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Cytoprotective and antioxidant activity studies of jaggery sugar

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ABSTRACT

Jaggery and other sugars namely white, refined and brown sugars were evaluated for cytoprotectivity on NIH 3T3 fibroblasts and erythrocytes, DPPH radical scavenging activity, reducing power and DNA protection. In addition, total phenol content and phenolic acid composition were also determined. Results indicated a total phenolic content of 26.5, 31.5, 372 and 3837 μg GAE/g for refined, white, brown and jaggery, respectively. The HPLC analysis revealed the presence of different phenolic acids in brown sugar and jaggery. On NIH 3T3 cells oxidation, at 4 mg/ml concentration, jaggery showed 97% protection compared to brown sugar, and both sugars effectively reduced erythrocyte oxidation. A dose dependent reducing power and DPPH radical scavenging activity was also observed for jaggery and brown sugar. An EC_{50} of 7.81 and 59.38 μg/ml were observed for jaggery and brown sugar in the DPPH scavenging assay. In DNA oxidation studies, higher protection was observed in jaggery followed by brown, white and refined sugar treated samples.

1. Introduction

Sugars, often called culinary sugars (used in cooking) are an important foodstuff consumed all over the world, and are manufactured either from sugarcane (70%) or sugar beet (30%). Its consumption remains high despite increase in synthetic sweeteners, and has become an essential nutrient in the world diet for its nutritional, sweetening and preservative properties (Chen & Chou, 1993). The culinary sugars are of different types based on their method of production, and also there is difference in the nature, size of the crystals, colour and taste of these sugars. The most common types of sugars that are widely consumed are white, refined, brown and raw sugar. White sugar, also called blanco directo is common in India and other south Asian countries, comes from precipitating many impurities out of the cane juice by using the phosphatation technique. Refined sugar is the most common form of sugar in North America as well as in Europe and is made by dissolving brown sugar and purifying it with a phosphoric acid method similar to that used for blanco directo. It is then further decolourised by filtration through a bed of activated carbon or bone char depending on where the processing takes place. Refined sugar is typically sold as granulated sugar, which has been dried to prevent clumping. Raw sugar is comprised of yellow to brown sugars made from clarified cane juice boiled down to a crystalline solid with minimal chemical processing, which helps in retaining more mineral salts and phytochemicals. Manufacturers sometimes prepare raw sugar as loaves called jaggery in India rather than as a crystalline powder. Brown sugar comes from late stages of sugar refining, when sugar forms fine crystals with significant molasses content or from coating refined sugar with cane molasses syrup. In terms of sucrose purity, refined sugar is more pure than blanco directo followed by brown sugar and jaggery sugars.

In recent years, plant and plant products have been the main focus in the search for nutraceuticals to combat oxidative stress induced diseases (Saxena, Venkaiah, Anitha, Venu, & Raghunath, 2007). Free radicals are generated during normal cellular metabolism and their effect is neutralised by antioxidant molecules present in the body. However, this balance between the oxidants and antioxidant molecules is disturbed by free radicals derived from exogenous sources like ozone, exposure to UV radiations and cigarette smoke (Gutteridge & Halliwell, 2000). The free radical production in cells can be significantly increased by certain toxic redox cycling compounds such as drugs and carbon tetrachloride (Wang, Ma, Liu, Tian, & Fu, 2007). Importantly, the main biomolecules like DNA, lipids and proteins are vulnerable to free radical damage resulting in cell destruction. Damaged cells lead to abnormal functioning and results in oxidative stress induced diseases. A potent scavenger or quencher of these free radical species may serve as a possible preventive measure for free radical mediated diseases.

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; EC_{50}, effective concentration for 50% radical scavenging activity; GAE, gallic acid equivalent; ABTS, [2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid); MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; PBS, phosphate buffer saline; SD, standard deviation.

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Apart from the nutritional and sweetening aspects of sugars, very little has been studied on their nutraceutical role. The interest in polyphenols, including flavonoids and phenolic acids, has considerably increased in recent years because of their possible role in the prevention of oxidative stress induced diseases such as cardiovascular complications, diabetes, ulcers and cancer (Halliwell, 2007; Repetto & Llesuy, 2002; Sachidanandam, Fagan, & Ergul 2005; Shah, Baliga, Rajapurkar, & Fonseca, 2007). Sugarcane (*Saccharum officinarum*) contains phenolic compounds (Fontaniella et al., 2003) and these compounds have also been found in sugar products such as syrup or molasses and in brown sugar (Palla, 1982). However, the presence of these phytochemicals in sugarcane juice is often undesirable, as they influence the quality and colour of final product sugar and hence these phytochemicals are removed through various purification procedures in the sugar industry. Jaggery and brown sugar are the least processed sugars containing polyphenols. The brown sugar was also known to possess ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (Payet, Cheong Sing, & Smadja, 2005; Takara, Matsui, Wada, Ichiba, & Nakasone, 2002). White and refined sugars undergo extensive purification procedures for the removal of phenolic compounds. The bioactivity of these sugars can be anticipated, as they contain phytochemicals to different extent depending on their manufacturing process. Jaggery is the main source of sugar in rural India and has been considered by many Ayurveda practitioners as a wholesome sugar. Indian Ayurvedic medicine considers jaggery to be beneficial in treating throat and lung infections. Sahu and Saxena (1994) have found that jaggery can prevent lung damage from particulate matter such as coal and silica dust in rats. However, there are no reports available in the literature on cytoprotective abilities of jaggery and other sugars and their comparative evaluation.

