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Antioxidant potential of sesamol and its role on radiation-induced DNA damage in whole-body irradiated Swiss albino mice

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ABSTRACT

Sesamol (SM) is a dietary phytochemical present in the processed sesame oil. In this present study we have evaluated the antioxidant potential of SM and its role in the protection of radiation-induced DNA damage in γ-irradiated mice. The antioxidant properties of SM were evaluated by using different *in vitro* antioxidant assays. SM shows scavenging effect against hydroxyl ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), nitric oxide, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation ($ABTS^{\bullet +}$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Our results demonstrate that SM exhibits strong antioxidant property in all the *in vitro* assays. When mice were exposed to 7 Gy γ-radiations there was an increase in % tail DNA, tail length, tail moment and Olive tail moment in blood lymphocytes. SM (100 mg/kg b.wt) pretreatment significantly decreased the % tail DNA, tail length, tail moment and Olive tail moment in irradiated mice lymphocytes. These results suggest that SM protects γ-radiation-induced DNA damage in mice lymphocytes, which may be attributed to its antioxidant property.

1. Introduction

Many of the biological consequences of ionizing radiation are generally attributed to DNA damage and other cellular macromolecules damage (Halliwell and Aruoma, 1991). Most of the cellular alterations induced by gamma radiation, one of the low-LET radiation, are indirect and is mediated by the generation of free radicals and related reactive species, mainly derived from oxygen (Maurya et al., 2006). It is well documented that gamma-radiation causes single-strand breaks, double-strand breaks, oxidative damage to sugar and base residues that leads to increased risk for numerous genetically determined diseases (Sankaranarayanan, 2006). Radiation-induced free radicals abstract the H atom from the C′ 4 position of the deoxyribose or attack the bases present in the DNA (Spotheim-Maurizot et al., 1992). The DNA damage within an individual cell induced by various genotoxic agents can be determined using the comet assay (Singh et al., 1988). The comet assay has recently received much attention and reports of a dose dependent increase in the level of nuclear DNA damage after exposure of cells to ionizing radiation (Cadet et al., 1997) have been documented. We used this technique to investigate the levels of nuclear DNA damage in whole-body irradiated mice lymphocytes.

The level of radiation-induced DNA damage can be modulated by treating animals or cultured cells with antioxidants such as naturally occurring compounds of plants or vitamins (Rajagopalan et al., 2002). Antioxidants prevent free radical induced cellular damage by scavenging them or by promoting their decomposition (Young and Woodside, 2001). Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as radiation-induced oxidative damages (Gulcin et al., 2004). Hence, the studies on natural antioxidants have gained increasingly greater importance. SM is a phenolic derivative with a methylenedioxy group, and like vitamin E is known to be an antioxidant contained mainly in processed sesame oil (Nagata et al., 1987). It has been known for many years that sesame oil is highly resistant to oxidative deterioration as compared to other edible oils (Mohamed and Awatif, 1998), possibly due to the presence of antioxidative compounds of lignans (low molecular weight compounds produced by oxidative coupling of para hydroxyphenylpropane), including sesamin and sesamolin. SM is formed from decomposition of sesamolin during the processing of sesame oil. In this present study, we studied the antioxidant potential of SM and its role on the modulation of radiation-induced DNA damage in whole-body irradiated Swiss albino mice peripheral blood lymphocytes.

2. Materials and methods

2.1. Free radical scavenging assay

2.1.1. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of sesamol was determined by the method of Halliwell et al. (1987). In this assay, $OH^{\bullet}$ is produced by reduction of H_2O_2

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by the transition metal (iron) in the presence of ascorbic acid. The generation of $OH^{\bullet}$ is detected by its ability to degrade deoxyribose to form products, which on heating with thiobarbituric acid (TBA) form a pink colour chromogen. Addition of sesamol competes with deoxyribose for $OH^{\bullet}$ and diminishes the colour formation. The incubation mixture in a total volume of 1 mL contained 0.1 mL of buffer, varying volumes of sesamol (10, 20, 30, 40 and 50 μg), 0.2 mL of 500 μM ferric chloride, 0.1 mL of 1 mM ascorbic acid, 0.1 L of 1 M EDTA, 0.1 mL of 10 mM H_2O_2 and 0.2 mL of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then 1 mL of 1% TBA (1 g in 100 mL of 0.05N NaOH) and 1 mL of 28% TCA was added. All the tubes were kept in a boiling water bath for 30 min. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of sesamol. The efficiency of sesamol was compared with various concentrations (10, 20, 30, 40 and 50 μg) of ascorbic acid as standard. Decreased absorbance of the reaction mixture indicates increased hydroxyl radical scavenging activity. The percentage scavenging was calculated as shown below:

$$\%\ \text{of scavenging}\ [OH^{\bullet}] = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sesamol or ascorbic acid.

2.1.2. Superoxide anion scavenging assay

Superoxide anion radical scavenging activity of sesamol was determined by the method of Nishimiki et al. (1972) with modifications. The assay was based on the oxidation of NADH by phenazine methosulphate (PMS) to liberate PMS_{red}. PMS_{red} convert oxidized nitroblue tetrazolium (NBT_{oxi}) to the reduced form NBT_{red}, which formed a violet coloured complex. The colour formation indicated the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of colour after addition of the antioxidant was a measure of its superoxide scavenging activity. 1 mL of NBT (100 μmol of NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH (468 μmol in 100 mM phosphate buffer, pH 7.4) solution and varying volumes of sesamol (10, 20, 30, 40 and 50 μg) were mixed well. The reaction was started by the addition of 100 μl of PMS (60 μmol/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30 °C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sesamol was used as blank. Ascorbic acid was used as a standard for comparison. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage scavenging was calculated as shown below:

$$\%\ \text{of scavenging}\ [O_2^{\bullet -}] = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of sesamol or ascorbic acid as standard.

