

Detection of Irradiated *Astragalus membranaceus* Bunge and *Havenia dulcis* Thumb Using DNA Comet Assay

– Research Note –

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Abstract

Ionizing radiation can be used to sanitize herbs contaminated by various microorganisms. However, health concerns related to irradiation damage to complex molecules in plants necessitate that methods be developed to monitor such damage. To elucidate DNA damage of herbs caused by irradiation, the DNA comet assay was used for *Astragalus membranaceus* Bunge and *Havenia dulcis* Thumb, irradiated at 1, 5, 7, and 10 kGy. With increasing irradiation doses, the tails of comets became longer with average tail length increasing from 17 (non-irradiated) to 124 (10 kGy) μm in *Astragalus membranaceus* Bunge. Above 7 kGy, some of the tails were separated from the heads of comets. Distribution patterns of the tail length of 100 comets selected randomly in the irradiated herbs were analyzed to quantify the DNA damage. These results clearly suggest that the DNA comet assay is an effective and inexpensive tool for the detection of irradiation damage to DNA in herbs.

Key words: comet assay, detection, herbs, irradiation

INTRODUCTION

Ionizing radiation can be used to sanitize herbs contaminated by various microorganisms. Herbal remedies have been used for treatment of many diseases in Asian countries and by traditional healers throughout the world. Irradiation sterilization of herbs has increasingly gained commercial interest, since herbs are subject to microbial spoilage during storage. Irradiation for sterilization purposes normally uses ionizing radiation from high-energy electrons or gamma-rays from ⁶⁰Co. In general, treatment of foods with ionizing radiation has become popular in many countries since it reduces food-borne illness. In order to monitor the use of irradiation, biological, chemical, and physical detection methods of food treated with ionizing radiation have been studied (1). Direct epifluorescent filter technique (DEFT) in combination with aerobic plate counts (APC) has been proposed as a screening method for irradiated spices (1,2). Turbidimetry combined with standard plate counts (SPC) was proposed as a detection method for irradiated spices contaminated with residual microflora (2). Differential scanning calorimeter (DSC) has been used to identify irradiated foods in many countries (3,4). Also, based on the production of free radicals in the irradiated foods, electron spin resonance (ESR) and thermoluminescence (TL) have been shown to be useful detection methods (1,5).

Ionizing radiation also induces DNA damage in cells, which can be detected with the comet assay using agarose

gel electrophoresis (6). The DNA comet assay, originally developed by Stling and Jahanson (6), is based on the DNA strand breaks caused by ionizing radiation. DNA and its fragments migrate in the gel by electrophoresis, forming a DNA comet visualized under a microscope after staining. Irradiated cells show comets with long tails, while non-irradiated cells have no tail or very short ones. Measuring DNA strand breaks is based on the fact that strand breaking reduces the size of the large duplex DNA molecule; and that electrophoresis causes stretching and migration of separated strands, which are observed in the comet assay. The shape of the comets is used to estimate the dose. As the dose increases, the end of the tail becomes wider and the tails are longer and sometimes separated from the head (7). The DNA comet assay offers great potential as a rapid tool to detect irradiated food, both of animal and plant origin (8). The advantage of the comet assay is simplicity and low cost. Klaude et al. (9) studied the mechanisms of the comet assay, observing that the use of neutral versus alkaline conditions affected the behavior of DNA. Neutral treatments resulted in comet tails consisting of relaxed loops, whereas samples subjected to alkaline treatments had tails made up from DNA fragments.

This study further elucidated and refined the DNA comet assay for use in the detection and quantification of irradiation in foods; using two typical medicinal herbs, *Astragalus membranaceus* Bunge and *Havenia dulcis* Thumb.

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MATERIALS AND METHODS

Materials

Astragalus membranaceus Bunge and *Havenia dulcis* Thumb were purchased from a local market in Daejeon and packed in polyethylene bags. Normal and low melting point agaroses were purchased from FMC Bio Products (Rockland, USA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO., USA).

Sample irradiation

Herbs packed in polyethylene bags were irradiated at 1, 5, 7, and 10 kGy using a ^{60}Co irradiator (AECL, IR-79, Ontario, Canada) at the Korea Atomic Energy Research Institute. The dose was measured by a ceric-cerous dosimeter.

Preparation of cell suspensions

Samples (1.5 g) were cut into slices and put into a beaker containing 5 mL ice-cold phosphate buffered saline (PBS) for 5 min and stirred for 5 min. The homogenate was filtered through 200 μm and 100 μm nylon sieve cloths, and stored at 4°C for 30 min. After centrifugation at 500 \times g, the supernatants were saved for assay.

