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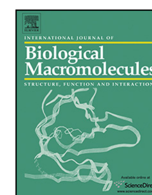

α-D-Glucosidase inhibitory activity of polysaccharide isolated from *Acacia tortilis* gum exudate

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ABSTRACT

The study aims to explore the antihyperglycemic activity of *Acacia tortilis* polysaccharide isolated from the gum exudate. α-D-Glucosidase from *Saccharomyces cerevisiae* and rat small intestine was used as in vitro model to assess α-D-glucosidase inhibitory activity of the polysaccharide against yeast and mammalian enzyme. The reduction in postprandial blood glucose level after carbohydrate rich diet fed to Albino Wistar rats was employed as in vivo model of α-D-glucosidase inhibition. The study revealed α-D-glucosidase inhibitory activity of the polysaccharide in both in vitro as well as in vivo models. Therefore, polysaccharide isolated from *A. tortilis* plant gum exudate may be a potential drug for diabetes mellitus and its complications.

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1. Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by persistent hyperglycemia; associated with abnormalities in carbohydrate, fat and protein metabolism and results in chronic complications including microvascular, macrovascular and neuropathic disorders [1]. The higher production and less utilization of glucose characterize type 2 diabetes mellitus. Diet and exercise remains the cornerstone of treatment, although pharmacological therapy is frequently necessary. Inadequate glycemic control with a single agent should prompt (i) the addition of second oral agent with addition of bedtime insulin, (ii) conversion to mixed-split insulin regime, or (iii) addition of third oral agent. Current oral treatment option can be subdivided into the hypoglycemic drugs (sulfonylurea and benzoic acid derivatives) and anti-hyperglycemic agents (biguanides, α-D-glucosidase inhibitors and thiazolidinediones) [2].

Mammalian α-D-glucosidase, located in the brush-border surface membrane of intestinal cells, is the key enzyme catalyzing the final step in the digestive process of carbohydrates. Hence, α-D-glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma blood glucose level and suppression of postprandial hyperglycemia [3]. Acarbose, miglitol and voglibose are competitive inhibitors of the intestinal α-D-glucosidase and reduce post-meal glucose excursions by delaying the digestion and absorption of starch and disaccharides in type 2 diabetes mellitus. The STOP-NIDDM (non-insulin dependent diabetes mellitus) trial demonstrated that α-D-glucosidase therapy in pre-diabetic persons successfully prevented a significant number of new cases of type 2 diabetes and helped in restoring β-cell function, concomitant with reduction of cardiovascular disease and hypertension. Intervention with acarbose also reduced cardiovascular events in diabetes. α-D-Glucosidase inhibitors are clinically criticized for their adverse effects such as flatulence, diarrhea, abdominal pain, increased hepatic enzyme and malnutrition [4].

Polysaccharides are the abundant biopolymers available in plants and the source of all biological energy. Natural polysaccharides in plants are mainly derived from seeds (e.g. guar gum, locust bean gum, tamarind gum, psyllium seed gum, etc.), exudates (e.g. gum arabic, gum karaya, gum tragacanth, gum ghatti, etc.), seaweeds (e.g. agar, alginates, carrageenan, furcellaran, etc.), and extracts (e.g. pectins, arabinogalactans, etc.). Polysaccharide based therapeutics is used extensively in cardiovascular and hematological treatments ranging from inflammatory diseases and antithrombotic treatment to wound healing. A large group of polysaccharides show biological activity against various chronic diseases and have strong immunomodulatory properties for human beings [5,6]. Recent polysaccharide investigations have played a decisive role in characterization of various antibiotic and antitumor

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agents [7]. Gum arabic, an exudate polysaccharide is used extensively for its anti-diabetic, immunostimulatory and wide range of other properties. Polysaccharide mechanism involved in anti-diabetic activity may include increased levels of serum insulin, reduction of blood glucose level and improved tolerance of glucose [8].

Among Acacia species, *Acacia tortilis* also known as Israeli Babool, family: Leguminosae (subfamily: Fabaceae) is widely distributed in Africa, Algeria, Egypt, Asia, Israel, etc. In India, it is found in the arid and semi-arid zone of Pali and Jodhpur in Rajasthan. Sharma and Bohra, 1979 studied the trypsin inhibiting activity of seeds of Italian desert plants including *A. tortilis* [9]. A number of compounds namely apigenin-6, 8-D-glucoside, n-hexacosanol, rutin, α-amyrin, β-amyrin, β-sitosterol, n-octacosanol, 3-acetyl-β-sitosterol, τ-sitosterol, betulin, friedelin, etc. have been reported from *A. tortilis*. Interestingly, despite wide activity of the *A. tortilis* gum exudate, the work is limited only toward identification of monosugars [10]. In the form of a solution or mucilage, *A. tortilis* gum exudate is frequently used in medicinal preparations for cough, cold, hoarseness, pharyngitis, gastric irritation and inflammation, diarrhea, dysentery, pulmonary tuberculosis, scalds of the mouth and alimentary canal, hemorrhage, relief from pain in burns etc. The gum exudate is used as vermifuge, possesses hypotensive, diuretic property and is used to cure skin diseases [11,12].