2.1.3. Nitric oxide radical inhibition assay

The nitric oxide radical inhibition activity of sesamol was measured by the method of Garrat (1964). The reaction mixture containing sodium nitroprusside (2 mL), PBS (0.5 mL) and various concentrations of sesamol and standard ascorbic acid (0.5 mL) was incubated at 25 °C for 15 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid and allowed to stand for 5 min for completing diazotization. Then 1 mL of naphthalene diamine hydrochloride was added, mixed and allowed to stand for 30 min at 25 °C. A pink colour chromophore was formed, the absorbance of which was measured at 540 nm. IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical production.

$$\%\ \text{of NO scavenging} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of sesamol or ascorbic acid.

2.1.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The stable free radical DPPH method is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Brand-Williams et al., 1995). Sesamol, at various concentrations ranging from 2 to 10 μg, were mixed in 1 mL of freshly prepared 0.5 mM DPPH ethanolic solution and 2 mL of 0.1 M acetate buffer at pH 5.5. The resulting solutions were then incubated at 37 °C for 30 min and measured at 517 nm in a Shimadzu UV-1601 spectrophotometer (Tokyo, Japan). $DPPH^{\bullet}$ radical scavenging activity of the sesamol was calculated from the decrease in absorbance at 517 nm in comparison with the negative control. IC_{50} value is the concentration of compound required to inhibit 50% of $DPPH^{\bullet}$ radical production.

$$\%\ \text{of}\ DPPH^{\bullet}\ \text{scavenging} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of sesamol or ascorbic acid.

2.1.5. ABTS radical scavenging assay

This method measures the capacity of different compounds to scavenge the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation ($ABTS^{\bullet +}$) (Arnao et al., 2001). The antioxidant activity was measured in a reaction mixture containing 0.5 mL of 15 μM H_2O_2, 0.5 mL of 7 mM ABTS and 50 mM sodium phosphate buffer, pH 7.5 and varying concentrations of sesamol (2–10 μg). The blank contained water in place of sesamol. The absorbance was read in spectrophotometer at 734 nm and compared with standard ascorbic acid. IC_{50} value is the concentration of sample required to inhibit 50% of $ABTS^{\bullet +}$ production.

$$\%\ \text{of}\ ABTS^{\bullet +}\ \text{scavenging} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of sesamol or ascorbic acid.

2.2. Experimental animals

Inbred Swiss albino mice, 6–8 weeks old, weighing 25–30 g each, from a stock purchased from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, were used for the experiment. They were housed in plastic bottom cages and maintained under controlled conditions of temperature and light (light:dark = 14:10 h). They were provided standard mice feed (supplied by Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The Institutional Animal Ethics Committee, Annamalai University, approved the experimental protocols.

2.3. Irradiation protocol

The Cobalt-60-Teletherapy Unit of the Cancer Treatment Centre, Dr. Kamakshi Memorial Hospital, Chennai, was used for irradiation. Animals were restrained in well-ventilated perpex boxes and exposed to whole-body γ-irradiation at a dose rate of 1.66 Gy/min so as to deliver 7 Gy as required by the study, 30 min after the treatment with SM.

2.4. Preparation of the drug and mode of administration

SM procured from Sigma Aldrich was dissolved in double distilled water (DDW) immediately before use and was administered intraperitoneally, at different doses at a volume of 10 ml/kg b.wt.

2.5. Single-cell gel electrophoresis (comet assay)

The comet assay was carried out under alkaline conditions, as described by Singh et al. (1988). Peripheral blood is collected by tail bleeding and the lymphocytes were isolated from the whole blood by using histopaque 1077 (Sigma–Aldrich). Damage to DNA in peripheral blood lymphocytes, after whole-body exposure of mice to 7 Gy γ- radiations, was studied at 24 h post-irradiation. Two slides per animal were prepared. Agarose gels were prepared on fully frosted slides coated with 1% normal melting point (NMP) agarose. 50 μl of mice peripheral blood lymphocytes was mixed with 0.5% low melting point agarose (LMP) placed on the slides and covered with layer of 0.5% LMP agarose. The slides were immersed for 2 h in freshly prepared ice-cold lysis solution. Denaturation and electrophoresis were carried out at 4 °C under dim light in freshly prepared electrophoresis buffer. After 20 min of denaturation, the slides were randomly placed in the horizontal gel-electrophoresis tank, facing the anode. Electrophoresis at 25 V and 300 mA lasted another 20 min. After electrophoresis, the slides were washed with a neutralization buffer. Slides were stained with ethidium bromide (20 μg/mL). For visualization of DNA damage, observations were made using a 20× objective on an epifluorescent microscope equipped with an excitation filter of 510–560 nm and a barrier filter of 590 nm. One or two hundred comets on duplicated slides were analyzed (for each group we prepared two comet assay slides and in each slide we scored 100 comets for analysis). Images were captured with a digital camera with networking capability and analyzed by image analysis software, CASP (http://casp.sourceforge.net). DNA damage was quantified by the torsional moment of the tail ('tail moment'), tail length (Olive et al., 1990), whose distribution was adjusted by a two-parameter, Weibull model (Ejchart and Sadlej-Sosnowska, 2003). OTM (Olive tail moment) is the product of the distance (in x direction) between the center of gravity of the head and the centre of gravity of the tail and the percent tail DNA.

2.6. Statistical analysis

All the values were expressed as mean ± S.D. of six determinations. Statistical analysis of the data was carried out by one-way ANOVA on SPSS (Statistical package for social sciences) and the group mean compared by Duncan's Multiple Range Test (DMRT). A value of $P < 0.05$ was considered to be significant.