Metformin-like effects of *Salvia officinalis*: useful in diabetes prevention?

Authors: *Cristovao F. Lima[†], Marisa F. Azevedo[†], Rita Araujo[†], Manuel Fernandes-Ferreira, Cristina Pereira-Wilson*

Address: *Department of Biology, Centre of Biology, School of Sciences, University of Minho, 4710-057 Braga, Portugal*

[†] *These authors contributed equally to the work.*

Corresponding author: Cristina Pereira-Wilson; telephone +351 253604318; fax +351 253678980; e-mail cpereira@bio.uminho.pt

Running title: Metformin-like effects of sage

Keywords: *Salvia officinalis L.*; diabetes; metformin; Rat hepatocyte; hypoglycaemic effects

Abstract

Common sage (*Salvia officinalis* L) is among the plants that are claimed to be beneficial to diabetic patients, and previous studies have suggested that some of its extracts have hypoglycaemic effects in normal and diabetic animals. In this study we purposed to verify the antidiabetic effects of the most common form of consumption of this plant as an infusion (tea). Replacing water with sage tea for 14 days lowered fasting plasma glucose in normal mice but had no effect on glucose clearance in response to an intraperitoneal glucose tolerance test. This indicated effects at level of the liver on gluconeogenesis. Hepatocyte primary cultures of healthy sage tea drinking rats showed, after stimulation, high glucose uptake capacity and a decreased gluconeogenesis in response to glucagon. Sage essential oil further increased hepatocyte sensitivity to insulin and inhibited gluconeogenesis. Overall these effects resemble those of the pharmaceutical drug metformin, a known inhibitor of gluconeogenesis used in the treatment and prevention of type 2 diabetes mellitus. In primary cultures of rat hepatocytes isolated from streptozotocin-induced diabetic rats none of these activities were observed. These results seem to indicate that sage tea does not possess antidiabetic effects at this level. However, its effects on fasting glucose levels in normal animals and the metformin-like effects on rat hepatocytes suggest that sage may be useful as food supplement in the prevention of type 2 diabetes mellitus by lowering plasma glucose of individuals at risk.

Introduction

Diabetes mellitus is a disease characterized by increased plasma glucose levels which is the result of an insufficient production (type 1 diabetes) and/or decreased tissue response to the pancreatic hormone insulin (type 2 diabetes). In type 1 diabetes there is an autoimmune disease in which the insulin secreting beta cells of the pancreas are destroyed by the individual's immune system. In type 2 diabetes, peripheral tissue insulin resistance strains insulin secretion which leads to subsequent failure of beta cells of the pancreas (Klover & Mooney, 2004). Type 2 diabetes accounts for the majority of cases (85-90% of cases) and it is likely to become even more prevalent over the coming decades because of the increasing rates of childhood and adult obesity and the generalisation to developing countries of western lifestyles (Williams & Pickup, 2004). Nowadays, diabetes mellitus is a major public health concern which has attained epidemic proportions in many countries.

Glucose is an essential nutrient for the human body and glucose homeostatic mechanisms aim at maintaining blood glucose within a narrow range, around 5-7 mmol/L (Williams & Pickup, 2004; Klover & Mooney, 2004). In healthy individuals blood glucose concentrations are maintained by the balance between glucose entry into circulation from intestinal absorption and glucose uptake into peripheral tissues such as muscle and adipose tissue. Circulating levels of insulin increase after meals stimulating GLUT-4 mediated glucose uptake by peripheral tissues, thereby preventing hyperglycemia. During periods of no intestinal glucose absorption, blood levels do not decrease drastically, because the liver releases glucose into circulation in response to the counter-regulation pancreatic hormone glucagon which stimulates both glycogen breakdown (glycogenolysis) and gluconeogenesis (i.e. the formation of new glucose from substrates such as glycerol, lactate and amino acids such as alanine). The anti-

hyperglycaemia effects of insulin include the suppression of glucose output from the liver, inhibiting both glycogenolysis and gluconeogenesis. In healthy individuals relatively low concentrations of insulin are needed to suppress hepatic glucose output (Roden & Bernroider, 2003; Williams & Pickup, 2004). However, in type 2 diabetes hyperglycemia exists not only post-prandialy, where it reveals insulin's inability to increase peripheral glucose uptake, but elevated blood glucose levels persist even during fasting due to increased liver gluconeogenesis (Roden & Bernroider, 2003; Klover & Mooney, 2004).

Before the establishment of the disease, those individuals more at risk of developing type 2 diabetes present the first signs of abnormal glucose metabolism such as impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) (Simpson et al., 2003). This provides an asymptomatic period at the beginning of the progression of type 2 diabetes, where preventive interventions can be applied. Previous studies have shown that changes to dietary habits and to sedentary behaviour can reduce the progression from impaired glucose tolerance to type 2 diabetes by 50-60% (Chiasson et al., 2002; Simpson et al., 2003). However, the difficulty in maintaining lifestyle changes over the long term justifies the need for pharmacotherapeutic support, and recent studies have shown beneficial effects of metformin and acarbose in the progression from IGT to type 2 diabetes (Simpson et al., 2003). Metformin mainly inhibits gluconeogenesis and acarbose reduces intestinal glucose absorption. However, any pharmacological intervention in an asymptomatic population raises ethical considerations in addition to practical and economic issues. Dietary supplements with glucose lowering properties could provide a culturally acceptable and economically viable alternative to pharmaceutical interventions at this stage. However, in spite of growing interest on the

effects of herbs and food supplements on glucose control in diabetes, information remains insufficient (Day, 1998; Yeh et al., 2003).

