ISSN: 2458-8989

Natural and Engineering Sciences

Supplement, 2019, 4(3): 25-31

-RESEARCH ARTICLE-

Assessment of DNA Damage by Comet Assay in *Trachinotus ovatus* Cells from Mersin Bay in the Northeastern Mediterranean

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Abstract

The aim of this study is to get database to elucidate the quantity of DNA damage in *Trachinotus ovatus* from Mersin Bay in the North-eastern Mediterranean. Primarily, we have used the single-cell gel electrophoresis, commonly known as Comet assay to detect the strand breaks in DNA. This technique was performed on liver and gill tissues of *T. ovatus* captured from the Mersin Bay. The single cell gel electrophoresis was executed under alkaline conditions. The slides were neutralized with ice cold 0.4 M Tris buffer (pH 7.5) and stained with 80 ml ethidium bromide (20 mg/ml) and imaged with attachment of Leica fluorescent microscope integrated CC camera. DNA damage levels were determined from 100 cells for each sample. As a result of the COMET analysis; tail length, tail ıntensity and tail migration were 39.718±6.826 µm, 32.752±9.281% and 30.089±9.930 in gill tissues while they were 29.440±9.889 µm, 30.010±6.222 % and 19.119±5.025 in liver tissues respectively. it can be concluded that COMET analysis in *T. ovatus* from Mersin Bay can be a useful tool for screening genotoxic pollutants in the recipient environment.

Keywords:
Comet assay, DNA damage, *Trachinotus ovatus*

Article history:
Received 04 September 2019, Accepted 27 December 2019, Available online 30 December 2019

Introduction

Over the past decades, the comet assay (single-cell gel electrophoresis) has become one of the methods of choice for the evaluation and measurement of DNA damage (Collins et al., 2008). In epidemiological molecular and biomonitoring studies, primary DNA damage, evaluated by the comet assay, is used as a biomarker of exposure that provides information on the biologically effective dose of various physical and/or chemical mutagens/carcinogens (Collins et al., 2000; Moller 2018). The comet assay is quite simple, quick, cost effective, accurate and reliable; in other

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words, it fulfils all the criteria for use in routine laboratory, field as well as clinical testing. The comet assay is a predictive test that allows for the detection of DNA alterations of diverse kinds, such as single-strand DNA breaks, double-strand DNA breaks, alkali-labile sites, altered bases, incomplete repair sites, and interstrand cross-links Collins et al., 2008).Quantification of DNA damage by lysing cells embedded in agarose gel under alkaline conditions was first introduced by Rydberg & Johanson (1978). To improve the sensitivity of the single-cell DNA damage detection, Östling & Johanson (1984) developed the micro-gel electrophoresis technique, also called the comet assay. In comet assay procedure, cells are embedded in an agarose matrix and lysed to produce nucleoids of supercoiled DNA attached to the nuclear matrix. Breaks in the DNA relax the supercoiling and allow DNA loops to expand, and on electrophoresis to move towards the anode. Along the way, the DNA path resembles the shape of a comet, which gave the assay its final name. This is followed by either visual (after classification into different categories based on tail length and shape), semi-automatic and/or automatic (which automatically recognize the extent of damage) analyses of stained DNA and calculation of fluorescence to determine the DNA damage extent. This is done by using appropriate software that enables commercially available image analysing systems to be connected through a camera to a fluorescence microscope, which facilitates the evaluation of DNA damage. Each trace resembles a comet with a brightly fluorescent head and a tail whose length and intensity are proportional to the frequency of DNA breaks present in the cell (Mcart et al., 2009; Shaposhnikov et al., 2009).

Physical agents, e.g. solar radiation, X-rays, and a variety of chemical compounds can damage the DNA of living cells. If not repaired these DNA lesions can initiate a cascade of biological consequences at the cellular, organ, whole animal and finally at the community and population level. DNA damage in a variety of aquatic animals has been associated with reduced growth, abnormal development and reduced survival of embryos, larvae and adults (Brenerman et al., 2014).

