating group of aquatic system (Jayaprakash, 2003). Increased disposal of tannery effluent in fresh water results in increased effluent residues in freshwater (Jayaprakash *et al*., 2005: Ganesan and Mazher Sultana, 2010). Fishes provide an excellent material for the study of mutagenic or carcinogenic potential of water samples, since they can metabolise, concentrate and store waterborne pollutants (Akcha *et al.,* 2003; Ateeq *et al*., 2005). Comet assay increases the flexibility and utility of this technique for detecting various forms of DNA damage and repair in virtually any eukaryotic cell. The comet assay is a rapid and sensitive method for detecting primary DNA damage at the cellular level. It combines a biochemical approach to detect DNA strand breaks and/or alkali labile sites with a single-cell approach typical for cytogenetic assays (Collins *et al*., 1997, Lee and Steinert, 2003). This technique has already been

successfully employed for monitoring DNA damage in laboratory and field studies with various fish species, both in freshwater and marine environments (Deveaux *et al*., 1997, 1998; Belpaeme *et al*., 1998; Pandey *et al*., 2006). There are scanty reports whether this phenomenon reflects in target tissues like liver which is the major metabolic site and testis which are involved in reproduction in freshwater fish *Channa striatus,* Therefore, the present investigation was based on continous exposure to tannery effluent, focusing on the assessment of oxidative stress response and their role in induction of genotoxicity.

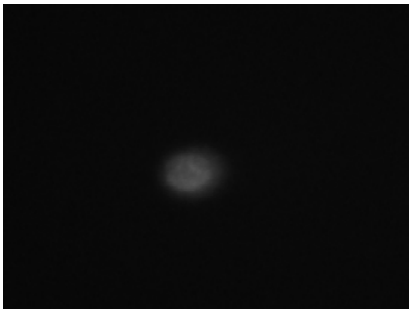
MATERIALS AND METHODS

Healthy adult male fishes weighing 150-200 g and about 20-25 cm in length were used as an experimental model to evaluate the effects of tannery effluent toxicity. The fishes were procured from Puzhal lake, outskirts of Chennai, brought to the laboratory and were acclimatized to laboratory conditions for a period of three weeks. The fishes were maintained in a rectangular plastic tubs (64 x 44 x 29.5cm) filled with 20 L of dechlorinated tap water. The tubs were disinfected with potassium permanganate solution and washed thoroughly prior to introduction of fishes to prevent fungal infection. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of experimental fishes to tannery effluent for 96 hr. The sublethal concentrations was determined and the fishes were maintained in 10 % of tannery effluent for 30 days. Group I served as the control, while group II was exposed to 10% concentration of tannery effluent for 30 days. Statistical analysis was performed using Student 't'test between control and experimental group.

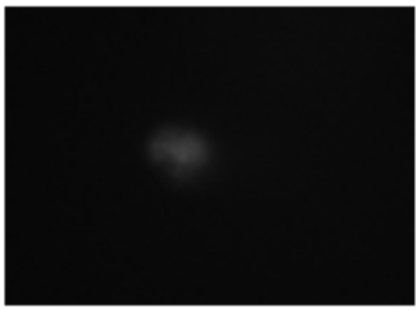
Comet assay: Comet assay to detect the DNA alterations and breaks was according to the method of Cintya *et al.,* 2009. The Comet assay also called Single Cell Gel Electrophoresis is a rapid and very sensitive fluorescent microscopic method to assess DNA damage and repair in individual cells (Olive and Bannath, 2006). This assay has critically important applications in the field of genetic toxicology. Liver and testicular tissues were removed and washed with saline and isolation of DNA was performed. The DNA samples were subjected to single cell gel electrophoresis (Comet assay).

RESULTS

DNA damage was confirmed by Comet assay in liver and testicular tissues. Genotoxicity was measured as DNA strand breakage. When compared to control tissues, DNA damage were significantly higher ($p<0.05$) in liver and testicular tissues of fishes exposed to tannery effluent for a period of 30 days as reaveled by comet percentage scores and tail length (Table 1; Fig 1a,b and 2 a,b).