For centuries plants have been used in folk medicine and their beneficial effects described. *Salvia oficinalis* L. (common sage) is among those which are reputed to possess antidiabetic properties (Baricevic & Bartol, 2000).

Recently, Alarcon-Aguilar and collaborators (2002) showed that a water-ethanolic extract from *S. officinalis* injected intraperitoneally had hypoglycaemic effects in fasted normoglycaemic mice and in fasted mildly alloxan-induced diabetic mice. In addition, Eidi and co-workers (2005) showed that a sage methanolic extract given intraperitoneally reduced significantly serum glucose in fasted streptozotocin-induced diabetic rats without changes in insulin levels. Sage has a high essential oil (EO) content (Giannouli & Kintzios, 2000). That has also been tested and proved to be hypoglycaemically active in normal and in alloxan-induced diabetic rats (Baricevic & Bartol, 2000 and references therein) but not in streptozotocin-induced diabetic rats (Eidi et al., 2005).

With this study we aimed at evaluating the hypoglycaemic properties of a sage infusion (hereafter referred to as a sage tea), the most common form of human sage consumption, and to shed some light on possible mechanisms of action. In mice and rats treated for 14 days with sage tea we evaluated: *in vivo* the response to an intraperitoneal glucose tolerance test (ipGTT); and, in primary cultures of hepatocytes isolated from normal and streptozotocin-induced diabetic rats the effects on responses to glucose, insulin and glucagon were examined. The *in vitro* effects of sage EO were also investigated.

Materials and methods

Chemicals

Collagenase (grade IV), William's Medium E (WME), Dulbecco's Modified Eagle's Medium (DMEM), Dexamethasone, Insulin, Glucagon, 1,1-Dimethylbiguanide hydrochloride (Metformin), streptozotocin (STZ) and Bradford reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucofix for glucose measurements was acquired from A. Menarini Diagnostics (Firenze, Italy). All others reagents were of analytical grade.

Plant material, preparation of sage tea, isolation of the EO and analysis of its constituents

Salvia officinalis L. plants were grown in an experimental farm located in Arouca, Portugal, and were collected in April, 2001. The aerial parts of plants were lyophilized and kept a -20ºC. The sage tea was routinely prepared as in a previous study by pouring 150 ml of boiling water onto 2 g of the dried plant material and allowing to steep for 5 min (Lima et al., 2005). This preparation produced a 3·5 ± 0·1 mg of extract dry weight per ml of infusion, with rosmarinic acid (362 μg/ml of infusion) and luteolin 7-glucoside (115·3 μg/ml of infusion) as a major phenolic compounds and 1,8-cineole, *cis*-thujone, *trans*-thujone, camphor and borneol as a major volatile components (4·8 μg/ml of infusion) (Lima et al., 2005). The EO was obtained by hydrodistillation and the compounds identified by GC and GC-MS in a previous work (Lima et al., 2004). The EO includes around 60 compounds, the most abundant being *cis*-thujone (17·4%), *alpha*-humulene (13·3%), 1,8-cineole (12·7%), *E*-caryophyllene (8·5%) and borneol (8·3%) (Lima et al., 2004).

Animals

Female Balb/c mice (8-10 weeks) and male Wistar rats (150-200 g) were purchased from Charles River Laboratories (Barcelona, Spain) and acclimated to our laboratory animal facilities for at least one week before the start of the experiments. During this period, the animals were maintained on a natural light/dark cycle at 20 ± 2ºC and given food and tap water *ad libitum*. The animals used in the experiments were kept and handled in accordance to our University regulations that follow the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985). To study the effects of sage tea drinking, sage tea was given to mice and rats *ad libitum* for 14 days in replacement of water as previously performed (Lima et al., 2005). The volumes consumed were found not to be significantly different between water and sage tea in both normal mice and rats (Lima et al., 2005). Diabetes was induced in rats by intraperitoneal injection of a freshly prepared streptozotocin solution (50 mg/kg in 0·1 M-citrate buffer, pH 4·5). Experiments with diabetic rats were carried out 1 week after STZ injection. During this period diabetes was well established with polydipsia, polyuria and non-fasting blood glucose levels >250 mg/dl. The animals were used in four different experiments.

Experiment 1

This experiment aims to evaluate the hypoglycaemic potential of the sage tea in normal mice where animals from two different groups (water and sage tea drinking) were used to perform an ipGTT. Twenty female Balb/c mice were randomly divided into two groups, given food *ad libitum* and either tap water or sage tea *ad libitum* for 14 days (beverage was renewed daily). On day 15 an ipGTT (ip injection of 300 g/l-glucose in physiologic saline in a dose of 5·83 ml/kg of mouse) was performed in 3 hours fasted mice (half of the animals of each group used as a vehicle group – ip injection of saline

alone). Blood samples were collected 45 min after the ip injection and plasma used for glucose measurements.

