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AN OPTIMIZED COMET ASSAY PROTOCOL FOR *DROSOPHILA MELANOGASTER*

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Abstract

Genotoxins can cause various disorders in chromosome and DNA structure such as gene mutations, chromosome abnormalities and DNA chain breaks. This situation has been associated with the concept of genetic toxicity. Genotoxicity tests have been developed to predict the possible effects of genotoxins on all living things in nature. The single cell gel electrophoresis assay (SCGE, also known as comet assay) is used as the fastest, easy and sensitive. The Comet method is based on distinctive migration characteristics of DNA molecules having different electrical charges and molecular weights at alkaline pH. According to this method, nucleolus' and cells can be visualized by staining with a fluorescent dye by first placing between the agarose layers and then running at alkaline electrophoresis buffer and lysis solution and lastly applying neutralization procedure. The displayed DNA fractures are measured and evaluated according to tail length. DNA images are graded according to the degree of damage. DNAs that are not damaged are evaluated as arbitrary units (AU) by scoring 0 and damage DNAs are scored from 1 to 4 according to the degree of damage. Comet assay is a useful method in evaluating the potential toxic effect. *Drosophila melanogaster* is the most important non-mammalian organism used in the fields of medicine and biology. *Drosophila melanogaster* has been used for many years as an *in vivo* model organism for potential toxicity and genotoxicity studies. It is important to determine the damage of genotoxins on DNA. It was preferred because it was easy to obtain and grow in the laboratory, the larvae were cheap and the life cycle was short-lived. Comet analysis using *Drosophila melanogaster* tissues to determine the damage of various genotoxins on DNA is an effective and easy method. This study aims to explain the alkaline Comet assay aimed to determine the effect of general various genotoxins on *Drosophila melanogaster* tissues.

Keywords: *Drosophila Melanogaster*, genotoxicity, comet assay.

1. Introduction

Contrary to biological incidents causing DNA damage (e.g. mutations and chromosome abnormalities, etc.), a range of techniques are used so as to identify DNA damage caused by substances which have genotoxic activity (Tice et al.,2000).

Researchers analyze DNA damage caused by various agents in their toxicology studies. These DNA damages include such damages as DNA adducts, DNA breaks, chromosomal abnormalities and gene mutations. Genotoxicity studies increase in importance day by day as genotoxicity causes various diseases such as cancer and aging. Genotoxicity tests are developed especially to measure carcinogenic potential of mutagenic and genotoxic substances (Sezginer & Dane, 2016). "Comet Assay" takes an important place among genotoxicity tests. With the use of plants, a wide range of terrestrial or aquatic species especially in environmental risk assessments, comet assay has been among the most widely conducted experiments (Langie et al., 2015).

In addition, *D. melanogaster* has become one of the most widely used model organisms in experimental studies on human health (Gaivão & Sierra, 2014; Langie et al., 2015). Various larvae (from brain, midgut, hemolymph and virtual disk) and pubescent forms of this model organism have been widely used in comet assay (Gaivão & Sierra, 2014). Comet assay was first tested on *D.melanogaster* in 2002. Since 2002, fly studies have been attracting more attention thanks to the assay. Many researchers point out that in *D.melanogaster*, the use of comet assay for toxicology studies is an important potential. Comet practice may clarify impact mechanisms of toxins which may considerably affect the limited research conducted on vertebrates. It is also considered that the use of comet assay for DNA stability assessment of flies will rapidly become more widespread in the future with a high degree of probability (Augustyniak et al.,2015).

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This study aims to introduce *D. melanogaster* model organism and comet assay and to clarify a comet assay protocol which is optimized for *D. melanogaster*.

2. *Drosophila Melanogaster*

D.melanogaster (phylum of Arthropoda, class of insecta, order of diptera, genus of *D. melanogaster*) is an invertebrate model organism which most resembles human beings. When compared to other model systems, this non-mammal model system has various advantages such as short life cycle, being easily cultured, ease of care, rapid sexual maturation, multiple molecular mechanisms and ease of experimental manipulation. Furthermore, they are the reason behind frequent use of *D.melanogaster* in toxicology studies. When *D.melanogaster* genome sequencing is analyzed, it is revealed that 70% of human genes have orthologs of *D.melanogaster* genes. In addition, most of the genes which regulate reproduction are those genes which are also protected in vertebrates. *D.melanogaster* is an excellent in vivo genetic model system for toxicity studies thanks to its 65-70% of functional homology with human beings as well as the proven genetics and developmental biology (Tiwari et al., 2011). Nucleotide sequencing of almost whole 120-megabase euchromatic portion of *D. melanogaster* genome has been completed to a large extent. Also, analyses reveal that 13,600 genes have been coded (Adams et al., 2000).

D. melanogaster is a key model in improving research on molecular mechanisms of ageing. Signal reduction through insulin and IGF-I (Insulin-like growth factor-I) in healthy aging has a critical role in ensuring evolutionary protection. It is possible to easily conduct research on how *D. melanogaster* may change the process of aging by taking into consideration the genetic findings (with the movements in intestines and muscles) thanks to its vulnerability throughout its life cycle and as it is possible to conduct external intervention (Piper & Partridge, 2018).

