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Structural characterization and evaluation of antioxidant, anticancer and hypoglycemic activity of radiation degraded oat (Avena sativa) β- glucan

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ABSTRACT

Oat β-D-glucan after extraction was degraded at doses of 3, 6, 9, 12 and 15 kGy. The average molecular weight decreased to 45 kDa at dose of 15 kGy from an initial value of 200 kDa in native sample. XRD analysis revealed no significant change in diffraction pattern of irradiated samples when compared with control, except a decrease in intensity of x-ray diffraction. The results of the antioxidant activity revealed decrease in EC50 values and corresponding increase in antioxidant activity of radiation degraded oat β-D-glucan. Results of the anticancer studies indicated that cytotoxicity of gamma irradiated oat β-D-glucan in cancer cell lines was highest against colo-205 and MCF7 cancer cells compared to T47D cell and no cytotoxicity was observed in normal cell lines at all concentrations used. Evaluation of hypoglycemic activity showed highest inhibition in α-glucosidase activity compared to α-amylase activity due to gamma irradiation of oat β-D-glucan. Comparison of the EC_{50} values of known standards and gamma irradiated oat beta-glucan samples indicates that radiation treatment significantly modified the biological activity of the beta-glucan samples. Therefore, it is suggested that gamma irradiation can be used for producing low molecular weight oat β-D-glucan; which can help in modifying the biological activities.

1. Introduction

Oats (*Avena sativa*) is a cereal grain and a good source of dietary fiber - both soluble and insoluble, antioxidants, proteins and unsaturated fat. Oats has been regarded as a health promoting food and can help prevent a range of ailments when consumed on a regular basis. Consumption of oatmeal can lower cholesterol levels by reducing the ability of blood cells to stick to the insides of the artery walls (Zhang and Daou, 2012). These beneficial effects of oats are chiefly due to the soluble fiber content of oats. The present interest in soluble oat fiber originated from the reports that showed that dietary oats can help in lowering cholesterol, postprandial blood glucose level as well as modifying immune response and reducing risk of various cancers (Regand et al., 2011).

The principal component of the soluble fiber in whole oats comprises a class of polysaccharides known as beta-D-glucan often referred as beta-glucan. Beta-glucan is composed of glucose units linked together by (1 − 3) linkage of cellotriosyl and cellotetraosyl units to form a long polymer chain. These β- glucans have immune-enhancing activities, which nutritionally potentiate and modulate an immune response (Borchers et al., 2004). The biological activities and immunemodulatory effects of beta-glucan are influenced by molecular weight, degree of branching, length of branch and higher order structure (Qi et al., 2005). Among these, molecular weight is one of the most important factors determining the biological activities of polysaccharides (Hou-Jin et al., 2016; Jiao et al., 2011; Wang et al., 2010). High molecular weight polysaccharides result in low solubility and processability, therefore limiting their penetration and accessibility into the cell to perform a function. Numerous reports are available in the literature regarding the molecular weight-activity relationship of polysaccharides (Hou-Jin et al., 2016; Jiao et al., 2011). Recently it has been reported that low molecular weight laminarin induce the expression of gene coding for immune response proteins and reduce apoptotic cell death (Kim et al., 2006). In another study, it has been reported that low molecular fucoidan promotes revascularization of hindlimb ischemia in rats, boosts osteoblast proliferation for bone regeneration, enhances human endothelial cell formation and increases cytotoxicity in cancer cells (Choi and Kim, 2013).

Various methods (acidolysis, enzymolysis, and gamma irradiation) are used to produce low molecular weight β-glucan. Gamma irradiation leads to the degradation of polysaccharides by the cleavage of glycosidic bonds. The basic advantage of degradation of polysaccharides by radiation include the ability of the process to promote changes reproducibly and quantitatively without the introduction of chemical

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reagents and minimizes structural changes and side reaction (Cho et al., 2003). There are several reports indicating that low molecular weight polysaccharides degraded by either chemical treatment or gamma irradiation enhance biological activities. Choi et al. (2011) reported that antioxidant activity of laminarin, a polysaccharide in seaweed increases after gamma irradiation. Similar beneficial biological effect has also been reported for low molecular weight fucoidan produced by gamma irradiation (Choi and Kim, 2013). Literature review also reveals that very few studies have been conducted on low molecular weight β-glucan. Byun et al. (2008) reported that β-glucan from black yeast could be efficiently degraded by gamma irradiation without changing functional groups. Preliminary study conducted by Shah et al. (2015) reported that low molecular weight barley β-glucan produced by gamma irradiation had good antioxidant activities. However, the effects of gamma irradiation on the health promoting effects of oat β-glucan have not been evaluated in detail and no comparison has been made between available standards and the irradiated beta glucan samples with respect to biological activities. Therefore the purpose of the present study was to investigate the effect of radiation processing on the structural changes of oat β-glucan and enhancement in biological activities and subsequent comparison with the standards.

2. Materials and methods

2.1. Oats samples

The Oats samples of *Sabzar* variety were purchased from the Department of Agriculture, Jammu and Kashmir, India. The Oats grains were subjected to milling and sieved through 0.50 mm mesh to obtain flour. The flour samples thus obtained were used for extraction of β-glucan.

