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Insight into anti-diabetic effect of low dose of stevioside

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ABSTRACT

Diabetes mellitus is a chronic disease characterized by abnormal carbohydrate, lipid and protein metabolism due to a lack of insulin or reduced target cell sensitivity to insulin. *Stevia rebaudiana* is an important source of biochemically active substances with proven anti-diabetic effect. The aim of this study was to determine anti-diabetic effects of the low dose of stevioside in *NMRI Haan* mice. Aqueous stevioside solution (20 mg/kg body weight) was administered by oral route of administration. Anti-diabetic effect of stevioside was estimated by oral glucose tolerance test, adrenaline test after a 10 day stevioside treatment, and alloxan induced hyperglycaemia in mice (two experimental groups, 10 day stevioside treatment before and after alloxan administration). Aqueous stevioside solution prevented significant increase in glycaemia in oral glucose tolerance test (9.22 ± 1.13 to 9.85 ± 1.32 mmol/l, $P < 0.05$), and not in adrenaline test. Significant difference in glycaemia was detected in mice pre-treated with saline and stevioside in alloxan induced hyperglycaemia (saline 23.32 ± 2.14, stevioside 14.70 ± 4.95 mmol/l, $P < 0.05$). In mice pre-treated with stevioside, smallest β cells loss was found compared to other alloxan treated groups. Preserved normal cytoarchitectonic arrangement in islets was detected. Based on the given results we presume there exist a potential therapeutic use of low dose stevioside in diabetes.

1. Introduction

Stevia rebaudiana (Bertoni) Bertoni is an herbaceous perennial plant of the family *Asteraceae* originally grown in Brazil and Paraguay, characterized by a presence of diterpenic steviol glycosides among which stevioside and rebaudioside A are most common [1]. Steviol glycosides are sourced from a sweet tasting plant which is known as Stevia or Honey leaf [2]. For a century Stevia plant, its extracts, and stevioside have been used as natural zero calories sweetener in South America and Asia [3]. Recently stevia has been approved as a sweetener by the Joint Food and Agriculture Organization and the World Organization Expert Committee on Food Additives [4]. *Stevia rebaudiana* is an important source of biochemically active substances which have great potential in therapy of many diseases, especially diabetes mellitus (DM) [1]. Increase in number of diabetic patients, has been followed by an increase in research focused on stevioside, glycoside which is characterized by antioxidant, hypoglycemic, antihypertensive and many other properties [1].

Diabetes mellitus is a disease of modern times which affects around 415 million people worldwide. It is characterized by a disorder of carbohydrate, fat and protein metabolism. Type 1 diabetes is caused by an autoimmune reaction, in which antibodies are directed against insulin-producing β cells in the pancreas. Consequently β cells are destroyed and insulin is no longer produced. Unlike type 1, in type 2 diabetes the pancreas produces insulin but the body becomes resistant and insulin is ineffective. In addition pancreas gradually loses capacity to produce sufficient amount of insulin. Published data suggest that oxidative stress may play key role in pathogenesis of insulin resistance and type 2 diabetes [5,6]. Permanent high blood glucose levels in diabetes lead to serious diseases affecting cardiovascular system, eyes,

Abbreviations: DM, diabetes mellitus; BW, body weight; ADI, acceptable daily intake; OGTT, oral glucose tolerance test; SC, subcutaneous; IP, intraperitoneal; SD, standard deviation.

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kidneys and nerves. Diabetes and its complications are major causes of death in most countries [7,8].

Chan et al. [9], state that the use of stevioside as a food sweetener has led to a decrease in blood glucose values and reduction of body weight in obese people. Consuming stevioside 250 mg/kg of body weight (BW) three times a day for a one year period significantly reduced blood pressure in hypertensive volunteers [9]. On the other hand the Joint FAO/WHO Expert Committee on Food Additives and EFSA (European Food Safety Authority) have established an Acceptable Daily Intake (ADI) for steviol glycosides of 4 mg/kg BW per day, expressed as steviol equivalents [10].

Best to our knowledge, doses of steviol glycosides tested for antidiabetic effect in previously published papers are higher than 4 mg/kg BW per day. Hence the aim of this study was to evaluate anti-diabetic effect of low dose of stevioside in mice, whereby dose administered corresponds to value below ADI.