Experiment 2

In this experiment, primary cultures of rat hepatocytes, from overnight fasted normal animals, in a medium with low concentrations of glucose and a gluconeogenic substrate (lactate) were used to evaluate the modulation by sage tea of hepatocyte glucose production. Eight male Wistar rats were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 days (beverage was renewed daily). Hepatocyte isolation was performed between 10.00 and 11.00 o'clock by collagenase perfusion as previously described by Moldeus (Moldeus et al., 1978) with some modifications (Lima et al., 2004) from overnight fasted normal animals. Cell viability was > 85% as estimated by the trypan blue exclusion test. Cells were suspended in DMEM (containing 5·6 mM-glucose) supplemented with 10 mM-lactate, 100 ml/l-FBS, 10^{-9} M-insulin and 10^{-9} M-dexamethasone and seeded onto 6-well culture plates at a density of 1×10^{6} cells/well. Cells were incubated at 37ºC in a humidified incubator gassed with 50 ml/l-CO_2/air. After plating (to allow for cell attachment), culture medium was replaced with DMEM supplemented with 10 mM-lactate, 100 ml/l-fetal bovine serum (FBS) and none, one or more of the following compounds: 10^{-7} M-glucagon, 10^{-3} M-metformin and/or 4 nl/ml-sage EO. After 24 hours of incubation the medium was recovered for glucose quantification. Metformin was used as positive control.

Experiment 3

In this experiment, primary cultures of normal rat hepatocytes in media with high (11 and 22 mM) concentrations of glucose (to mimic post prandial and diabetic conditions) were used to evaluate the modulation of the sage tea in the glucose consumption capacity of the cells. Eight male Wistar rats were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 days (beverage was renewed daily). Hepatocyte isolation from normal animals were performed as above and cells suspended in WME (containing 11 mM-glucose) supplemented with 100 ml/l-FBS, 10^{-9} M-insulin and 10^{-9} M-dexamethasone and seeded onto 6-well culture plates at a density of 1×10^{6} cells/well. Cells were incubated at 37ºC in a humidified incubator gassed with 50 ml/l-CO_2/air. After plating, culture medium was replaced with WME supplemented with 100 ml/l-FBS and none, one or more of the following compounds: glucose (to a final concentration of 22 mM), 10^{-7} M-insulin and/or 4 nl/ml-sage EO. After 24 hours of incubation the media were recovered for glucose quantification.

Experiment 4

In this experiment, primary cultures of hepatocytes from STZ-induced diabetic rats were used in media with low and high concentrations of glucose (both containing a gluconeogenic substrate - lactate) to evaluate effects of sage tea drinking on cell glucose production. Eight STZ-induced diabetic rats (male Wistar) were randomly divided into two groups and given food *ad libitum* with either tap water or sage tea *ad libitum* for 14 days (beverage was renewed daily). Due to polydipsia of diabetic rats, sage tea drinking animals were pair-fed with the non diabetic animals given diluted sage tea, in order to ensure a similar intake of tea dry weight as rats of experiments 2 and 3. Hepatocyte isolation from diabetic animals were performed as above and cells suspended in DMEM

either containing 5·6 mM or 22 mM-glucose, supplemented with 10 mM-lactate, 100 ml/l-FBS, 10^{-9} M-insulin and 10^{-9} M-dexamethasone and seeded onto 6-well culture plates at a density of 1×10^{6} cells/well. The culture plates were incubated at 37ºC in a humidified incubator gassed with 50 ml/l-CO_2/air. After plating, culture medium was replaced with DMEM supplemented with 10 mM-lactate, 100 ml/l-FBS and none, one or more of the following compounds: glucose (to a final concentration of 22 mM), 10^{-7} M-insulin, 10^{-7} M-glucagon, 10^{-3} M-metformin and/or 4 nl/ml-sage EO. After 24 hours of incubation the medium was recovered for glucose quantification. Metformin was used as positive control.

Plating periods of 24 hours were used in cell cultures from normal fed animals for cell attachment. In an attempt to preserve altered physiological conditions, introduced both by fasting and STZ-induced of diabetes, cells were platted for 3 hours before exposure to the different test conditions. In the fasted condition, results from plating of 24 hours are also presented for comparison.

In all experiments with rat hepatocytes LDH activity was measured in the media to ensure no toxicity of the treatment to the cell layer.

Biochemical analysis

Glucose measurement - The content of glucose in mice plasma and culture media were measured using a colorimetric enzymatic method – Glucofix – following the manufacturer specifications.

LDH activity - The determination of lactate dehydrogenase activity in culture media was used as an indicator of hepatocyte plasma membrane integrity. The activity of the enzyme was measured at 30ºC by quantification of NADH consumption by continuous

spectrophotometry on a plate reader (Spectra Max 340pc, Molecular Devices, Sunnyvale, CA, USA) (Lima et al., 2005).

Protein - Protein content was measured with the Bradford Reagent purchased from Sigma using bovine serum albumin as a standard.

Statistical Analysis

Data are expressed as means and standard errors of the means (SEM). Two-way ANOVA followed by the Student-Newman-Keuls *post hoc* test (SigmaStat, version 2.03; SPSS Inc., San Rafael, CA, USA) was employed in experiment 1 to compare the effects of *in vivo* beverage (water versus sage tea) and the ipGTT (saline ip versus glucose ip). In the experiments 2, 3 and 4 (where 2 replicates were used for each experimental condition), the same statistical test was employed to compare the effects of *in vivo* beverage (water versus sage tea) and the *in vitro* treatments (in this case when a significant effect was obtained, a paired *t*-test was employed to find differences between each treatment). *P* values $\leq 0{\cdot}05$ were considered statistically significant.

