Antidiabetic potential of *Salvia fruticosa* infusion: effects on intestinal glucose transporters

University of Minho

School of Sciences

Azevedo, M.; **Lima**, C. F.;
Fernandes-Ferreira, M.; **Almeida**, M. J.; **Pereira-Wilson**, C.

Department of Biology, School of Sciences, University of Minho - Campus de Gualtar, 4710-057 Braga, Portugal

INTRODUCTION

Diabetes mellitus type 2 (DMT2) or Non-Insulin Dependent *Diabetes mellitus* (NIDDM) is a disease characterized by a deficient control of blood glucose, that is currently estimated to affect over 100 million people worldwide and is predicted to double by 2010. NIDDM is the most prevalent in populations with western-type lifestyles and diets. Complications such as vision loss, renal failure, nerve damage and cardiovascular diseases are common.

Glucose represents a large proportion of the carbohydrate present in western diets and the consequent high plasma glucose levels are thought to contribute to the hyperinsulinism and subsequent insulin resistance seen in DMT2 patients. Glucose is absorbed through the enterocyte via two types of glucose transporters: the SGLT1, a sodium dependent glucose transporter in the apical brush border membrane, and the facilitated glucose transporter GLUT2 located mainly on the basolateral membrane of the enterocyte [1] (Fig.1).

Intestinal monosaccharide transport capacity of diabetics is 3 to 4 times higher [1] than that of healthy individuals - which aggravates (post-prandial) hyperglycemia. Therefore, control of both carbohydrate digestion and intestinal absorption of resulting products would improve blood glucose levels and help reduce diabetic complications.

At present, management of diabetes relies mainly on dietary manipulation and use of agents to lower circulating glucose levels. However, the epidemic proportions of DMT2 justifies the search for new drugs effective in the treatment and preferably also in the prevention of this disease. Folk medicine has empirically identified plants with antidiabetic effects that provide a good source material for the search of novel active compounds.

In this study we tested the effects of *Salvia fruticosa* extracts on the expression of the intestinal glucose transporter SGLT1 and on the *in vitro* activity of α-amylase.

Wistar rats (5 weeks) were fed normal rat chow and given water (water group) or *S. fruticosa* tea (tea group) for 14 days. Plasma glucose was assessed after an overnight fast as well as 45 min. after an *ip* injection of 30% glucose (Table1).

Effects on intestinal SGLT1 expression were analysed by Western blotting (Fig. 3 and 4) and Immunohistochemistry (Fig.5). Changes in the activity of α-amylase by *in vitro* incubations with *S. fruticosa* water extract (tea) and essencial oil fraction shown in Table 2.

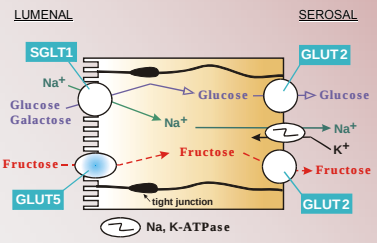

Fig. 1 – A schematic representation showing hexose transporter systems in the rat small intestine enterocyte. The brush-border membrane (BBM) is the area of membrane exposed to the lumenal contents. The basolateral membrane (BLM) is the remaining plasma membrane. Arrows indicate routes of monosaccharide absorption.

Table 1 – Plasma glucose levels (mM) of rats 45 min. after intraperitoneal injection of saline solution and glucose solution respectively.

	PLASMA GLUCOSE	
In vivo beverage	*Saline ip injection*	*30% (w/v) Glucose ip injection*
Water	6.10 ± 0.43	9.98 ± 0.61
Tea	5.28 ± 0.51	8.31 ± 1.46

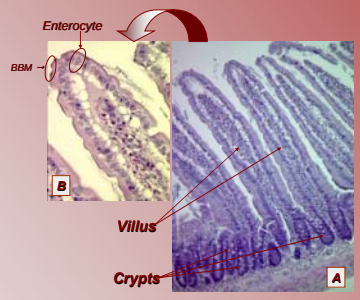

Fig. 2 – Representative images of rat small intestine sections.
(A) intestinal villus and crypts, amplified 100x.
(B) magnification of one villus tip showing enterocytes and brush-border membrane (BBM), amplified 400x.

RESULTS

Western blotting

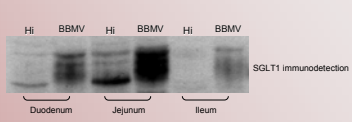

Fig. 3 – Western blot analysis of SGLT1 expression in three intestinal mucosa segments: duodenum, jejunum and ileum; crude homogenate (Hi) and purified brush-border membrane vesicles (BBMV).

- These results indicate that:

1. the antibody used (rabbit anti-SGLT1, Chemicon) cross-reacts with SGLT1 because the signal is in the region of 75kDa (which corresponds to SGLT1 molecular weight); the signal increases in the BBMV fraction relative to the crude homogenate which is in agreement with the brush border membrane localization of SGLT1.
2. the highest relative abundance of SGLT1 is in the jejunal segment of the intestinal mucosa.

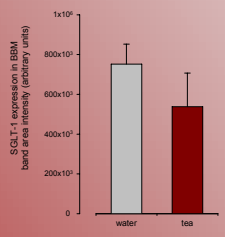

Fig. 4 – Western blot analysis of SGLT1 expression in rat jejunum BBMV of water and *Salvia fruticosa* tea drinking animals.

