Inhibitory effect of the plant proteins mixture on the specific sucrase activity in intestine of diabetic mice

Abstract

Background and Purpose: Increased activity of sucrase, one of the intestinal alpha-glucosidase found in diabetes mellitus. Inhibition of sucrase activity plays a major role in preventing rise in postprandial glucose level in diabetics.

On peer-reviewed literature could be found regarding investigation the effect of mixture plant proteins, Mw 3 – 15 kDa (MPP), isolated from Astragali radix – Astragalus membranaceus Fisch., Foenugraeci semen – Trigonella foenum graecum L., Cichorii radix – Cichorium Intybus L. and Urticae radix and herba – Urtica dioica L. on sucrase activity. This plants are used in traditional medicine of treatment of diabetes mellitus. The aim of this study was to determine activity of sucrase in small intestinal homogenates of NOD diabetic mice on feeding with and without MPP in chow.

Materials and Methods: In mice diabetes was induced by i.v. injection of aloxan-monohydrate (75 mg/kg b.m.) seven days before treatment with MPP.

The proteins (Mw 3 – 15 kDa), were isolated from ethanol extract, each plants separately, by gel filtration method on Sephadex G-25 column. Eluted fraction which highest absorbance on 280 nm were pooled, dialyzed, lyophilized and mixed (MPP) and before treatment in mice solvent in sterile PBS. After seven days of treatment diabetic NOD mice with MPP (1,8 g/d), the small intestine was removed and divided into three segments, from pylorus to duodenum, and two equal lengths of the jejunum and ileum and homogenized in cold 0.14M KCl. Specific sucrase activity was determined using method of Dahlquist et. al., by sucrose as substrate.

Results and Conclusion: We confirmed the increased specific sucrase activity in intestine of diabetic NOD mice. Our results also indicate that MPP have strongly inhibitory potential on intestinal sucrase activity (p<0.05) in diabetic mice. Conclusions drawn from this study should be further supported and our future experiments will be focused on determining the amino acid sequence of each protein from MPP.

INTRODUCTION

In digestion of carbohydrates, dietary disaccharides such as sucrose, lactose, and maltose are hydrolyzed to their constituent – monosaccharides, by a series of specific disaccharidases: sucrase, maltase, and lactase, named α – glucosidases, which are attached to the small intestinal brush-border membrane (7). These monosaccharides are absorbed across the wall of the duodenum and ileum by an active, energy-requir-
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Robert Petlevski et al.

MATERIAL AND METHODS

2.1. Preparation of extracts

Plant material used in this study: Astragali radix – Astragalus membranaceus Fisch., Foenugraeci semen – Trigonella foenum graecum L., Cichorii radix – Cichorium Intybus L. and Urticae radix and herba – Urtica dioica L. were identified at the Ruder Bošković Institute, Department of Molecular Medicine, Zagreb, by testing of the external matter of plants (31).

7 g of each dry plant material extracted with 100 mL 60 % ethanol, on room temperature. After seven days, the macerate was filtrated and ethanol evaporated on a rotator evaporator (Büchi R-144, Switzerland), at a temperature of 45°C and frozen on the −20°C until use.

2.2. Isolation and purification of the proteins from plant extract

a) Gel filtration

Gel filtration separates molecules according to differences in size as they pass through a gel filtration medium packed in a column. Sephadex G-25 (Pharmacia, LKB, Uppsala, Sweden) is recommended for the majority of group separations involving globular proteins. This medium is excellent for removing salt and other small contaminants away from molecules that are greater than Mr 5 000.

Freeze-dried plant material of each plant was dissolved in distilled water (1 mg/mL), then loaded in a Sephadex G-25 gel filtration column, pre-equilibrated and eluted with Tris-HCl buffer. Eluted fractions (1 mL per tube) were collected. In eluted fractions, the proteins were measured by spectrophotometer at 280 nm. Fractions with the highest absorbance at 280 nm were pooled (Fig. 1a-1d).

b) Dialysis and lyophilisation

Pooled fractions (No. 8, 9 and 10 or No. 9, 10 and 11) of each plant were dialyzed using a 35 kDa cut off cellulose membrane (Spectrum Medical Industries, CA, USA) against destilled water at 4°C over night. The molecules less of 3.5 kDa can free move across membrane, but greater molecules remained inside.