Hence, in the present investigation, the protective effect of jaggery in comparison with white, refined and brown sugars on free radical induced damage of NIH 3T3 fibroblasts, erythrocytes and DNA were assessed in addition to 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability and reducing power. Further, the total phenol content and various phenolic acids present in these sugars were also determined.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, ascorbic acid, tris–HCl, glutaraldehyde, agarose, ethidium bromide, cell culture media (RPMI 1640), fetal bovine serum, L-glutamine, penicillin, streptomycin, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), *tert*-butyl hydroperoxide, phenolic acid standards such as caffeic, *p*-coumaric, ferulic, gallic, gentisic, 4-hydroxyphenylacetic acid, protocatechuic, cinnamic, syringic and vanillic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Lambda phage DNA was procured from Bangalore Geni, Bangalore, India. NIH 3T3 fibroblast cells were purchased from National Center for Cell Sciences, Pune, India. The other chemicals such as ferric chloride, trichloroacetic acid and solvents used in the experiment were purchased from Sisco Research Laboratories, Mumbai, India.

2.2. Sample

Refined, white, and brown sugars were procured from three local sugar factories (Mandya, Karnataka, India) and jaggery was purchased from a local market (Mysore, Karnataka, India). All the samples ($n = 3$) were preserved in dry condition at room temperature.

2.3. Determination of total phenol content

The total phenol content of refined, white, brown and jaggery sugars were determined colorimetrically using the Folin–Ciocalteu method (Singleton & Rossi, 1965). A sample aliquot of 100 μl was added to 900 μl of water, 5 ml of 0.2 N Folin–Ciocalteu reagent and 4 ml of saturated sodium carbonate solution (100 g/l) and mixed in a cyclo mixer. The absorbance was measured at 765 nm in Shimadzu UV-160 spectrophotometer (Kyoto, Japan) after incubation for 2 h at room temperature. The total phenolic content was expressed as micrograms of gallic acid equivalent (GAE) per gram sample.

2.4. Extraction of phenolic acids

The phenolic acids of various sugars were extracted as per the protocol followed by Liyana-Pathirana and Shahidi (2006) with slight modification. Two grams of sugar sample was solubilised in 50 ml distiled water (in triplicates, $n = 3$) at room temperature (25 ± 2 °C) with constant stirring. The solution was then centrifuged at 4000*g* for 20 min (Sigma 3-16K, USA) and supernatants were collected and combined. The solution was acidified to pH 2 with 6 M hydrochloric acid and extracted six times with diethyl ether. The ether extracts were then combined and evaporated to dryness at 30 °C under vacuum (Buchi 011, Switzerland). The extracted phenolic acids were dissolved separately in 2 ml of methanol and stored at –20 °C until used within 1 week.

2.5. HPLC analysis of phenolic acid extracts

The phenolic acid extracts of jaggery and other sugars were analysed on a HPLC (Model LC-10A. Shimadzu Corporation, Kyoto, Japan) using a reversed phase Shimpak C_{18} column (4.6 × 250 mm) using a diode array UV-detector (operating at 280 nm). A solvent system consisting of water/acetic acid/methanol (Isocratic, 80:5:15) was used as mobile phase at a flow rate of 1 ml/min (Subba Rao & Muralikrishna, 2002). Phenolic acid standards such as caffeic, *p*-coumaric, ferulic, gallic, gentisic, 4-hydroxyphenylacetic acid, protocatechuic, cinnamic, syringic and vanillic acid were used for identification of phenolic acids. The identified phenolic acids were quantified on the basis of their peak area and comparison with a calibration curve obtained with the corresponding standards.

2.6. Cytoprotective effect on cultured NIH 3T3 fibroblast cells exposed to tert-butyl hydroperoxide

Cytoprotective ability of different sugars was carried out using NIH 3T3 fibroblast cells. The cells were subjected to oxidative stress according to the method reported by Nardini et al (1998). NIH 3T3 fibroblast cells (1×10^6 cells/ml, maintained at 37 °C under 5% CO_2 and 95% air in complete medium (RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin) were used for the assay. NIH 3T3 fibroblast cells (2.8×10^4 cells/ml) were cultured with or without sugar samples (20 μL, 0–20 μg/ml) dissolved in PBS in a 96 well microplate (180 μl suspension/well). After 30 min of incubation, cells were treated with 500 μM tert-butyl hydroperoxide and incubated for 3 h. Cell viability was assessed by microculture tetrazolium assay (Hansen, Nielsen, & Berg, 1989). Twenty-five microlitres of MTT solution (5 mg/ml) were added to each well, and the cells were incubated at 37 °C for 4 h. Then, 100 μl of lysis buffer were added to each well and the cells