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Effect of γ-irradiation on antioxidant and antiproliferative properties of oat β-glucan

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HIGHLIGHTS

- Irradiation leads to depolymerization of oat β-glucan and reduced viscosity.
- Irradiated oat β-glucan exhibited increased antioxidant activity as compared to non-irradiated oat β-glucan.
- Irradiated oat β-glucan being more potent antiproliferative activity than non-irradiated β-glucan.

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ABSTRACT

The present study was undertaken to examine effect of γ-rays on the antioxidant and antiproliferative potential of β-glucan isolated from oats. Irradiation doses of 0, 2, 6 and 10 kGy were given to extracted β-glucan. The samples were characterized by Fourier transform infrared spectroscopy, Gel permeation chromatography and quantitative estimation by (1-3) (1-4) β-D-Glucan assay kit (Megazyme). The average molecular weight of non-irradiated β-glucan was 199 kDa that decreased to 70 kDa at 10 kGy. Both FT-IR spectrum and chemical analysis revealed that the extracted β-glucan was pure having minor impurities. Antioxidant activity was evaluated by DPPH, lipid peroxidation, reducing power, metal chelating ability and oxidative DNA damage assays. Results revealed increased antioxidant activity of β-glucan with the increase in irradiation dose. Irradiated β-glucan also exhibited dose dependent cancer cell growth inhibition with irradiation doses. The study revealed that the low molecular weight β-glucan with increased antioxidant and antiproliferative activities can be produced by irradiation treatment.

1. Introduction

Oats (*Avena sativa*) belongs to the family Gramineae and in world its production ranks sixth within cereals. In India about 0.1 million ha of land is utilized for cultivation of oat and the average yield is 42 t/ha (ICAR, 2006). Although oats was previously cultivated as a fodder crop, there has been a significant change and now it is extensively investigated as a human health food (Tiwari and Cummins, 2009).

In recent years, natural antioxidants extracted from plant material have gained a lot of research impetus for their use in foods or medicinal material as a substitute for synthetic antioxidants (Kinsella et al., 1993). β-Glucan is one of the polysaccharides which also possess antioxidant capacity (Johansson et al., 2004). Cereals particularly barley and oats are rich sources of soluble fiber especially β-glucans. Use of β-glucan in the prevention of cancer and other diseases are well reported (Chen and Seviour, 2007). Generally biological activities of polysaccharide depend on the molecular structure which includes molar mass, degree of branching and branch length (Qi et al., 2005). Problems with high molecular weight β-glucan are its high viscosity resulting in low permeability into cell and to counter these problems many degradation methods are being used (Methacanon et al., 2011). Previously we reported that gamma-irradiation leads to the fragmentation of polysaccharide by breaking of glycosidic bonds (Gani et al., 2012a, 2012b, 2014; Wani et al., 2015; Ashwar et al., 2014). The advantages of the irradiation as a degradation process are that it does not make use of chemical agents and no further purification steps are needed. Recently, effects of radiation on carbohydrates such as sodium alginate, chitosan, carrageenan, and cellulose has shown to enhance their bioactivities without causing environmental pollution (Chmielewski et al., 2005). Degradation of yeast β-glucan by gamma irradiation without changing functional groups has already been reported (Byun et al., 2008). However, the effects of irradiation on antioxidant and antiproliferative activity of β-glucan have not been evaluated. In the present study our aim was to irradiate oat β-glucan so as to enhance its nutraceutical potential.

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2. Materials and methods

2.1. Materials

The indigenous variety of oats (Sabzaar) was procured from Sher-e-Kashmir University of Agriculture & Technology of Kashmir, India. Whole grains were pulverized in a laboratory mill and there after sieved through 0.50 mm screen in order to obtain flour.

2.2. Reagents and β-glucan extraction

DPPH, ferrozine and linoliec acid were procured from Sigma–Aldrich (Poole, UK). All reagents and chemicals used were of analytical grade. β-Glucan extraction was carried out according to Temelli (1997). 50 g of whole oat flour (dry weight basis) was extracted in water, pH lowered to remove proteins. Then β-glucan was precipitated by ethanol and air dried.

2.3. β-Glucan irradiation

The oat β-glucan samples were packed in double layered polyethylene bags and were irradiated at room temperature (20 ± 2 °C) in a cobalt-60 (^{60}Co) source irradiator. Doses of 2, 6 and 10 kGy at the dose rate of 2 kGy h^{-1} were used.

2.4. Chemical analysis of β-glucan

Moisture, starch, fat and ash contents of β-glucan from oats were determined according to AACC (2003), nitrogen contents by the Kjeldahl method AACC (1990). β-Glucan assay was conducted according to the method of McCleary and Glennie-Holmes (1985) using the (1-3) (1-4) β-D-Glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). β-Glucan content was reported on moisture-free basis.