Results

Experiment 1

Fourteen days of sage tea drinking significantly lowered fasting (3 h + 45 min) plasma glucose concentration from 8·8 mM to 6·8 mM ($P \leq 0{\cdot}01$) in normal mice (table 1). In response to an ipGTT, a significant increase of plasma glucose was observed at 45 min in both groups (table 1) although no differences were observed between water and sage tea drinking groups.

Table 1

Experiment 2

When hepatocytes of overnight fasted rats were plated with 5·6 mM-glucose and 10 mM-lactate containing medium there was a release of glucose (mainly due to gluconeogenesis) to the medium. Hepatocyte glucose production increased in response to glucagon (fig. 1A) in cells from water drinking animals but not statistically significant in cells from sage tea drinking animals (figs. 1A and 1B). In general hepatocyte glucose production (fig. 1A) was lower in cells isolated from sage tea drinking rats when compared with water drinking controls ($P \leq 0.05$), and the difference became significant in the glucagon + EO groups (fig. 1A). When incubated with sage EO, a significant decrease in hepatocyte glucose production was observed in both drinking groups (fig. 1A). In co-incubations with glucagon, sage EO significantly decreased the glucose production response to glucagon (fig. 1A). Fig. 1

Metformin (a known inhibitor of gluconeogenesis) decreased significantly the hepatocyte glucose production capacity, even when co-incubated with glucagon, both in cells from water and tea drinking animals (fig. 1B).

Experiment 3

Hepatocyte glucose consumption, measured after 24 hours incubation was higher in 22 mM-glucose medium (fig. 2B) than in 11 mM-glucose medium (fig. 2A), and increased in response to insulin. Glucose consumption was significantly higher ($P \leq 0{\cdot}01$) in cells isolated from tea dinking rats under all tested circumstances. Fig. 2

When cells were incubated with sage EO, no significant differences were obtained in hepatocyte glucose consumption, although consumption was higher in cells isolated from the sage tea drinking animals. In co-incubations with insulin, sage EO significantly potentiates the hormone's effects on glucose consumption (figs. 2A and 2B).

Experiment 4

In contrast with what was the case for cells from healthy animals, when hepatocytes from STZ-induced diabetic rats were plated with 22 mM-glucose (and 10 mM-lactate) containing medium, glucose production was observed (and not comsumption). Insulin stimulation of glucose consumption did not occur (fig. 3). Sage tea drinking did not modify this situation. Also EO did not inhibit hepatocyte glucose production. Only metformin was able of reducing glucose production of hepatocytes isolated from diabetic water and sage tea drinking rats (fig. 3). Fig. 3

When hepatocytes from STZ-induced diabetic rats were plated with 5·6 mM-glucose (and 10 mM-lactate) containing medium glucose production was similar in cells isolated from both water and sage tea drinking rats (fig. 4). Glucagon was not able to further stimulate glucose production (fig. 4). As above, no effect was observed for EO. Once again, metformin significantly reduced hepatocyte glucose production in cells from both water and sage tea drinking rats by about 60% (fig. 4). Fig. 4

All the treatments in the primary cultures did not induce LDH release to the medium, an indicator that there was not cell toxicity in any of *in vitro* treatments.

Discussion

The present work shows that sage tea drinking significantly reduces fasting plasma glucose level in mice. This suggested an inhibition of gluconeogenesis and/or glycogenolysis in the liver. In agreement with this rat overall hepatocyte glucose production was lower in cells isolated from sage tea drinking animals when compared to controls. Furthermore, stimulation with glucagon did not increase gluconeogenesis significantly in cells from sage tea drinking animals. Sage EO, although not as effective

293 as metformin, produced a significant decrease in hepatocyte gluconeogenesis. In
294 addition, hepatocyte reponse to insulin was significantly increased by sage EO. This
295 data suggests a metformin-like effect for sage tea and in particular for the EO fraction of
296 *Salvia officinalis*. These effects were, however, not observed in hepatocytes isolated
297 from STZ diabetic animals where only metformin, a drug used in the treatment and
298 prevention of type 2 diabetes, was effective in reducing glucose production. The effects
299 of metformin were not modified by sage tea drinking which seems to imply that sage tea,
300 although not effective in diabetics, would not interfere negatively with metformin
301 therapy.

302 Although using a different extract and experimental methodology,
303 hypoglycaemic effects of sage have previously been reported by others (Alarcon-
304 Aguilar et al, 2002; Eidi et al., 2005). Alarcon-Aguilar and collaborators (2002)
305 showed that, 4 hours after an ip injection of a sage water ethanolic extract, blood
306 glucose decreased significantly in fasted normal mice and in fasted mildly alloxan-
307 diabetic mice but not in fasted severely alloxan-diabetic mice. Although the authors
308 stated that insulin may have mediated the hypoglycaemic effect of the extract, once the
309 animals were tested in the fasted condition, it seems likely that an inhibition of
310 gluconeogenesis was the cause of the observed effects in their study as indeed suggested
311 by our results. Additionally, Eidi and co-workers (2005) showed that, 3 hours after an ip
312 injection of a sage methanolic extract, blood glucose decreased significantly in fasted
313 STZ-diabetic rats but not in fasted normal rats. This effect was not accompanied by an
314 increased release of insulin (Eidi et al., 2005).