In principle, any cell type can be used for genotoxicity testing with the comet assay The sensitivity of the comet assay can be significantly increased by introducing an additional digestion step with specific restriction endonucleases that recognize one or several DNA lesions and convert unrepaired lesions to additional DNA strand breaks. (Tice et al., 2000). Comets can be identified and scored by visual inspection or by using image analysis software packages (Jha, 2008). Visual scoring gives a simple qualitative indication of DNA damage. However, it is very subjective. In contrast, the use of comet analysis software provides quantitative and reproducible measurements. The currently available comet analysis tools can be broadly classified as manual or automated. Manual analysis typically requires an expert to set threshold brightness values separating the background, to select the nucleus, and to mark the comet head (Konca et al.,2003). Automated tools employ image analysis techniques to recognize and measure comets, and are generally much faster than manual scoring. Due to the efficiency gained through automation, one can typically afford to measure larger sample sizes, which is critical for statistically significant results (Konca et al., 2003). The aim of this study is to get database to elucidate the quantity of DNA damage in *Trachinotus ovatus* from Mersin Bay in the North-eastern using comet analysis software (automated tools).

Material and Methods

Fish Samples

In this study, six *Trachinotus ovatus* specimens (mean weight 69.56±1.063 g, mean length 23.5±0.745 cm) were sampled from the Aydıncık coast (33°23′36″−33°32′57″N; 36°07′00″−36°09′39″E) western entrance of the Mersin Bay (North-eastern Mediterranean) using a commercial bottom trawl net in December 2017 (Figure 1).

Figure 1: *Trachinotus ovatus,* (Orginal image).

Alkaline Comet Assay

Formation of DNA damage was assayed by the alkaline comet assay essentially as described by Mayer et al., (2002). The test for sensitivity of the comet assay as used in this study is described in the supplement and indicated that DNA damage caused by gamma irradiation of 0.2 Gy is clearly detectable**.** Subsequently, slides were transferred to a horizontal electrophoresis unit (High Throughput comet assay tank COMPAC-50, Cleaver Scientific Ltd) filled with alkaline electrophoresis buffer. After 20 min of DNA unwinding, electrophoresis (power pack CS300, Cleaver) was performed at 4°C at 0.8 V/cm for 20 min. Slides were rinsed for 20 min first in neutralisation buffer, then for 20 min in water and subsequently air dried and stained with 50 µl SYBR Green solution (Molecular Probes). The comets per slide area were selected at random and evaluated by fluorescence microscopy (Schmeiser et al., 2019). The DNA damage levels were determined in head length (HL, µm), tail length (TL, µm), head intensity (% H-DNA), tail density (% T-DNA), tail moment (TM) and tail migration (TM) from the photographed images of 100 cells for each sample using Comet Software (3.0). All values were given as mean ± standard deviation of mean (SD). All analyses were carried out in triplicates for each tissues of *T. ovatus.*

Results

The DNA damage parameters as Head length (µm), Tail length (µm), percent DNA in head, percent DNA in tail, tail Moment (µm) and tail migration (TMi) are given in Table 1. In gill cells, tail length, tail ıntensity and tail migration were 39.718±6.826 µm, 32.752±9.281% and 30.089±9.930 while in liver cells they were 29.440±9.889 µm, 30.010±6.222 % and 19.119±5.025 respectively.

Tablo 1**.** The DNA Damage parameters in different tissues of *Trachinotus ovatus,* (Data are shown as mean± SD)

	Gill	**Liver**
Head lenght (µm)	22.699±6.444	20.724±5.849
Tail lenght (µm)	39.718±6.826	29.440±9.889
Head Intensity (%)	67.248±4.159	69.990±12.151
Tail Intensity (%)	32.752±9.281	30.010±6.222
Tail Moment (µm)	7.789±1.015	5.288±1.211
Tail Migration	30.089±9.930	19.119±5.025

Fig. 2 show the DNA damage in gill and liver cells of *T. ovatus* respectively. In both the tissues medium damage was observed according to the criteria (Mitchelmore et al., 1998). In gills tail ıntensity (%) was 32.752±9.281% whereas in liver ıt was 30.010±6.222 % respectively. According to Mitchelmore et al. criteria (1998) they created in the light of their studies, the% T-DNA used to determine DNA damage is less than <10%, minimal damage, low damage between 10-25%, medium damage between 25-50%, high damage between 50-70% and > 70% if the criteria for extreme damage.

