EVALUATION OF DNA DAMAGE OF THE FISH *CLARIAS BATRACHUS* EXPOSED TO SAGO EFFLUENT USING THE COMET ASSAY

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ABSTRACT

In this study, the DNA damages were observed by comet assay carried out in the blood samples of the fish Clariasbatrachus exposed to control and different concentrations of treated Sago effluent. The concentration chosen were 25%, 50% and 100%.The DNA damage was determined by the length of comet tail. The control fish showed normal structure whereas the effluent exposed individuals registered alterations in the comet tail. The length of the comet tail was increased with increasing the concentration of the effluent.

Keywords:Sago effluent, *Clariasbatrachus*, comet assay.

INTRODUCTION

Contamination of aquatic resources is one of the most worrying subjects of humankind. Domestic and industrial effluents are the principal responsible for the contamination of aquatic environment (Claxton *et al*., 1998; White and Rasmussen, 1998). Fish have been commonly used as bio indicators of the aquatic ecosystems because they play an important role in the food chain, bioaccumulation toxic substances directly and indirectly through ingestion of both compounds dissolved in water and previously contaminated organism (Cavas and Ergene – Gozukara, 2005; Biagini*et al*., 2009). Fish is an excellent model for detection of mutagenicity or carcinogenicity since they metabolize, concentrate and store water borne pollutants (Al-sabti, 1995).

One of the ways to evaluate the genotoxicpotential of physical, chemical and biological substances is the comet assay. This method is used to detect small DNA damage (Single strand breaks, double strand breaks and alkali-labile sites of individual cells) (Singh *et al*., 1998). Comet assay was first applied to ecotoxicology about 15 years ago and it has become one of the popular tests for detecting strand breaks in aquatic animals through *in*

vitro, *invivo* and *in situ*exposures (Mitchelmore and Chipman, 1998; Cotelle and Fierard, 1999; Lee and Steinert, 2003; Rajaguru*et al*., 2003 and Ohe*et al*., 2004).

Comet assay is considered sensitive, fast, cheap and requires small number of cells to be performed (Mitchelmore and Chipman, 1998; Sasaki *et al*., 1997; Kosz-Vnenchak and Rokosz, 1997) and it has been applied, with great success in erythrocytes of many species of the fishes (Nacci*et al*., 1996; Belpaemee*et al*., 1996) exposed to several genotoxic agents. Genotoxic tests can detect compounds capable of promoting primary damage in the DNA of exposed organisms and is therefore a warning sign of future environmental problems.

Hence in the present study an attempt is made to study the DNA damage of the fish *Clariasbatrachus* exposed to different concentrations of treated sago effluent.

MATERIALS AND METHODS

The Sago industry effluents were collected from a private Sago industry, situated at Poonachi near Ammapet of Erode District, Tamil Nadu, India. The effluent from the industry was collected and transported to the laboratory and used for further experiments. Fingerlings of healthy *Clariasbatrachus* were brought to the laboratory and acclimatized for 15 days. The fish were well fed during the acclimatized period. Feeding was stopped one day before commencement of the experiment.

Blood samples from each specimen of *Clariasbatrachus* were obtained by cardiac puncture using heparinized syringes. The comet assay was performed according to Singh *et al*. (1998). This protocol describes the fluorescent, ethidium bromide staiming methodology and non-fluorescent, silver staining technique (Cerad*et al*., 1997), which is now being routinely used in the laboratory.

RESULTS

In the present study DNA comet assay was done by using blood sample of the fish *Clariasbatrachus* exposed to control, 25%, 50% and 100% concentrations of treated sago effluent. The DNA damage was determined by the length of comet tail. The control fish did not show any abnormalities in the comet tail whereas the effluent exposed individuals registered alterations in the comet tail. The length of the comet tail was increased with increasing the concentration of the effluent. The results are shown in Fig. 1.

DISCUSSION

Cassava sago industry is an increasingly important agro-based industry. Cassava starch is a major raw material in food, textile and pharmaceutical industries. Cassava tubers contain about 20-30% starch which is distributed in the cellulose matrix. Extraction of starch from Cassava consists of washing of tubers, mechanical peeling, rasping, grinding, sieving, regrinding, and dewatering.

The recovery of starch from tubers is not complete; some amount of starch along with fibrous wastes is discharged as residues. The waste water coming out of the settling tanks contain unextracted starch, cellulose, carbohydrates, nitrogenous compounds and cyanoglucosides. It has been customary to discharge the effluents from the factory to rivers, lakes, ponds, drainage channels and fields.

The effluents have a high BOD, COD, cyanide content and pose serious threat to environment. The ground water sources near the factories are also polluted with the cyanoglucosides. The untreated waste water causes damage to crop growth which is grown near to factories.

The physiochemical characteristics of cassava sago starch factory effluents were studied in detail. Wide variations of physical and chemical constituents of untreated and treated effluents obtained from cassava sago starch factory.

DNA damage within the cells can be one of the earliest signs of a whole range of health problems, including disease, diet and exposure to occupational or environmental toxins. The comet assay is a simple and inexpensive method for measuring this damage.The technique is a major advance over other methods as it enables DNA damage from disease, exposure to toxins or diet to be identified significantly earlier than before.

It works by determining the number of breaks in the strands of DNA within the cell. Cells are embedded in gel on a microscope slide and washed to remove the cell membrane, soluble cell contents, and the histones (proteins associated with DNA) from the nucleus. An electric field is then activated across the remaining clumps of 'supercoiled' DNA, which is attracted to the anode.

If the DNA is intact there is little movement, if there are breaks in the DNA strands, however, loops of DNA are pulled toward the anode. Fluorescence microscopy then shows the image which gives the technique its name: a clump of undamaged DNA (the head) with the loops pulled away, forming a tail, the proportion of DNA in the tail enables the number of strand breaks to be determined, either visually or by computerized image analysis.

Raja Guru *et al*., (2003) have studied the genotoxic properties of water and sediment collected from Noyyal River, which is polluted with industrial effluent and sewage by exposing the fish (*Cyprinuscarpio*) and earthworms (*Eiseniafoetida*) using the alkaline comet assay. Upon electrophoresis, extensive damage, measured as the DNA length width ratio of the DNA mass was observed in erythrocytes, liver and kidney cells of the fish exposed to polluted water samples and the amount of damage increased with the duration of exposure.

Lima *et al*., (2006) have conducted the comet assay in the blood samples of the fish *Oreochromisniloticus* exposed to swine industry effluent to determine the DNA damage. The fish exposed sub chronically and chronically to effluent showed consistently greater DNA damage.

Cavas and Konen (2005) and Hoshina*et al*.,(2008) found that the final effluent from the oil refineries induced high frequencies of micronuclei and nuclear abnormalities in the gills and erythrocytes of *Oreochromisniloticus*, showing the harmful effects of waste from the petrochemical industry on the genetic material of aquatic organisms.Souza and Fontanetti (2007)using the comet assay studied the influence of petroleum refinery effluent in *Oreochromisniloticus* and observed a significant increase in comet score.

Similar findings have been observed in the present investigation. The fish reared in natural condition showed normal structure whereas the fish exposed to sublethal concentrations showed damages in their structure. The damages were increased with the increase in concentration of the effluent. This study reveals that the effluent discharged after treatment may still contain certain amount of genotoxic and mutageneic compounds.

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Figure.1. Results of the comet assay performed with *Clariasbatrachus* blood samples. Control – without damage; 25% treated – small damage; 50% treated – medium damage and 100% treated – large damage.