315 In humans, the abnormal glucose metabolism observed both in pre-diabetic
316 states as well as in overt type 2 diabetes results in part from a deregulation of glucose
317 production by the liver, which is caused mainly by unrestrained glucagon stimulation of

gluconeogenesis. Therefore, in these individuals gluconeogenesis is active even when plasma glucose concentrations already are elevated which further aggravates hyperglycaemia (Roden & Bernroider, 2003). By analogy with the effects of the drug metformin, used in the prevention and treatment of diabetes, the observed decrease in hepatocyte glucose production of sage tea drinking animals could be favourable by preventing the liver's contribution to hyperglycaemia in groups at risk. Metformin is a derivative of guanidine, the active compound of goat's rue (*Galega officinalis*) (Williams & Pickup, 2004). It acts by reducing liver glucose production and by increasing insulin action (Chiasson et al., 2002). Also in ours experiments, metformin showed these effects both in hepatocytes isolated from normal and STZ diabetic rats.

Generally, sage tea drinking increased rat hepatocyte glucose consumption, decreased fasting gluconeogenesis and inhibited glucagon's stimulation of hepatic glucose production. However, in spite of decreasing plasma glucose, after an *in vivo* ipGTT in mice, sage tea did not improve glucose clearance which suggests sage tea did not increase insulin response *in vivo*. Also *in vitro* insulin's stimulatory effects on glucose consumption were observed only in co-incubations with EO. This suggests a role for EO in the increase of insulin's sensitivity. Increased insulin sensitivity has been suggested as a possible mechanism of action of other plant extracts with attributed hypoglycaemic activities (Li et al., 2004; Saxena & Vikram, 2004; Qin et al., 2004; Han et al., 2005). The presence of low amounts of EO in sage tea, about 4·8 µg/ml (Lima et al., 2004), could explain the lack of an increase in insulin's sensitivity observed after tea drinking both *in vivo* and *in vitro*. It cannot be excluded that higher doses of the tested compounds administered either by gavage or ip injection could produce similar effects as *in vitro*.

342 Also in STZ-induced diabetic rat hepatocytes, sage tea drinking and EO *in vitro*
343 showed no significant improvement on responses to insulin. Normally, the liver (and
344 hepatocytes) should suppress glucose release in response to insulin (Klover & Mooney,
345 2004). In STZ rat hepatocytes insulin administration failed to suppress glucose
346 production. Previous studies have also indicated that insulin was incapable of
347 stimulating glucose utilisation *in vitro* by hepatocytes from STZ-induced diabetic rats
348 (Salhanick et al., 1983; Amatruda et al., 1984; Hussin & Skett, 1988). The insulin
349 resistance imposed by STZ treatment was not reverted by sage tea and/or EO. The lack
350 of effect of sage tea/EO on STZ hepatocytes seems to indicate that sage requires an
351 intact insulin signalling pathway to produce its effects. In STZ diabetic rat hepatocytes
352 stimulation with glucagon did not enhance gluconeogenesis. Others have also failed to
353 significantly stimulate the gluconeogenesis *in vitro* in hepatocytes from STZ-induced
354 diabetic rats (Dunbar et al., 1989). A possibility for the lack of glucagon stimulation of
355 gluconeogenesis in diabetic hepatocyte is that gluconeogenesis, *in vivo*, had been
356 maximally stimulated. Cells are, however, metabolically competent and respond to
357 metformin with a decrease of glucose production. In STZ treated rats, insulin deficiency
358 increases gluconeogenesis through enhanced lactate and pyruvate uptake and flux
359 through the enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Large & Beylot,
360 1999). Metformin has been shown to reduce substrate flux through PEPCK (Large &
361 Beylot, 1999) and to inhibit PEPCK gene expression (Cheng et al., 2001; Yuan et al.,
362 2002), thereby decreasing gluconeogenesis. This gene expression inhibition seems to be
363 mainly through an insulin-independent pathway (Yuan et al., 2002). This agrees with
364 the possibility that sage tea and/or sage EO requires an intact insulin signaling pathway
365 to produce its effects that were observed only in normal rats.

The reduction in fasting plasma glucose shown in healthy animals indicates a possible type 2 diabetes preventive potential of sage extracts through a metformin-like effect, mainly in people at risk of developing it as is the case of those who present IGT and IFG. Taking into consideration the high worldwide and increasing prevalence of type 2 diabetes and the high costs involved in its treatment, the primary prevention of this disease arises as an important issue (Lai, 2002; Costacou & Mayer-Davis, 2003; Jermendy, 2005). Considering that there is now substantial evidence that type 2 diabetes could be considered as a preventable disease through changes in lifestyle that include, among others, dietary factors (Costacou & Mayer-Davis, 2003; Schernthaner, 2003; Simpson et al., 2003; Stoeckli & Keller, 2004; Jermendy, 2005), the search for preventive strategies should be actively promoted. Sage products can easily be considered functional foods or food supplements that could have a beneficial impact in low cost prevention strategies of diabetes. In addition there are health benefits from the use of plant extracts as sources of antioxidants. In particular on the liver, sage tea has been shown not to have toxic effects and to improve liver glutathione levels (Lima et al., 2005). Among other effects, this may indirectly improve the liver mediated insulin response *in vivo* (Guarino et al., 2003). Currently, we are undertaking a pilot study with human volunteers to test the effects on the control of glycaemia in humans at risk of developing diabetes. Experiments with animal models of type 2 diabetes, such as the Zucker rat (Sreenan et al., 1996), could also provide additional information on the therapeutical effects of sage tea.

Acknowledgements

CFL and MFA were supported by the Foundation for Science and Technology, Portugal, grants SFRH/BD/6942/2001 and SFRH/BD/12527/2003, respectively. This work was

391 supported by the Foundation for Science and Technology, Portugal, research grant
392 POCTI/AGR/62040/2004.