2.5. Viscosity measurement

Determination of viscosity 1% β-glucan (w/v) was dispersed in deionized water and heating mixtures at 100 °C for 10 min. It was followed by adjusting pH at 7.0 and stirring for 2 h on magnetic stirrer at 30 °C. Viscosity was determined using a Brookfield Viscometer.

2.6. Molecular weight determination

Gel Permeation Chromatography was performed using separation module (Waters 2690), refractive index detector. Empower software (System Software, Empower option GPC, Waters Co.), and PL aquagel-OH mixed (7.8–300 mm) column. The injection volume was 200 μL (10 mg/mL β-glucan), and calibration was carried out using various standard dextrans (0.1%, w/w).

2.7. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectrum of β-glucan was recorded on a Perker Elmer FT-IR spectrometer using spectrum software version 10.3.2 in the wave number range 4000–450 cm^{-1} at a resolution of 4 cm^{-1} 32 co-added scans. The β-glucan powder was mixed with KBr powder before measurement.

2.8. Sample preparation

Irradiated oat β-glucan (20 mg) was suspended in 1 mL of water and sonicated for 30 min. The aqueous solutions were used for determining antioxidant and antiproliferative activity.

2.9. DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

The *DPPH* scavenging activity was determined according to the method of Bilos (1958) with certain modifications. In the first series different irradiated doses of the β-glucan (2 mL) mixed with 1 mL DMSO solution containing 0.2 mM DPPH. In the second series 1 mL DMSO solution was mixed with each sample. The reaction mixture was kept in the dark at 20 °C for 40 min after vortexed for 1 min. Absorbance was measured spectrophotometrically at 517 nm against the blank. DPPH scavenging activity was calculated from the equation $[1-(A_i-A_j)/A_c] \times 100$ where A_i was the absorbance DPPH solution mixed with extract, A_j was the absorbance of DMSO solution mixed with extract and A_c was the absorbance of blank-1 mL of DPPH solution mixed with 2 mL of DMSO.

2.10. Reducing power

The reducing power was determined using a modification of the Zou method (Oyetayo et al., 2009). 100 μL of irradiated β-glucan was mixed with 0.2 M sodium phosphate buffer (pH 6.6, 2.5 mL) and 1% (w/v) aqueous potassium ferricyanide (2.5 mL). The mixture was incubated for 20 min at 50 °C. 10% (w/v) of trichloroacetic acid (2.5 mL) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was diluted with deionized water (2.5 mL) and 0.1% (w/v) ferric chloride (0.5 mL) was added. The absorbance was measured at 700 nm against a blank and compared to unirradiated oat β-glucan as a standard.

2.11. Inhibition of lipid peroxidation

The antioxidant activity of the oats β-glucan irradiated at different doses was determined spectrophotometrically with minor modifications to the thiocyanate method (Osawa and Namiki, 1981). Oat β-glucan was added to 1 mL of linoleic acid (0.1 g in 100 mL of pure ethanol), 0.2 mL of H_2O_2 (30 mM), 0.2 mL of ascorbic acid (100 mM) and 0.2 mL of ferric nitrate (20 mM). This was followed by incubation at 37 °C in water bath for 1 h. The reaction was stopped by the addition of 1.0 mL of trichloroacetic acid (TCA, 10% w/v), following with 1.0 mL of thiobarbituric acid (TBA, 1% w/v). All the tubes were placed in a boiling water bath for 20 min and then centrifuged at 5000 rpm for 10 min. The amount of malonaldehyde formed in each of the sample was determined by measuring the absorbance of the supernatant at 535 nm against a blank.

Percentage inhibition was calculated by using the following formula:

$$\%\ \text{Inhibition} = A_{\text{control } 535} - A_{\text{sample } 535}/A_{\text{control } 535} \times 100$$

where $A_{\text{control } 535}$ is the absorbance of the control and $A_{\text{sample } 535}$ is the absorbance of the extract.

2.12. Determination of metal chelating activity

Chelating ability was determined according to the method described by Dinis et al. (1994). The reaction mixture, containing β-glucan, 0.5 mL of ferrous chloride (2 mM), 0.25 mL of ferrozine (5 mM) and volume adjusted to 1 mL with distilled water. Vortex and incubate for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank. The ability of all samples to chelate ferrous ion was calculated using the following equation:

$$\text{Chelating effect } (\%) = A_{\text{control } 562} - A_{\text{sample } 562}/A_{\text{control } 562} \times 100$$

2.13. Antioxidant activity against oxidative damage to DNA

Oxidative damage to DNA was performed according to the method of Ghanta et al. (2007) with some modifications. The