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Comet assay for rapid detection of oxidative DNA base Damage in foods

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تحليل الكومت للكشف السريع لإضرار قاعدة الحامض النووي الرائبوزي منقوص الأكسجين DNA المتأكسدة في الاغذية

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الملخص:

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تتصف تقنية الترحيل الكهربائي الهلامي للخلايا المنفردة- تحليل الكومت، بكونها طريقة سريعة وحقيقية وحساسة لكسور أشرطة الحامض النووي الرائب زي منقوص الاكسجين (الدنا) DNA المنفرد والمزدوج، والكشف عن مواقع االارتباط المتفلورة وموقع إصلاح الحذف في الخلايا المنقسمة. في السنوات الاخيرة، تستخدم هذه الطريقة بشكل واسع في دراسات إصلاح الدنا، علم السموم الوراثية والمؤشرات الحيوية البيئية، لذلك تعطي هذه التقنية كأداة مهمة للكشف عن أضرار الدنا في الكائنات الحية وازداد استخدامها في الاختبارات الوراثية لتأثير الكيميائيات

الصـناعية والملوثـات البيئيـة. يهـدف هـذا البحث في تقييم تأثيرات العوامل التأكسدية الناتجـة مـن التعـرض للملوثـات باسـتعمال تقنيـة التحليـل الكومـت. اسـتخدم البحـث خمـس عينـات لكـل نمـوذج مـن النمـاذج (اللحـم، الـدجاج، رز، فواكـه، خضـروات والشـاي) لتقيـيم التـأثيرات الوراثيـة عنـد التعـرض للتلـوث بالملوثـات البيئيـة. تؤكـد المعلومـات المستحصـلة مـن التجـارب بـان مؤشرات ضرر الدنا(طول الـذيل، عـرض الـذيل) والـذي وجـد بمعـدلات عاليـة عنـد التعـرض الـى التلـوث بالمقارنـة مـع نسـب طـول الـذيل إلـى عـرض الخلايـا التـي أظهـرت عـدم وجـود هجـرة عنـدما تصـل النسـبة الـى واحـد(1). نسـبة وتوزيـع لهـذه الخلايا المتعرضة للملوثات قد ازدادت مـع زيادة قيم التعرض. نستنتج من هذا البحـث إمكانية استخدام هذه التقنية كتقنيات حساسة

والتي من الممكن استعمالها في الكشف عن خطورة العوامل البيئية بصورة مبكرة (مراحلها الأولية).

Abstract

Single cell gel electrophoresis (S C G E) or comet assay technique a sensitive, reliable and rapid method for DNA double & single strand break, alkali- labile sites and delayed repair site detection in individual cells. In recent years, this method has been widely used for studies of DNA repair, genetic toxicology, and environmental biomonitoring; however, this technique serves as an important tool for detection of DNA damage in living organisms and is increasingly being used in genetic testing of industrial chemicals, environmental agent's contaminations. This Research paper helps to evaluate

the oxidant agent's effects of exposure to organic pollutants by using comet assay technique. This study used five samples of each food sample (Meat, chicken, Rice, Fruits, Vegetables and Tea) to evaluate the genotoxic effects of exposure to environmental agent's pollutants. The experimental data suggest that the DNA damage parameters (Tail length, Tail width l) were found higher value in exposed population when compared with the ratio of the length to width that cells exhibiting no migration having a ratio of; 1. The percentage and distribution of cells in exposed population also increases with the increase in values. This study demonstrates that, using sensitive techniques, it is possible to detect environmental agent's risks at an early stage.

Key Words- Comet assay, DNA damage, oxidative stress.

Introduction

production of reactive oxygen species (ROS), such as the superoxide anion (O2 •2), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH), are an inevitable consequence of utilizing inspired molecular oxygen for oxidative phosphorylation [1]. It has been widely hypothesized that the production of ROS, localized particularly around mitochondria (the major site of oxygen consumption and ROS production), results in lipid peroxidation, protein oxidation, and nucleic acid damage (1– 2). Ionizing radiation -IR undoubtedly can damage DNA by directly ionizing DNA itself and by indirect processes in which DNA reacts with numerous radiolytic reactive products including •OH, H•, O2•-, and H2O2, that are generated in aqueous fluid surrounding DNA (3). pesticides have been associated with pathological and chromosomal damage in humans. There are also epidemiological links with cancer (4). Pesticides possibly induce oxidative stress leading to the generation of free radicals and alteration in antioxidant/free radical scavenging enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione

reductase and glutothione transfers (5). During the last few years, there has been a great interest in developing rapid and simple tests to identify the effects of exposure to environmental agents that can affect the health of individuals due to DNA damage. One of these methods is the comet assay, which is a rapid and sensitive technique to measure sites sensitive to basic pH and DNA breaks in individual cells (6). The comet assay is sensitive to damage above about 50 breaks per diploid mammalian cell and will lose sensitivity above about 10,000 breaks per cell (7). 8 (1984) were the first to develop a microgel electrophoresis technique for detecting DNA damage at the level of the single cell. The advantages of comet assay include the applicability to various tissues and special cell types, sensitivity for detecting low levels of DNA damage, requirement for small number of cells per sample, general ease of test performance, the short time needed to complete a study and relatively low cost (9).

Result& discussion:

All slides, including those of the positive and negative. The methods used for quantifying DNA migration by this assay have varied almost as much as the number of scientists using the technique.

The most flexible approach for collecting comet data involves the application of image analysis techniques to individual cells. The simplest method for collecting comet data is based on determining the proportion of cells with altered migration. However, this approach is generally limited to electrophoretic conditions Fig. (1).