Immunohistochemistry

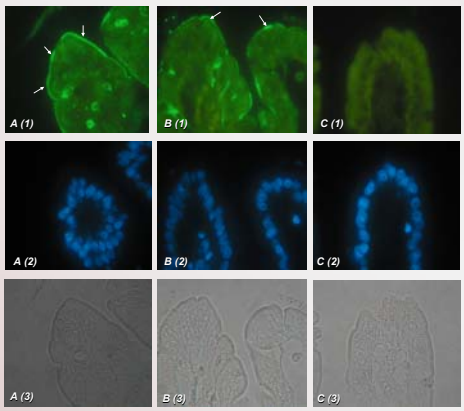

Fig. 5 - Representative photomicrographs of rat jejunum showing SGLT-1 immunolocalization using the rabbit anti-SGLT1 antibody + goat anti-rabbit Alexa Fluoro 488 green stain).
A (1) rat who drunk water; B (1) rat treated with infusion of *S. fruticosa* and C (1) normal rabbit serum, control for immunohistochemistry showing background fluorescence.
A (2), B (2) and C (2) – The same sections stained with DAPI – nuclei stain.
A (3), B (3) and C (3) – The same sections observed by Phase Contrast microscope.
Arrows indicate the signal of SGLT1 on the BBM of the enterocytes.

FINAL REMARKS

- Western blotting results suggest that *Salvia fruticosa* tea represses SGLT1 expression on BBM (Fig.4).

- Immunohistochemistry results (Fig.5) showed the localization of SGLT1 on the BBM. SGLT1 expression was decreased in tea drinking rats (Fig. 5 – B (1)) relative to water drinking controls (Fig.5 – A(1)).

- In what concerns the α-amylase assay, the results obtained, although preliminary, seem promising given that we obtained some inhibition of α-amylase activity, by *S. fruticosa tea* (0.05mg/mL) and by UA and EO (abundant constituents of *S. fruticosa*) (Table 2).

- These results indicate an effect of *S. fruticosa* tea on intestinal glucose absorption that deserves further investigation.

Alpha-Amylase Assay

Table 2 – Effects of *S. fruticosa* tea, Ursolic Acid (UA), *S. fruticosa* essential oil (EO *S. fr*) and *S. officinalis* essential oil (EO *S. of*) on porcine pancreatic α-amylase activity.

Compounds	*Effect on α-amylase activity* Mean ± SD
S. fruticosa tea_0.5mg/mL	+ 5.4 ± 4.0
S. fruticosa tea_0.05mg/mL	- 4.6 ± 4.1
UA_50μM	- 6.0 ± 3.7
UA_250μM	- 9.5 ± 0.5
EO *S. fr*_100nl/mL	- 2.4 ± 2.3
EO *S. fr*_10nl/mL	- 7.9 ± 2.1

METHODS

PREPARATION OF BRUSH-BORDER MEMBRANE VESICLES (BBMV)

BBMV were prepared from rat intestinal sections of jejunum, duodenum and ileum (Fig. 3), using a combination of cation precipitation (with $MgCl_2$) and differential centrifugation as described in [2]. The final purified BBMV were suspended in a buffer containing 300mM mannitol and 5mM Tris-HCl, pH 7.4.

WESTERN BLOTTING

The abundance of SGLT1 protein was measured by quantitative Western blotting as described in [3]. The BBMV proteins were separated on an 8% polyacrylamide gel containing 0.1% (w/v) SDS and electrotransferred to PVDF membranes. The membranes were blocked and incubated with the antibody to SGLT1 (1:2000 in 1%BSA+0.05%Tween 20 in PBS pH7.4) for 1h (Chemicon, AB1352), washed in TPBS, and incubated for 1h with the secondary antibody, goat anti-rabbit horseradish peroxidase-linked whole antibody diluted 1:30000 in TPBS. Cross-reactive bands were detected by ECL (Amersham). The exposed film was scanned and the resulting digitised images were analysed using densitometry software (Sigma Scan).

IMMUNOHISTOCHEMISTRY

Jejunal tissue was fixed in 3%PFA/PBS, processed for paraffin, embedding and 5μm sections were mounted on glass slides and dewaxed in Clear-Rite.

After rehydration in TPBS (PBS with 0.5% Tween 20), the slides were incubated for 20 min in blocking reagent (5% normal goat serum/1% bovine serum albumin in TPBS pH7.4) followed by primary antibody to SGLT1 (1:200) overnight. Sections were then rinsed in TPBS and incubated with secondary antibodies (goat anti-rabbit, Alexa Fluoro 488) for 1h at 37ºC. After rinsed in TPBS the sections were coversliped using a fluorescent mounting media. Negative controls were processed on the same slide in a identical manner, replacing the primary antibody with normal rabbit serum. (Fig. 5 (C1/2/3)).

Alpha - AMYLASE ASSAY

Activity of porcine pancreatic α-amylase (EC 3.2.1.1 - Sigma) was measured as described in [4] by determination of the reducing groups arising from hydrolysis of soluble starch.

Test compounds (in DMSO and/or Sörensen buffer, pH6.9), starch (0.2% (w/v)) were mixed and the reaction started by addition of the enzyme (α-amylase 0.05U/mL) (reaction volume=2mL). After 10 min at 30ºC the reaction was stopped by addition of 1mL of DNS (1% w/v). The mixture was heated for 10 min at 100ºC. After cooling absorbance was measured at 540nm against the reagent blank.

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