DNA comet assay

Slides (76 \times 26 mm) were soaked in methanol for 24 hrs to remove impurities and then pre-cooled to facilitate adhesiveness of agarose. Each slide was layered with warm agarose solution, air-dried for 30 min, and stored in a dust proof box. One hundred micro-liters of cell suspension was mixed in 1 mL of warm agarose solution. The mixture was then layered on a coated slide and mounted with a cover glass (24 \times 50 mm), avoiding air bubbles. The slide was placed on ice for 5 min until a gel was formed and the cover glass removed. The prepared slide was put into a lysis solution (1.0% SDS in 45 mM Tris-borate, 1 mM EDTA, pH 8.4) at room temperature for 5 min, and then washed in TBE buffer for 5 min. Electrophoresis (Owl Scientific Inc., USA) was performed at 2.3 V/cm for 2 min. The slide was then washed in cold distilled water for 5 min and air-dried. Silver staining was performed as described by Cerda et al. (8).

Comet analysis

The comet images were analyzed using an image analyzer (Hi-Vision HR-303, Han-Ra Precision Eng Co., Korea). The DNA damage was evaluated by measuring tail length between comet head and tail. The tail lengths of 100 comets selected randomly from each sample were analyzed.

RESULTS AND DISCUSSION

Fig. 1 shows the DNA comet pattern of irradiated *Ha-*

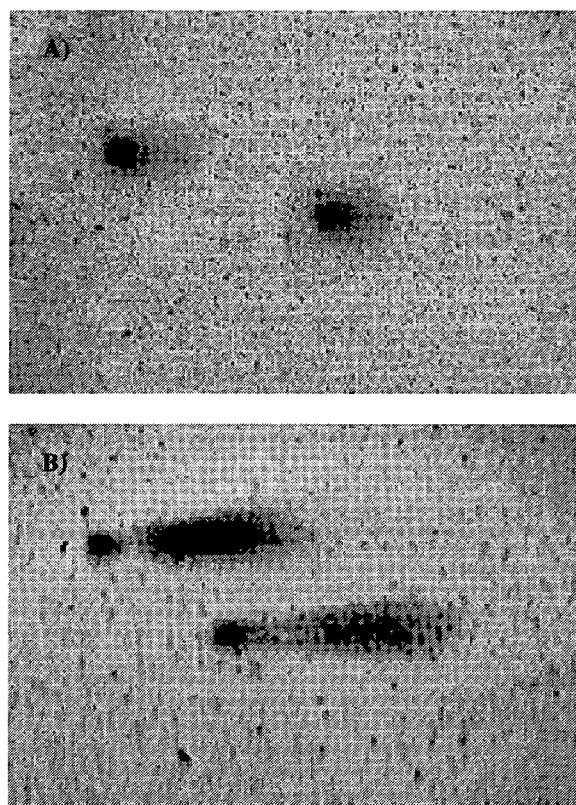


Fig. 1. DNA comet pattern of irradiated *Havenia dulcis* Thumb (20 \times objective). A) 0 kGy, B) 10 kGy.

venia dulcis Thumb. In the non-irradiated sample the tails can be seen, but the DNA comets are short; whereas the comets from irradiated samples have longer tail lengths that lengthen further as irradiation doses increase. At 10 kGy, the tails were even separated from the heads, an observation that was also reported in other studies (7,10). For *Astragalus membranaceus* Bunge, the same pattern was observed. To further examine the tail length of the comets, 100 comets were randomly selected from each sample and observed under an image analyzer (200 \times objective), and their average lengths compared at various irradiation doses. The average tail lengths of comets from non-irradiated *Astragalus membranaceus* Bunge was 17 μm , as compared to 73 μm in samples irradiated at 7 kGy and 124 μm at 10 kGy. Samples irradiated at 5 kGy or less had only slightly elongated comets (Fig. 2A), therefore, samples irradiated at 7 kGy or higher have significantly increased comet tail lengths that are easily identifiable. These observations clearly demonstrate that tail length measurements, from DNA comet assays, are a viable way to detect irradiated herbs. For *Havenia dulcis* Thumb, the same trend was observed (Fig. 2B). The tail length of the non-irradiated samples averaged 20 μm , but increased to 75 μm at 7 kGy and 177 μm at 10 kGy. It is clear that increasing irradiation dose increases the tail length of comets and that distribution