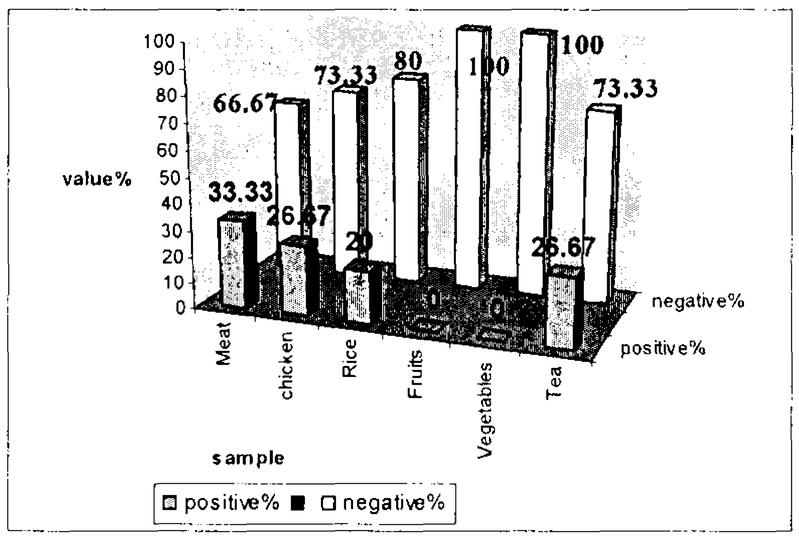

Fig.1: show proportion of cells with altered migration of different samples.

A more useful approach classifies comets into several categories, based on the length of migration and/or the perceived relative proportion of the DNA in the tail; (15).By assigning a numerical value to each migration

class, the average extent of DNA migration among cells within a culture or animal can be calculated. The metric most commonly used in Comet studies is the length of DNA migration, presented generally in microns. Migration length is generally believed to be related directly to fragment size and would be expected to be proportional to the level of single strand breaks-SSB and Alkaline libeled site- ALS, and inversely proportional to the extent of DNA cross-linking. This metric has been measured using a variety of approaches, including by micrometer in the microscope eyepiece, by ruler on photographic negatives/positives of cell images or on a camera monitor, and by image analysis. The criteria used to identify the trailing and leading edge of the migrating DNA seem to be investigator. Furthermore, some investigators use the term tail length to describe image length while others apply the term to migrated DNA only. A variant of this metric is to present the ratio of the length to width (16) or width to length (17), with cells exhibiting no migration having a ratio of; 1. As the use of computerized image analysis systems to collect comet data has increased, a metric based on the percentage of migrated DNA (18) has become used more

frequently. This metric assumes signal linearity in quantifying the amount of DNA ranging over multiple orders of magnitude and that the efficiency of the fluorescent dye in staining migrated and non migrated DNA is equal. Neither assumption has been validated for all imaging systems and dyes used (19). which metric of DNA migration is used will depend on the resources of the investigator and the experimental design. The expert panel did not consider any single metric to be without usefulness. However, when using a derived metric such as tail moment, data on tail length and the percentage of migrated DNA should be provided also (19) table (2), (fig. 2, 3, 4).

Table2: A variant of this metric is to present the ratio of the length to width of different sample.

sample	ratio of the length to width				
Meat	2.7±0.29	2.5±0.89	3.8±0.89	2.6±0.89	1.7±0.69
chicken	1.3±0.07	1.7±0.03	1.23±0.19	1.8±0.29	
Rice	1.5±0.06	1.23±0.05	1.34±0.29		
Fruits	0.3±0.02	0.6±0.02			
Vegetables	0.5±0.03	0.8±0.01			
Tea	1.04±0.09	1.26±0.09	1.44±0.59	1.28±0.69	

Values are presented as means ±SE.

(n= 5).

Fig3: Comet assay of beef cells
Fig2: Comet assay of chicken cells

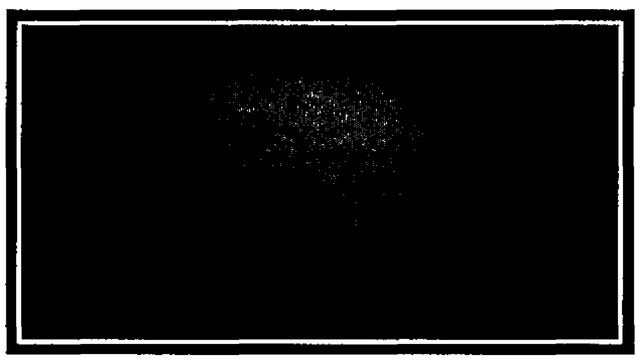

Fig4: Comet assay of rice cells

The potential of ROS to damage macromolecules has led to their production being linked to senescence and the degenerative diseases associated with aging, e.g., heart disease, Parkinson's disease, diabetes mellitus, and mitochondrial diseases (20). This linkage between ROS production and senescence forms the basis of Harman's free radical theory of aging

(9). Irradiation damages food by breaking up molecules and creating free radicals. The free radicals kill some bacteria, but they also bounce around in the food (11)., damage vitamins and enzymes, and combine with existing chemicals (like pesticides) in the food to form new chemicals, called unique radiolytic products (URPs). Some of these URPs are known toxins (benzene, formaldehyde, lipid peroxides) and some are unique to irradiated foods. Scientists have not studied the long-term effect of these new chemicals in our diet. Therefore, we cannot assume they are safe.

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