2. Material and methods

Study approval was obtained from the Ethics committee of the University of Novi Sad. Stevioside hydrate (Sigma, 98% purity, catalog no. S3572) was used in this study. The male mice (*Mus musculus*, *NMRI Haan* strain), body weight 23–29 g, were orally administered aqueous stevioside solution (2 mg/ml) at a dose of 20 mg/kg BW, once a day, for a period of 10 days. Administered dose equals to human dose of approximately 1,63 mg/kg [11]. During the study all animals had free access to food and water with a 12 h light/dark cycle. The influence of stevioside isolated from the *Stevia rebaudiana* plant was examined through oral glucose tolerance test, adrenaline test and alloxan induced diabetes. Experimental animals were divided in 8 groups of 6 mice: G1a – saline 20 mg/kg BW, once a day for 10 days, oral glucose tolerance test (OGTT) on 10th day; G2a – stevioside 20 mg/kg BW, once a day for 10 days, oral glucose tolerance test OGTT on 10th day; G1b – saline 20 mg/kg BW, once a day for 10 days, adrenaline test on 10th day; G2b–stevioside 20 mg/kg BW, once a day for 10 days, adrenaline test on 10th day; G3–saline 20 mg/kg BW, once a day for 10 days, alloxan (150 mg/kg BW) on 10th day; G4–stevioside 20 mg/kg BW, once a day for 10 days, alloxan (150 mg/kg BW) on 10th day; G5–alloxan (150 mg/kg BW), saline 20 mg/kg BW, once a day for 10 days; G6–alloxan (150 mg/kg BW), stevioside 20 mg/kg BW, once a day for 10 days. At the end of the experiment animals were anesthetized with urethane and decapitated, pancreatic tissue specimens were harvested for histological examination.

Glycaemia in mice was measured in capillary blood sampled by incision near the tip of the tail, test strip opening is drawn closer to touch blood drop until it is absorbed enough to begin the test (ACCU CHEK Active, Roche) [12].

2.1. Oral glucose tolerance test(OGTT)

Before glucose administration blood glucose is measured; glucose 500 mg/kg BW is administered by oral route of administration; glycaemia is measured 30 min after glucose administration [12].

2.2. Adrenaline test

Before adrenaline administration blood glucose level is measured; adrenaline 0.2 mg/kg BW is administered by subcutaneous route of administration (SC); glycaemia is measured 45 min after adrenaline administration [12].

2.3. Alloxan diabetes induction

Before alloxan administration blood glucose is measured; alloxan 150 mg/kg BW is administered by intraperitoneal (IP) route of administration; glycaemia is measured 24 h after alloxan administration; if glycaemia is below 15 mmol/l, another dose of alloxan is administered; it is considered that diabetes has successfully developed if glycaemia is above 15 mmol/l [12].

2.4. Histological analysis

2.4.1. Immunohistochemical analysis

Pancreata were harvested from each of the experimental animals. The splenic lobe of harvested pancreatic tissue was placed in Bouin's solution for 24 h at 4 °C. After appropriate dehydration, the samples were embedded in paraffin (Histowax, the Netherlands) and cut on a rotary microtome (Leica, Germany) at 5 μm. Two serial sections were immunohistochemicaly stained for each of the pancreatic tissue samples. Methods for immunohistochemical staining included primary antibodies: mouse anti–insulin Ab-6 in a 1:200 dilution (Lab Vision; Thermo Scientific, Rockford, IL), and rabbit anti–glucagon in a 1:100 dilution (Lab Vision; Thermo Scientific), using the appropriate visualization system: UltraVision LP Detection System HRP Polymer & AEC Chromogen (Lab Vision; Thermo Scientific). Glucagon immunostaining required antigen retrieval using citrate buffer (pH 6.0) in a microwave oven at 850 W for 20 min. All the antibodies were applied for 30 min at room temperature. Visualization was performed using AEC Chromogen (Lab Vision; Thermo Scientific). Mayer's hematoxylin was used as a counterstain for immunohistochemistry followed by mounting and coverslipping (Bio-Optica, Italy) for slides. Prepared slides were analyzed using a Leica DMLB microscope (Leica, Germany) and photographed on a Leica MC 190 HD camera (Leica, Germany).

Table 1
Glycaemia values in OGTT and adrenaline test. Glucose values were measured after 10 day treatment with saline (20 mg/kg BW) and aqueous stevioside solution (20 mg/kg BW).

	10 day saline treatment (20 mg/kg BW)	10 day stevioside treatment (20 mg/kg BW)
Glycaemia before oral glucose load (mmol/l)	8.10 ± 0.95	9.22 ± 1.13
Glycaemia 30 min after oral glucose load (mmol/l)	10.00 ± 1.16*	9.85 ± 1.32
Δ	1.9 ± 1.71	0.63 ± 0.85
Glycaemia before s.c. adrenaline administration (mmol/l)	7.43 ± 1.29	7.78 ± 0.90
Glycaemia 45 min after s.c. adrenaline administration (mmol/l)	14.82 ± 2.83	13.38 ± 1.67
Δ	7.37 ± 1.96	5.60 ± 1.05

* $P < 0.05$ refers to the comparison of the input and output values in the group treated with saline in OGTT.