References

Alarcon-Aguilar FJ, Roman-Ramos R, Flores-Saenz JL & Aguirre-Garcia F (2002) Investigation on the hypoglycaemic effects of extracts of four Mexican medicinal plants in normal and alloxan-diabetic mice. *Phytother Res* **16**, 383-386.

Amatruda JM, Salhanick AI & Chang CL (1984) Hepatic insulin resistance in non-insulin-dependent diabetes mellitus and the effects of a sulfonylurea in potentiating insulin action. *Diabetes Care* **7(Suppl 1)**, 47-53.

Baricevic D & Bartol T (2000) The biological/pharmacological activity of the *Salvia* genus. In *SAGE - The Genus Salvia*, pp. 143-184 [SE Kintzios, editor]. Amsterdam: Harwood Academic Publishers.

Cheng JT, Liu IM, Chi TC, Su HC & Chang CG (2001) Metformin-like effects of Quei Fu Di Huang Wan, a Chinese herbal mixture, on streptozotocin-induced diabetic rat. *Horm Metab Res* **33**, 727-732.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A & Laakso M (2002) Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* **359**, 2072-2077.

Costacou T & Mayer-Davis EJ (2003) Nutrition and prevention of type 2 diabetes. *Annu Rev Nutr* **23**, 147-170.

Day C (1998) Traditional plant treatments for diabetes mellitus: pharmaceutical foods. *Br J Nutr* **80**, 5-6.

Dunbar JC, Schultz S, Houser F & Walker J (1989) Regulation of the hepatic response to glucagon - role of insulin, growth-hormone and cortisol. *Horm Res* **31**, 244-249.

Eidi M, Eidi A & Zamanizadeh H (2005) Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *J Ethnopharmacol* **100**, 310-313.

Giannouli AL & Kintzios SE (2000) Essential oils of Salvia spp: examples of intraspecific and seasonal variation. In *SAGE - The Genus Salvia*, pp. 69-80 [SE Kintzios, editor]. Amsterdam: Harwood Academic Publishers.

Guarino MP, Afonso RA, Raimundo N, Raposo JF & Macedo MP (2003) Hepatic glutathione and nitric oxide are critical for hepatic insulin-sensitizing substance action. *Am J Physiol Gastrointest Liver Physiol* **284**, G588-G594.

Han YM, Oh H, Na M, Kim BS, Oh WK, Kim BY, Jeong DG, Ryu SE, Sok DE & Ahn JS (2005) PTP1B inhibitory effect of abietane diterpenes isolated from *Salvia miltiorrhiza*. *Biol Pharm Bull* **28**, 1795-1797.

Hussin AH & Skett P (1988) Lack of effect of insulin in hepatocytes isolated from streptozotocin-diabetic male-rats. *Biochem Pharmacol* **37**, 1683-1686.

Jermendy G (2005) Can type 2 diabetes mellitus be considered preventable? *Diabetes Res Clin Pract* **68 Suppl 1**, S73-S81.

Klover PJ & Mooney RA (2004) Hepatocytes: critical for glucose homeostasis. *Int J Biochem Cell Biol* **36**, 753-758.

Lai LC (2002) Prevention of type 2 diabetes. *Malays J Pathol* **24**, 71-76.

Large V & Beylot M (1999) Modifications of citric acid cycle activity and gluconeogenesis in streptozotocin-induced diabetes and effects of metformin. *Diabetes* **48**, 1251-1257.

Li WL, Zheng HC, Bukuru J & De Kimpe N (2004) Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol* **92**, 1-21.

Lima CF, Andrade PB, Seabra RM, Fernandes-Ferreira M & Pereira-Wilson C (2005) The drinking of a *Salvia officinalis* infusion improves liver antioxidant status in mice and rats. *J Ethnopharmacol* **97**, 383-389.

Lima CF, Carvalho F, Fernandes E, Bastos ML, Santos-Gomes PC, Fernandes-Ferreira M & Pereira-Wilson C (2004) Evaluation of toxic/protective effects of the essential oil of *Salvia officinalis* on freshly isolated rat hepatocytes. *Toxicol In Vitro* **18**, 457-465.

Moldeus P, Hogberg J & Orrenius S (1978) Isolation and use of liver cells. *Methods Enzymol* **52**, 60-71.

Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y & Sato Y (2004) Gosha-jinki-gan (a herbal complex) corrects abnormal insulin signaling. *Evid Based Complement Alternat Med* **1**, 269-276.

Roden M & Bernroider E (2003) Hepatic glucose metabolism in humans - its role in health and disease. *Best Pract Res Clin Endocrinol Metab* **17**, 365-383.

Salhanick AI, Konowitz P & Amatruda JM (1983) Potentiation of insulin action by a sulfonylurea in primary cultures of hepatocytes from normal and diabetic rats. *Diabetes* **32**, 206-212.

Saxena A & Vikram NK (2004) Role of selected Indian plants in management of type 2 diabetes: a review. *J Altern Complement Med* **10**, 369-378.

Schernthaner G (2003) Progress in the prevention of type 2 diabetes. *Wien Klin Wochenschr* **115**, 745-757.

Simpson RW, Shaw JE & Zimmet PZ (2003) The prevention of type 2 diabetes - lifestyle change or pharmacotherapy? A challenge for the 21st century. *Diabetes Res Clin Pract* **59**, 165-180.