2.2. Extraction of β-glucan

β-glucan extraction was carried out according to the method of Asif et al. (2010). 1 kg of Oat flour was refluxed with 80% ethanol for 1 h followed by mixing with 1 M NaOH in a ratio of 1:7. The contents were stirred on hot plate for 90 min at 45 °C and centrifuged at 15,000*g* for 15 min at 20 °C. The supernatants were adjusted to pH 3.5 with citric acid and again centrifuged for 20 min at 4 °C. The supernatants were mixed with 80% ethanol in 1:2 ratio and held for 15 min followed by centrifugation at 3500 g at 4 °C for 20 min. The precipitated β-glucan samples were air dried.

2.3. Analysis of β-glucan

β-glucan assay was conducted according to the method of McCleary and Holmes (1985) using β-D-glucan enzymatic assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). B-D-glucan content was reported on moisture free basis.

2.4. Gamma irradiation of β-glucan

The dried oat β-glucan samples were packed in polyethylene pouches and irradiated at doses of 3, 6, 9, 12, and 15 kGy using Co-60 gamma-ray source (GC-5000, BARC, Mumbai). The samples were irradiated at a dose rate of 4 kGy/h. The radiation treatment was carried out under normal light conditions and at room temperature. To ensure that samples received the exact dose, the dosimeters were placed along with the samples. A ceric-cereous dosimeter was used to measure the absorbed dose of gamma irradiation by the samples. The samples that received no radiation treatment were considered as control. For all the irradiation treatments including control, the sample size used was approximately 15 − 20 g of β-glucan in triplicates. After irradiation treatment, the samples were kept at 10 ± 2 °C till further use.

2.5. Molecular weight determination

Gel permeation chromatography (GPC) was performed using the following system: separation module (Waters 2690), refractive index detector, Empower software (System software, Empower option GPC, Waters Co.), and a PL aquagel-OH mixed column (7.8 × 300 mm). The mobile phase used was 0.1 M sodium nitrate at the flow rate of 1 ml/min and the analysis was performed at room temperature. The injection volume was 200 μL (10 mg/ml β-D-glucan) and the calibration was carried out using standard dextrins at concentration of 0.1% (w/v) (Byun et al., 2008). Due to the lack of glucan standards, calibration was performed using dextrans (source: *Leuconostoc mesenteroids;* Sigma Aldrich, St. Louis, MO, USA) and therefore the resulting molecular weights of glucans are approximate values only.

2.6. Viscosity measurement

For viscosity measurement, 1% (w/v) dispersion of β-D-glucan in deionized water was prepared by heating mixtures at 100 °C for 10 min followed by stirring on a magnetic stirrer at 30 °C for 2 h and adjusting the pH at 7.0. Viscosity was measured with the help of Brookfield Viscometer (DV-II+ pro, Brookfield Engineering Laboratories, MA, USA).

2.7. Solubility and water absorption capacity (WAC) measurement

Solubility of control and gamma irradiated β-D-glucan sample was determined by the standard method of Subramanian et al. (1994), whereas WAC was determined according to the method described by Mishra and Rai (2006) with a few modifications.

2.8. Fourier transform infra red (FTIR) spectroscopy

To explore the structural features of native and gamma irradiated β-D-glucan, FTIR spectrum was recorded on a Perkin Elmer FTIR spectrometer using spectrum software version 10.3.2 in the wave number range of 4000 – 450 cm^{-1} at a resolution of 4 cm^{-1} with 32 co-added scans using the KBr pellet method which is prepared as follows. KBr was placed in a mortar and grinded to fine powder. Mix small amount of sample (about 0.1 - 2% of the KBr) with the KBr powder. Subsequently grind the mixture for 3 – 5 min. Following grinding, assemble the 7 mm die and place the powder on the 7 mm silicone collar. Put the die together with the powder into the Qwik Handi-press. Press the powder for 2 min to form a pellet. Disassemble the die set and take out the 7 mm collar. Put the collar together with the pellet on the sample holder and record the spectra.

2.9. X-ray diffractometry (XRD)

X-ray diffraction patterns of the control and irradiated β-D-glucan samples (approx. 20% moisture) were taken with a Rigaku RPT 300PC X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan) equipped with CuKα radiation. An accelerating voltage and current of 40 kV and 100 mA, were used, respectively. The diffractograms were recorded in a 2θ between 2° and 49° with a scanning rate of 2.0°/min.

2.10. Antioxidant assays

DPPH (1, 1- diphenyl-2-picryl-hydrazyl) radical scavenging activity of control and gamma irradiated β-D-glucan samples was measured according to the previous method with slight modifications (Brand-Williams et al., 1995). Ferric reducing/antioxidant power (FRAP was determined according to the method of Oyaizu (1986)). Ferrous ion chelating activity (FICA) of control and gamma irradiated β-D-glucan samples was measured according to the method of Suter and Richter (2000). The inhibition of lipid peroxidation of control and irradiated β-