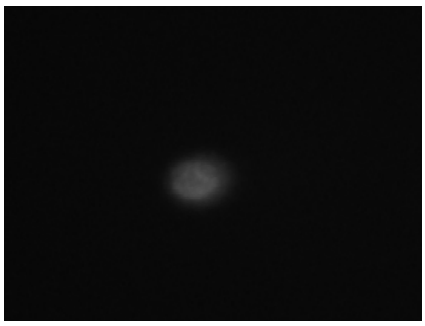


a. Control Liver



b. Tannery effluent treated Liver

Fig. 1a and b. Comet assay of DNA of control and tannery effluent treated liver tissue of *Channa striatus*



a. Control Testis

b. Tannery effluent treated Testis

Fig. 2a and b. Comet assay of DNA of control and tannery effluent treated testicular tissue of *Channa striatus*

Table 1. Effect of tannery effluent on DNA damage in liver and testis of freshwater fish *Channa striatus*

Tissues	Tail Length	% DNA damage
Control - Liver	2.01 ± 0.14	1.6 ± 0.28
Tannery effluent treated liver	13.7 ± 1.10	14.26 ± 1.07
Control -Testis	1.13 ± 0.02	0.88 ± 0.28
Tannery effluent treated testis	12.6 ± 0.3	12.1 ± 1.7

Values are represented as Mean ± SD (n=3)

DISCUSSION

Pollution causes changes to the structure of the fish community and can be fatal to many species. It is known that rare and vulnerable species of fish are particularly threatened by long-term water pollution. Fish in the aquatic environment can be subjected to multiple pollution state and the occurrence of sequential exposure is an important aspect of eco-toxicological research. Sediments also are a sink for anthropogenic contaminants and may act as a pollution source

for bottom-dwelling organisms. Cotelle and Ferard (1999) suggested that sediment-feeders are especially suitable and recommended for biomonitoring studies. DNA damage were shown associated with aquatic pollutants. Comet assay was confirmed as an initial indicator of general, nonspecific DNA damage/genotoxicity and an effective biomarker for environmental monitoring (Mitchelmore and Chipman 1998 a, b; Frenzilli *et al*., 2004). It has great potential to estimate DNA damage in fish because metaphases stage of cell division or karyotyping of the chromosome numbers are not required (Belpaeme *et al*., 1998), as it involves the analysis of single cells, inter-cell variability in responses may be also evaluated. It is known that complex mixtures such as wastewaters and surface waters are composed of a multitude of chemical substances (Reifferschied and Grummt, 2000). Many pollutants, including potential genotoxic polyaromatic and chlorinated substances, have been detected in water and sediment samples (Buschini *et al*., 2004). Synergistic effects from a combination of chemicals cause DNA damage and possibly also have an effect on immune response. DNA integrity relies on complex intrinsic processes in the organism or cell, it is also significantly affected by many external factors, particularly the exposure to pollutants. Exposure to genotoxic chemicals in lower animals results in neoplasia, but frequently with a variety of symptoms known as a genotoxic disease syndrome. These manifestations include impairments in enzyme function, altered protein turnover, impairments in general metabolism, production of initiators of cytotoxic injuries, inhibition of growth, degenerative processes and atrophy in tissues and organs, impairments in immune response, reproduction and adaptation, decrease in survival and succession and ultimately the extinction of species (Kurelec, 1993).

Interaction of genotoxic agents with DNA forms strand breaks but also alkali labile adducts and other modifications, which due to enzymatic removal of damaged nucleotides can contribute to an increased level of DNA strand breaks as observed in the liver and testicular tissues *of Channa striatus* exposed to tannery effluent for 30 days. Chromiun compounds in the tannery effluent may also be a contributing factor for alterations in DNA in the present study. Cadmium and mercury associated genotoxicity in fish has been repoted (Ayllon and Garcia - Vazquez 2000; Risso - de Faverney *et al*., 2001). Genotoxic and carcinogenic effects of arsenic and copper have also been well documented (Reifferschied and Grummt, 2000; Gabbianelli *et al*., 2003). Fish are recognized as ideal indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels and are of varying size and age (Gabbianelli *et al*., 2003). The metabolism of several pollutants, heavy metals among them, generates reactive oxygen species that can attack cellular macromolecules DNA, lipid and proteins leading to serious damage (Livingstone, 2001; Gabbianelli *et al*., 2003; Mamaca *et al*., 2005). Although the majority of fish species avoid long-term exposure to pollutants dissolved in the water at a particular site by actively swimming, some territorial species have a more stationary behaviour and thus may be exposed for longer time. The estimation of genotoxic effects is essential to any comprehensive study of pollutants in the aquatic environment (Van der Oost *et al*., 2003). Although based on a relatively small data, our results confirmed high sensitivity of the comet assay for the detection of DNA damage in liver and testicular tissue. They are also promising for further standardization and the use of comet assay on fish in environmental risk assessment. The present study showed a considerable DNA damage pattern thus proving this species is a potential bio-indicator of genotoxicity. Data obtained could be utilized to estimate the degree of genetic susceptibility as well as to minimize threats to species and improve strategies for its protection in future.

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