In a study conducted with *D.melanogaster*, it is observed that vitamin C leads to a substantial increase in survival, development and copulating success of flies in their life cycles. Consequently, it gives rise to thought that vitamin C may suppress the induction of oxidative stress (Ong et al., 2014).

3. Comet Assay

Comet assay is known as single cell gel electrophoresis experiment. It is a method used in single cell eukaryotes so as to identify DNA damage in qualitative and quantitative terms. It has become one of the most popular and most common methods for -especially *in vivo*- genotoxicity studies over the past decade. The assay has become popular thanks to its simplicity and the availability of various tissue cells, and as it requires few cells (Carmona et al., 2011). Comet assay is a method used to identify DNA strand break in individual cells and this technique has both alkaline and neutral versions (Fairbairn et al., 1995). In this study, it is observed that alkaline version of Comet assay is a suitable method for *D.melanogaster*.

3.1 Comet Assay Protocol Conducted with *Drosophila Melanogaster*

- 0.5% Agarose which has a low melting point (LMA: Low Melting Agarose) is melted for comet analysis and kept in a heater at 40°C so as to ensure that it is not frozen.
- 1% Agarose which has a normal melting point (NMA: Normal Melting Agarose) is prepared and covered by slides.
- After fly specimens are completely minced in HBSS Solution (20mM EDTA (Ethylenediaminetetraacetic acid)/10% DMSO (Dimethyl sulfoxide)), 50 µl is taken from the mixture and 100 µl is taken from 0.5%-LMA and they are mixed, which is added to slide covered by NMA and kept until frozen. It is kept at 4°C for 5 minutes.
- Preparations (2.5 M NaCl (Sodium Chloride), 100 mM EDTA, 10 mM Tris base are arranged at pH.10 and 1% Triton X-100 and 10% DMSO are freshly added) are kept in lysis solution for 1 hour at 4°C.
- Preparations (10N NaOH (Sodium Hydrochloride), 200mM EDTA, pH>13.0) are kept in electrophoresis buffer at 4°C for 15 minutes.
- Alkaline electrophoresis 24 V and 300 mA are performed for 40 minutes.
- Preparations are neutralized in 0.4 M Tris buffer (pH 7.5) for 5 minutes.
- Afterwards, preparations are stained with ethidium bromide (10 µl/ml) and fluorescence microscope (Zeiss, Germany) is used to analyze them (Dhawan et al., 2009).
- 100 comets on each slide are visually classified in a way to be in one of five classes which are predefined in accordance with tail density and each scored comet is given a value of 0, 1, 2, 3, or 4.

Among 100 cells, those who are damaged are scored from undamaged (0) to maximally damaged (4) (Olive & Banáth, 2006).

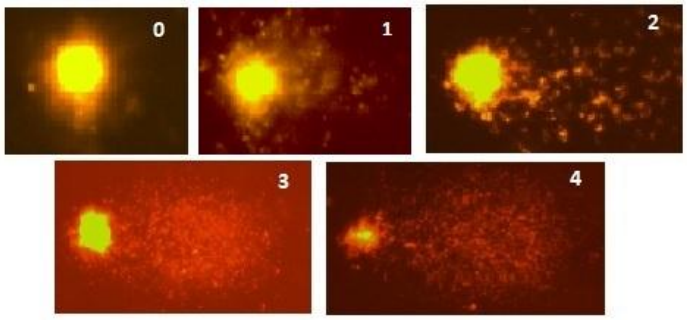

Figure 2. Image of comet assay in gel. (Bartin University, Molecular Biology and Genetic Lab.)

4. Discussion and General Conclusions

When studies conducted and test protocols used are analyzed, the test protocol suitable for *D.melanogaster* species is compiled in this study thanks to trials carried out. The use of *D.melanogaster* model organism in comet assay increases in importance day by day. To illustrate, in a study conducted with *D.melanogaster*, it is found that there is a significant positive correlation between chlorpyrifos and production of ROS (recative oxygen species), apoptosis and DNA damage. (i) It is thought that ROS may cause apoptosis and DNA damage in *D. melanogaster* larvae which are exposed to genotoxins such as chlorpyrifos, and (ii) it is expressed that *D. melanogaster* can be used as an alternative for *in vivo* animal model for xenobiotic risk assessment (Gupta et al., 2010). In another study, it is observed that medaka (Oryzias latipes) fish are exposed to genotoxins such as 4-NP for 15 days and it is revealed through comet assay that it causes the highest DNA damage at the highest dose (100 µg/l) (Sayed et al., 2018). According to the studies conducted, it can be assumed that distributed image (Figure 2) of DNA, the damage of which is detected through comet assay, also represents apoptotic cells (Collins, 2004). It is important to learn the impact of substances -to which creatures are exposed due to environmental pollution increasing with the advancement of technology- on DNA damage so as to diagnose in advance diseases such as cancer, aging, etc.

Finally, the results obtained by the alkaline *in vivo* comet assay in *D.melanogaster* tissue clearly demonstrate that this is a suitable *in vivo* assay for the detection of DNA damage caused by chemical and physical mutagens (Carmona et al.,2011). Briefly, in this review, the comet assay steps optimized for *D.melanogaster* tissues are described in detail for researchers working with comet analysis on *D.melanogaster*.

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