The conventionally used drug acarbose is an oligosaccharide and used as an anti-hyperglycemic agent due to its α-D-glucosidase inhibition activity. Thus, with the same hypothesis, the present study was designed to explore the anti-hyperglycemic activity of the polysaccharide isolated from *A. tortilis*.

2. Experimental

Exudate gum was collected from stands of *A. tortilis* at Central Arid Zone Research Institute (CAZRI) research farm in Jodhpur, Rajasthan, India in the month of March 2011 under the Network project on 'Harvesting, processing and value addition of natural resins and gums'. The material was authenticated by Dr. J.C. Tewari, Principal Scientist (Forestry), CAZRI, Jodhpur, India.

2.1. Materials

4-Nitrophenyl-α-D-glucopyranoside, α-D-glucosidase from *Saccharomyces cerevisiae* (Sigma–Aldrich, Milwaukee, USA), sucrose, casein, DL-methionine, choline bitartrate (S.D. Fine-Chem., Ltd., Mumbai, India), acarbose (as a free sample from Biocon, Ltd., Bangalore, India) and all other chemicals were of analytical grade. Accu-check (Roche model 360°) was used for estimation of fasting and postprandial blood glucose level. Commercially available kit for determination of lipid profile, Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase SGPT, and glycated HbA1% was purchased from Abbott India, Ltd., Mumbai.

2.2. General methods of analysis

Solutions were concentrated at or below 40 °C in a rotary evaporator under reduced pressure. All melting points were uncorrected. Optical rotation was determined on Autopol-II, automatic polarimeter (Rudolph Research, Flanders, NJ, USA) at 589 nm, D-lines of sodium. Paper chromatography was carried on Whatmann 1 and 3 mm filter paper sheets using the solvent systems: n-butanol:ethanol:water (4:1:5, S_1) upper layer; n-butanol:pyridine:water (6:4:3, S_2) and ethyl acetate:acetic acid:n-butanol:water (4:3:2:2, S_3). Spots were identified using ammoniacal silver nitrate (R_1) and aniline phthalate spray (R_2) separately. Detection with ammoniacal silver nitrate (R_1) was done by treatment with three reagents (a), (b) and (c) which were prepared as follows: (a) to silver nitrate solution (12.5 g in 10 ml water), 1 l acetone was added with continuous shaking. Distilled water was added drop wise with stirring until the white precipitate completely dissolved to form a clear solution. (b) Sodium hydroxide (20 g) was dissolved in 400 ml of ethanol. (c) Aqueous ammonia solution by dissolving 20 ml ammonia solution (30%) in 100 ml distilled water. The dried chromatograms were dipped and passed through reagent (a) for about 5 min, dried at room temperature and passed through reagent (b); when the dark brown spots were visualized, the paper was dipped in reagent (c) for 5–10 min with shaking. Finally the chromatograms were washed with water and dried in air [13]. In the aniline phthalate spray (R_2) method, aniline phthalate solution (0.93 g aniline and 1.66 g of phthalic acid were added to 100 ml of butanol saturated with water) was sprayed on the paper chromatogram and heated at 105 °C for 10 min in an oven.

2.3. Isolation of polysaccharide

Gum exudate was glossy reddish brown in color and ground to fine particles using laboratory grinder. Gum material (100 g) was stirred vigorously in distilled water (200 ml) for 6 h at room temperature and centrifuged to remove water-insoluble part. The supernatant solution was decanted off and concentrated. The concentrated aqueous solution was poured into 3 times its volume of ethanol with constant stirring. The polysaccharide was precipitated out in the form of a fluffy precipitate. The precipitate was again dissolved in water and reprecipitated with ethanol. The polysaccharide thus obtained was treated successively with dry solvent ether and acetone. It was filtered under vacuum and dried in vacuum desiccator (7.6 mm Hg) at ambient temperature [13].

2.4. Complete hydrolysis

The pure polysaccharide was subjected to hydrolysis with sulfuric acid (2 N) for 18 h on steam bath. The hydrolysate was cooled, neutralized by addition of saturated solution of barium carbonate till the pH of the solution reached at 7, it was filtered and the residue washed with water. The combined filtrate was concentrated at or below 40 °C in rotary evaporator under reduced pressure. The hydrolyzed mass was used for paper chromatography.

2.5. Physicochemical characterization of polysaccharide from gum exudate

The isolated dried polysaccharide was examined for percentage yield and subjected to physicochemical characterization such as solubility, weight loss on drying, pH, optical density and ash value. The nitrogen content of the pure polysaccharide was determined by Kjeldhal method. The viscosity of the pure polysaccharide was determined at 25 °C using a Brookfield Digital Viscometer 'RVTD', Soughton, USA. The spindle used was SCF4-21. The apparent viscosity was calculated using the following equation:

$$\eta = \frac{\tau}{D}$$

where η is the apparent viscosity in centipoises (cps), τ is the shearing stress (dyne/cm^2) and D is the rate of shear (s^{-1}).

2.6. Experimental protocol

2.6.1. Explorative phase (in vitro study)

2.6.1.1. α-D-Glucosidase (from S. cerevisiae) inhibitory activity and half inhibition concentration (IC_{50}). The α-D-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenyl α-D-glucopyranoside (4-NPGP) according to Tunsaringkarn et al.,

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