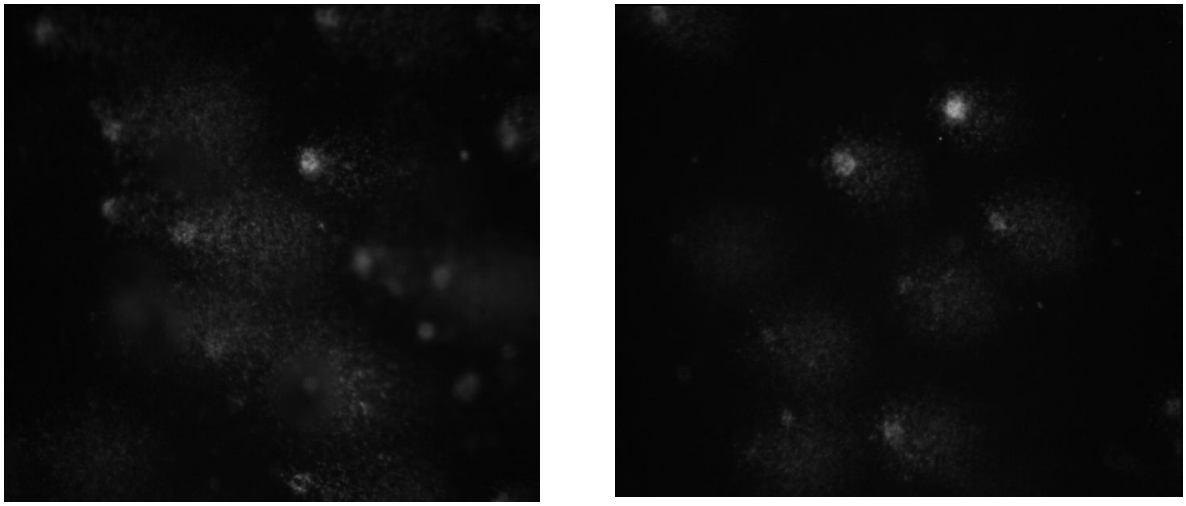

Figure 2. Comet images of gill (left) and liver (right) tissue of analysed *Trachinotus ovatus.*

Discussion

Due to growing number of agricultural, commercial and industrial chemicals, the rate of genetic disorders, diseases and mortality of exposed organisms in the natural habitats has increased significantly (Livingstone, 2001). This needs to study the impacts of these chemicals on integrity and functioning of cellular DNA in organisms. DNA plays a key role in life processes because it carries genetic information. Most likely, the genotoxicity of xenobiotics/agrochemicals/drugs is modulated through cellular distribution of bound DNA (Ahmad et al., 2016). In this study, we found medium DNA damage of gill and liver cells in *T. ovatus* according to Mitchelmore et al., 1998. Similarly, Ameur et al., (2012) observed signi*fi*cant increase in DNA damage (as % tail DNA) 89.23% and 93.97% respectively in mullet and sea bass from Bizerte Lagoon, Tunisia. Genetic

damage is of high concern as it can lead to changes at gene level resulting in mutagenicity or carcinogenicity in *fi*sh tissues owing to chronic exposure to heavy metals or PAH etc. in marine ecosystem (Udroiu, 2006).

Being a vertebrate model, fish is the best accessible to estimate potential risks, due to their ability to metabolize and accumulate contaminants in their bodies (Diekmann et al., 2004). In the detection of DNA damage in fish, various tissues such as gill, liver, sperm, muscle, kidney, brain can be examined extensively and gill and liver are among the most commonly observed tissues (Klobuˇcar et al., 2010; Fatima et al., 2015). Frenzilli et al. (2009) reported that liver is one of the most frequently observed tissues due to its direct effect on metabolic activities and contaminants in fish. During present investigation, *T. ovatus* appeared sensitive to exhibit DNA damage and the DNA damage detected as high damage was observed in gill than liver. Few other workers have also noticed high DNA fragmentation in liver cells from *fi*eld studies (Rajaguru et al., 2003) and from induced studies (Akter et al., 2008; Ahmed et al., 2013). The comet assay has a well-deserved popularity, as it is simple and economical to perform. The assay has been used under a variety of exposures, including in vitro, in vivo and in situ. DNA damage in aquatic animals collected from contaminated field sites was associated with effects on growth, reproduction and population dynamics (Lee et al., 2003).

Consequently, it can be concluded that COMET analysis in *T. ovatus* from Mersin Bay can be a useful tool for screening genotoxic pollutants in the recipient environment. Additionally, the data generated by this study provides an overview of the health of ecosystem which is highly useful in risk assessment studies.

Acknowledgment

This is presented in the International Next Generation Biometry Workshop and Course held on 04-06 October 2019 in İskenderun, Hatay, Turkey.

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