After dialysis, isolated fractions were lyophilized (Wkf L-501), so as to stabilize and preserve the proteins.

2.3. Animals

Three-month old, male mice of NOD strain, body mass 23 – 35 g, devided in three groups, were used in the
The mice were housed in metabolic cages on a 12-h light/dark cycle at a temperature of 22–24°C. All mice were fed ad libidum with standard laboratory chow (Mucedola, Italy), and had free access to water. Diabetes was induced by i.v. injection of Alloxan monohydrate (Sigma, St Louis, MO, USA) in Hank’s solution (pH=7.0) in a dose of 75 mg/kg body mass, 7 days before the treatment with MPP. There were three groups of NOD mice, six animals in each group: (1) control (C) mice; (2) diabetic (D) mice fed with standard chow, free of MPP; (3) diabetic (D/MPP) mice fed with standard chow containing MPP (1.8 g/d). They were fed for 7 days. Chows with MPP were prepared daily. Body weight was measured after 7 days of feeding. All experiments were carried out in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, under ether anesthesia, between 9:00 and 10:00 hours, without fasting.

### 2.4. Intestinal homogenates

Intestine was immediately excised, cut longitudinally, washed in ice-cold saline and then blotted on tissue paper. The small intestine was divided into three segments, from pylorus to duodenum, and two equal lengths of the jejunum and ileum. These tissues were homogenizated (100 g/L) in cold 0.14 M KCl using a Teflone homogenizator (Janke and Kunkel, Staufen, Germany). Homogenates were centrifuged at 12 000 g for 30 min in a Mistral 2L refrigerated centrifuge (Measuring and Scientific Equipment Ltd, Crawley, UK). Supernatants were stored at –20°C until analysis.

### 2.5. Sucrase activity

Sucrase activity was determined with 0.056 M sucrose in 0.1 M sodium maleate buffer, pH 6.0 (9). Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as standard (17). Sucrase activity was expressed as specific activity (U/g proteins).

### 2.6. Statistical analysis

Data are shown as mean ± standard deviation (S.D.). The significance of the effect of various treatments was assessed by use of Students t-test. A value of \( p < 0.05 \) was considered statistically significant. SIGMASTAT program for Windows, version 3.0, Jandel Co. (San Rafael, CA, USA) was used for statistical analysis (20).

### RESULT

#### 3.1. Effect of MPP treatment on body weight and intestinal weight of NOD mice

Body weights of the experimental mice were measured after 7 days of feeding with standard chow or chow with...
MPP, (1.8 g/d) on a electric balance (SAC-62, 0.1g Scaltec). Body weight of aloxan-induced diabetic mice (D) were significant decreased when compared with control group of animals (C) (p < 0.05) as a characteristic feature of diabetic status (Table 1).

Intestine weights of the control and diabetic mice on normal feeding as well as on feeding with MPP in chow, all measured immediately after excision, were not significantly different.

3.2. Effect of MPP treatment on sucrase activity in intestine of control and diabetic mice

It was found that activity of sucrase in the interested intestinal regions (duodenum, jejunum, and ileum) of diabetic mice was significantly higher than sucrase activity in normal control mice (Fig. 2.). Compared with normal, control mice, diabetes resulted in 2–3 fold increase in sucrase activity of duodenum, jejunum, and ileum, respectively. MPP treatment almost restored sucrase activity in intestinal regions of diabetic mice to the level near of normal, control mice. All these results demonstrated that diabetes induced significant increase in intestinal sucrase activity, and MPP treatment reversed the increase in sucrase activity under diabetic states.

DISCUSSION

In the present study we investigated the activity of mixture plant proteins (3 – 15 kDa) (MPP) isolated by gel filtration method from given plants against mice intestinal sucrase.

Diabetes is possibly the world’s fastest growing metabolic disease, and as knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies.

