Salvia fruticosa tea drinking reduces the expression of sodium/glucose cotransporter 1 in enterocytes’ brush-border membrane of streptozotocin-induced diabetic rats

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by elevated plasma glucose concentrations, resulting from insufficient insulin secretion, insulin resistance or both. The prevalence of Type 2 diabetes mellitus is increasing worldwide and 366 million diabetic people are expected by 2030 [1]. Considering this increase in the prevalence of this disorder and a lack of efficient treatment, there is a growing interest on the research of new bioactive compounds. One of the features of type 2 diabetes mellitus (T2DM) is an increase in Na+-dependent glucose transporter (SGLT1) expression in the brush-border membrane (BBM) of the enterocyte. This increases intestinal glucose absorption and aggravates hyperglycaemia [2]. Moreover, secretion of glucagon-like-peptide-1 (GLP-1), one of the enteroendocrine incretin hormones, and levels of insulin are reduced under a diabetic condition [3]. Salvia fruticosa is a medicinally used plant whose bioactive properties have been extensively investigated [4,5]. In our previous results suggested an effect on control of blood glucose by reducing SGLT1 expression in brush-border membrane vesicles (BBMV) of rats after diet manipulation (unpublished data). In an attempt to characterise the antidiabetic effects of sage tea at the levels of intestinal epithelium and insulin secretion, normal and streptozotocin (STZ)-induced diabetic rats were used. Salvia fruticosa was given ad libitum, instead of water, for 14 days to the treated animals. Enterocyte SGLT1 and facilitative glucose transporter 2 (GLUT2) expression were evaluated by Western blotting. Effects on GLP-1 and islet regeneration (β-cell insulin expression) were assessed by immunohistochemistry.

RESULTS

Plasma insulin and blood glucose

Figure 1 – Effect of S. fruticosa tea drinking on blood glucose levels, during the experimental period.

Figure 2 – Effect of S. fruticosa tea drinking, for 14 days, on plasma insulin concentration. *P<0.05 and **P<0.01 when compared with normal water group. The STZ group did not significantly differ from the respective normal water group.

SGLT1 and GLUT2 expression

A

B

C

D

E

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L

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P

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R

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X

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Z

Figure 3 – Effect of S. fruticosa tea drinking on the number of GLUT2 expressing enterocytes’s β-cells (A), and on the density of the GLP-1 signal in islet endocrine cells (C) assessed by immunohistochemistry. (A) – Representative photomicrographs of immunostained β-cells showing GLP-1 signal among groups: diabetic water group (1); normal water group (3); S. fruticosa tea drinking group (4) – diabetic tea group (5) – Islet cells of S. fruticosa group. (B) – Bar chart showing the respective normal control group. (C) – Average intensity of insulin signal in pancreatic β-cells (normal group) and β-cells (diabetic group). (D) – Presence of S. fruticosa tea drinking significantly reduced the increase of GLP-1 expression (P<0.001), although GLP-1 intensity was significantly higher in STZ-diabetic rats (P<0.005).

Effects on pancreatic islets of Langerhans

Figure 4 – Effect of S. fruticosa tea drinking on pancreatic islet regeneration. A – Representative photomicrographs of rat pancreatic islet showing insulin immunostained β-cells using the rabbit anti-insulin (ProGen) visualized with Alexa Fluor 488 green stain: (a) normal group, (b) diabetic group. B – Intensive signal in β-cells in normal group; C – Average intensity of insulin signal in pancreatic β-cells (normal/diabetic) of the animals, **P<0.01 and ***P<0.001 compared with the respective normal control group.

METHODS

PREPARATION OF BRUSH-BORDER MEMBRANE VESICLES (BBMV)

BBMV were prepared from rat intestinal sections of jejunum, using a combination of homogenization (with collagenase and different concentrations of collagenase as described in 4). The brush-border BBMV were incubated in buffer containing a cocktail of proteinase inhibitors. Immunoblotting (rabbit anti-SGLT1, rabbit anti-GLUT2, 1:5000 dilution) and ELISA (SGLT1, insulin, 1:5000 dilution) were performed.

WESTERN BLOTTING

The membrane proteins were separated on 10% SDS-PAGE gels and electroblotted onto nitrocellulose membranes. The membranes were blocked and incubated with the antibody to SGLT1 or GLUT2 (1:1000 in TMBL buffer in 5% non-fat dry milk, 0.1% Tween 20) overnight at 4°C. Blots were then washed with TMBL buffer and incubated with peroxidase conjugated donkey anti-rabbit or anti-mouse antibody (1:5000 in TMBL buffer) for 1 hour. Blots were washed and the signal visualised with ECL reagent visualised with Fuji autoradiography film. Positions of the bands were then cut out, excised, washed with distilled water and processed for autoradiography.

IMMUNOSTAINING/HISTOLOGY

Diabetic rats were killed by cervical dislocation (for intra-peritoneal fluid samples) or exsanguination (for tissue samples). The pancreas and gut tissues were removed and fixed in 4% paraformaldehyde for 24 h, pre-fixed in 25% sucrose in PBS for 2 days and then embedded in Tissue-Tek OCT compound. Sections were cut at 6μm thickness. Aqueous mounting media containing anti-fade agents were used to mount sections. Negative controls were processed on the same slide in identical conditions using the secondary antibodies alone. Sections were coversliped using an aqueous mounting media containing anti-fade agents.

REFERENCES


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FINAL REMARKS

Western blotting results showed that Salvia fruticosa tea drinking significantly reduced the increase in SGLT1 expression in BBMV in response to hyperglycaemia induced by STZ (Fig.3).

Salvia fruticosa tea drinking seems to have an effect, although small, on insulin secretion (looking to the plasma-insulin concentration associated with a slight increase in islet regeneration of sage tea STZ-diabetic rats) (Fig. 2 and 6).

These effects of Salvia fruticosa tea seem to be beneficial in a diabetic condition, where an increased intestinal glucose transport (SGLT1 reduction to decrease plasma insulin concentration) – essentially hyperglycaemia mainly by reducing SGLT1 expression in apical membrane of enterocytes.

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