Sreenan S, Sturis J, Pugh W, Burant CF & Polonsky KS (1996) Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin and troglitazone. *Am J Physiol* **271**, E742-E747.

Stoeckli R & Keller U (2004) Nutritional fats and the risk of type 2 diabetes and cancer. *Physiol Behav* **83**, 611-615.

Williams G & Pickup JC (2004) *Handbook of Diabetes*, 3rd ed. Malden: Blakwell Publishing.

Yeh GY, Eisenberg DM, Kaptchuk TJ & Phillips RS (2003) Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* **26**, 1277-1294.

Yuan L, Ziegler R & Hamann A (2002) Inhibition of phosphoenolpyruvate carboxykinase gene expression by metformin in cultured hepatocytes. *Chin Med J (Engl)* **115**, 1843-1848.

Abbreviations:

DMEM - Dulbecco's Modified Eagle's medium

EGP - endogenous glucose production

EO - essential oil

FBS - fetal bovine serum

IFG – impaired fasting glucose

IGT – impaired glucose tolerance

ipGTT - intraperitoneal glucose tolerance test

PEPCK - phosphoenolpyruvate carboxykinase

STZ - streptozotocin

WME - William's medium E

Results (tables)

Table 1 – Plasma glucose concentration in mice in response to an ipGTT (45 minutes) performed on 3 hours fasted mice previously treated with or without sage tea for 14 days. Values are means ± SEM, n=5. * $P \leq 0{\cdot}05$ and ** $P \leq 0{\cdot}01$ when compared with the water + ip vehicle group. ††† $P \leq 0{\cdot}001$ when compared with the sage + ip vehicle group.

Group	*In vivo* beverage	Plasma glucose (mM)	
		Mean	SEM
ip vehicle	Water	8·8	0·6
	Sage tea	6·8 **	0·4
ipGTT	Water	10·5 *	0·3
	Sage tea	10·4 †††	0·4

Results (figures)

Figure 1 – Hepatocyte glucose production (24 hours) by primary cultures of rat hepatocytes (isolated from overnight fasted animals). Effects of previous *in vivo* treatment with sage tea (for 14 days) on hepatocyte responses to glucagon (10^{-7} M), EO (4 nl/ml) and metformin (10^{-3} M). Initial medium glucose concentration was 5.6 mM and experiments performed on 24 hours (A) or 3 hours (B) after plating. Values are means ± SEM, n=4, (A - water drinking rats: 100% = 6·4 ± 0·6 μmol glucose/mg prot., sage tea drinking rats: 100% = 6·2 ± 0·6 μmol glucose/mg prot.; B - water drinking rats: 100% = 8·2 ± 1·2 μmol glucose/mg prot., sage tea drinking rats: 100% = 9·5 ± 0·8 μmol glucose/mg prot.). * $P \leq 0{\cdot}05$ and ** $P \leq 0{\cdot}01$ when compared with the respective control group. † $P \leq 0{\cdot}05$ and †† $P \leq 0{\cdot}01$ when compared with the respective glucagon group. ‡ $P \leq 0{\cdot}05$, ‡‡ $P \leq 0{\cdot}01$ and ‡‡‡ $P \leq 0{\cdot}001$ when compared with the respective EO group (A) or metformina group (B). § $P \leq 0{\cdot}05$ between water and sage tea groups.

Figure 2 – Glucose consumption (24 hours) by rat hepatocytes in primary cultures. Effects of previous *in vivo* treatment with sage tea (for 14 days) on hepatocyte responses to insulin (10^{-7} M) and EO (4 nl/ml). Initial medium glucose concentrations were 11 mM (A) and 22 mM (B). Values are means ± SEM, n=4. * $P \leq 0{\cdot}05$ and ** $P \leq 0{\cdot}01$ when compared with the respective control group. † $P \leq 0{\cdot}05$ and †† $P \leq 0{\cdot}01$ when compared with the respective insulin group. ‡ $P \leq 0{\cdot}05$ and ‡‡ $P \leq 0{\cdot}01$ when compared with the respective EO group. § $P \leq 0{\cdot}05$ between water and sage tea groups. ‖ Almost significant ($P = 0{\cdot}0573$) when compared with the respective control group.

Figure 3 – Hepatocyte glucose production (24 hours) by primary cultures of rat hepatocytes (isolated from streptozotocin-induced diabetic animals). Effects of previous *in vivo* treatment with sage tea (for 14 days) on hepatocyte responses to insulin (10^{-7} M), EO (4 nl/ml) and metformin (10^{-3} M). Initial medium glucose concentrations were 22 mM and experiments performed on 3 hours after plating. Values are means ± SEM, n=4, (water drinking rats: 100% = 8·4 ± 1·7 μmol glucose/mg prot., sage tea drinking rats: 100% = 8·7 ± 0·9 μmol glucose/mg prot). * $P \leq 0{\cdot}05$ when compared with the respective control group. † $P \leq 0{\cdot}05$ when compared with the respective insulin group.

Figure 4 – Hepatocyte glucose production (24 hours) by primary cultures of rat hepatocytes (isolated from streptozotocin-induced diabetic animals). Effects of previous *in vivo* treatment with sage tea (for 14 days) on hepatocyte responses to glucagon (10^{-7} M), EO (4 nl/ml) and metformin (10^{-3} M). Initial medium glucose concentrations were 5.6 mM and experiments performed on 3 hours after plating. Values are means ± SEM, n=4, (water drinking rats: 100% = 7·9 ± 1·0 μmol glucose/mg prot., sage tea drinking rats: 100% = 7·6 ± 0·3 μmol glucose/mg prot). ** $P \leq 0{\cdot}01$ and *** $P \leq 0{\cdot}001$ when compared with the respective control group. † $P \leq 0{\cdot}05$ and †† $P \leq 0{\cdot}01$ when compared with the respective glucagon group.