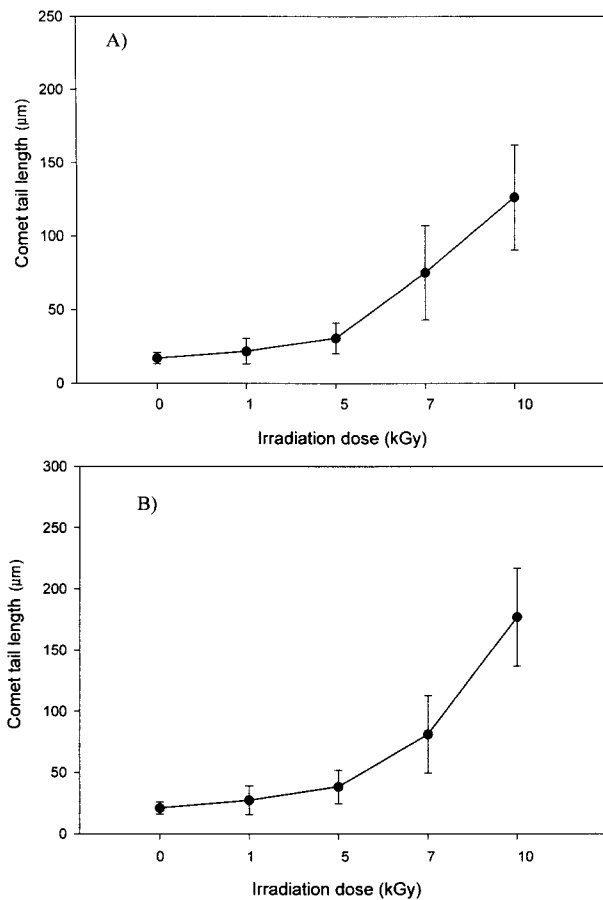


Fig. 2. Effect of irradiation dose on the average tail length of the comets in irradiated *Astragalus membranaceus* Bunge (A) and *Havenia dulcis* Thumb (B).

pattern of comet tail lengths is an important method for determination of irradiation doses in herbs.

To quantify DNA damage, Olive (11) used the term 'tail moment', as the product of the percentage of DNA in the tail and the displacement between the center of mass of the head and the center of mass of the tail. Kent et al. (12) further developed the 'tail moments' concept based on the moment of inertia of a plane figure of the comet image. However, a more rapid and simpler method to detect the irradiation dose is needed. Distribution patterns of tail length of comets can be considered to be another reliable method to quantify DNA damage. Fig. 3 shows the distribution pattern of the tail lengths of comets. For the non-irradiated *Astragalus membranaceus* Bunge, comets having tail lengths less than 25 μm were dominant. However, at 5 kGy, comets having tail lengths between 26 and 50 μm became dominant, and at 10 kGy half of the comets tails were above 100 μm. These results clearly demonstrate that comets having longer tails became dominant with increasing irradiation dose. In the case of *Havenia dulcis* Thumb, the difference was even greater. At 5 kGy, the most dominant tail length was between 26 and

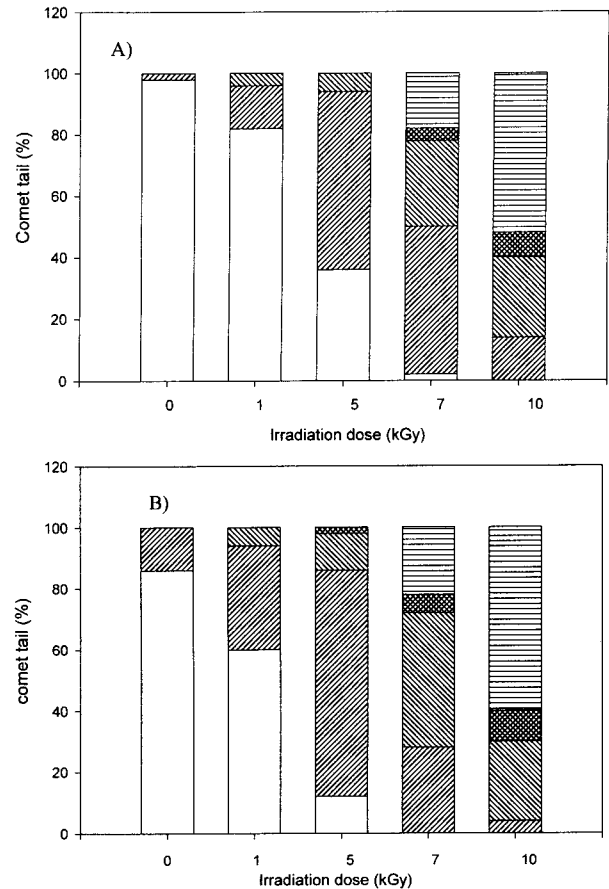


Fig. 3. Distribution pattern of the tail length of 100 comets selected randomly in irradiated *Astragalus membranaceus* Bunge (A) and *Havenia dulcis* Thumb (B). □ under 25 μm, ▨ 26~50 μm, ▩ 51~75 μm, ▤ 76~100 μm, ▥ above 100 μm.

50 μm, and at 10 kGy there were few tails whose length was below 50 μm and most tails were 100 μm or longer. The distribution pattern clearly suggests that DNA comet assays are a reliable and rapid tool for the detection of irradiated herbs.

In summary, the comet assay is a rapid and inexpensive method for reliable detection of irradiated *Astragalus membranaceus* Bunge and *Havenia dulcis* Thumb.

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