Carbohydrates are digested into glucose by intestinal alpha-glucosidase (one of them is sucase), which directly induces the increase in postprandial blood glucose levels. Structural and functional changes take place in the alimentary tract during diabetes, resulting in increased activities of intestinal disaccharidases (6, 32).

Finding effective mammalian alpha-glucosidase inhibitors from natural sources can be beneficial in the prevention and treatment of diabetes mellitus (1, 13, 15).

Astragalus Membranaceus Fisch. is used by practitioners of traditional Chinese Medicine to strengthen or tone the body’s overall vitality, improve digestion, and support the spleen. Astragalus is a good source of the essential trace

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Body weight (g) Mean ± S.D.</th>
<th>Intestine weight (g) Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Duodenum</td>
<td>Jejunum</td>
</tr>
<tr>
<td>Control (C)</td>
<td>6</td>
<td>34.0 ± 0.80</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td>Diabetic (D)</td>
<td>6</td>
<td>29.0 ± 1.84*</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Diabetic group (D/MPP)</td>
<td>6</td>
<td>28.0 ± 1.50</td>
<td>0.35 ± 0.09</td>
</tr>
</tbody>
</table>

*P < 0.05 significantly different vs.C.
mineral selenium. Selenium represents a constituent part of biologically important proteins which are essential for effective functioning of antioxidant mechanisms in cells. Analysis shows that Astragalus contains polysaccharides, monosaccharides, flavonoid, alkaloid, including choline and betaine, folic acid, various amino acids, mucicinic, gum and cellulose (8, 18).

Another plant is Trigonella foenum graecum L. – Foenu- græci semen. The seeds of Trigonella foenum graecum L. have been reported to have antidiabetic and hypocholes- terolaemic properties in both animal models and humans. Activity has been attributed largely to fenugreek’s saponin and cellulose and betaine, folic acid, various amino acids, mucoitin, monosaccharides, flavonoid, alkaloid, including choline and microbiotite. A series of cinnamic acid derivatives and their inhibitory activity on intestinal α-glucosidase. J Enzym Inh & Med Chem 24(5): 1194–2000. Inhibitory effect of the plant proteins mixture on the specific sucrase activity in intestine of diabetic mice Roberta Petlevski


REFERENCES

Urtica dioica L. is a plant shown to reduce blood glucose levels upon oral ingestion. Authors Domola et al. were described one active fraction of Uricae extract, termed UD-1. Component UD-1 was due to one or more structurally related cyclical peptides that facilitate glucose uptake by forming unique glucose permeable pores (10). Oral et al., 2005 described inhibition of alpha-glucosi- dase by aqueus extracts of some potent antidiabetic me- dicinal herbs (including Urtica dioica) (22).

Hypoglycemic and hypolipidemic properties of an ethanolic extract of Cichorium intybus L. was investigated from authors Pushparaj et al.. Authors concluded that administration of Cichorium extract produced a significant reduction in serum glucose, triglycerides and total cholesterol in STZ-induced diabetic rats without any effects on insulin secretion (25).

Increased specific activity of sucrase in the intestine, especially in jejunum and ileum of alloxan diabetic mice (Fig. 2), proved that experimental diabetes causes an increase in the digestive and absorptive functioning of sug- ars in the brush border membranes of intestinal epithel- cells (I3).

Feeding diabetic mice with MPP (1.8 g/mice/day (D/ MPP)) mixed with chow for 7 days led to significantly decreased (p < 0.05) specific activity of sucrase in duodenum, jejunum and ileum compared with diabetic mice on standard feeding (D) (Fig. 2). The decrease in the specific sucrase activities is the most prominent in jejunum and ileum.

In conclusion, the present study showed that diabetes mellitus increased intestinal sucrase activity. MPP (3 – 15 kDa) treatment reversed the increase induced by diabetes while MPP suppressed specific sucrase activity (p< 0.05) in diabetic NOD mice. It is clear, that the study of tradi- tional remedies for diabetes mellitus yields an excellent return in potential new sources of antidiabetic drugs.

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