Figure 1 (Lima *et al*.)

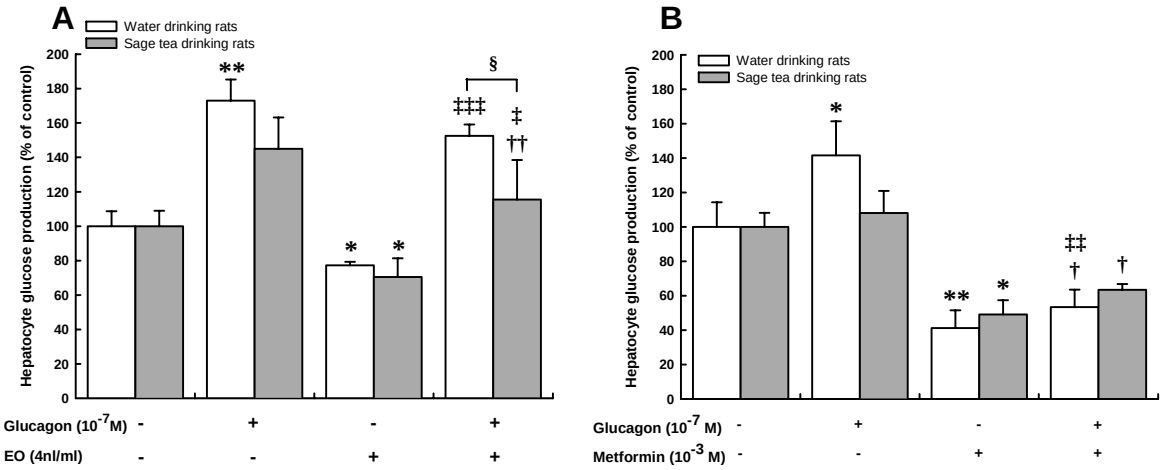

Figure 2 (Lima *et al*.)

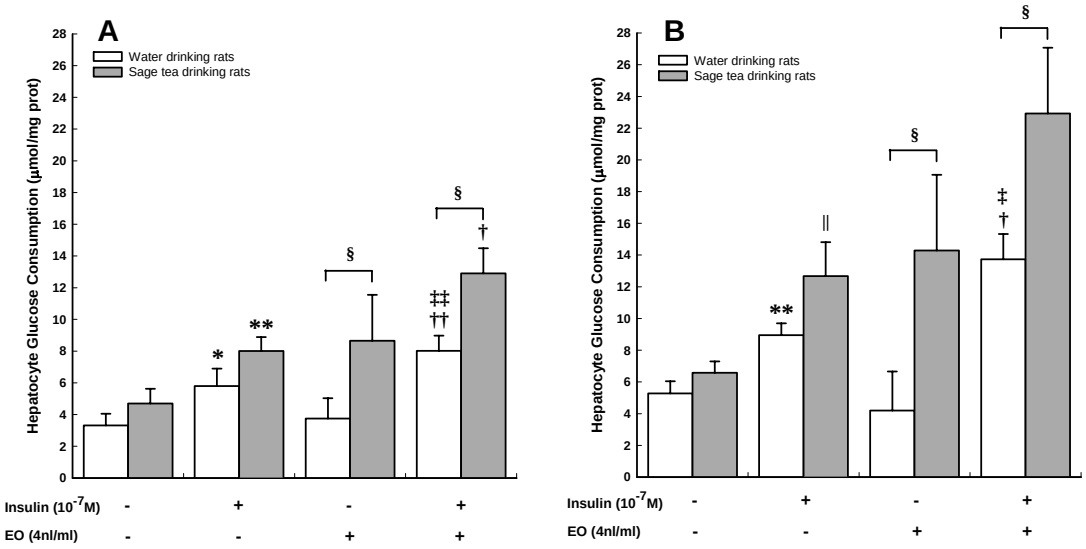

Figure 3 (Lima *et al*.)

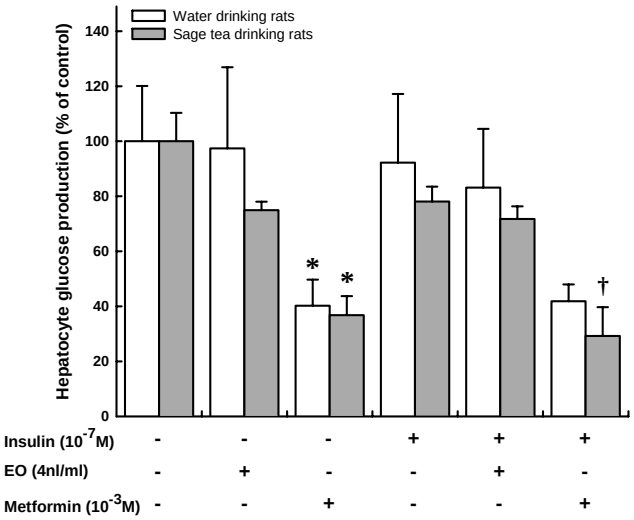

Figure 4 (Lima *et al*.)