



# Effectiveness and safety of *Stevia rebaudiana* dried leaves as an adjuvant in the short-term treatment of type 2 diabetes: A randomized, controlled, cross-over and double-blinded trial

Ángela Pallarés<sup>1\*</sup>, Genís Carrasco<sup>2</sup>, Youssef Nava<sup>2</sup>, Oriol Pallarés<sup>2</sup>, Isabel Pérez<sup>1</sup>, Rosa Rifá<sup>1</sup>, Miriam Rodríguez<sup>1</sup> and *Group of Research on Lifestyles and Health*<sup>1</sup>

<sup>1</sup>Faculty of Health Sciences, Blanquerna Foundation, Ramon Llull University, 326-332 Padilla Street, 08025 Barcelona, Spain.

<sup>2</sup>Radjem Health Center, Marché des Chameux sn, 70000 Laâyoune, Morocco.

## Article History

Received 14 April, 2015  
Received in revised form 31 May, 2015  
Accepted 10 June, 2015

## Keywords:

*Stevia rebaudiana*,  
Stevioside,  
Rebaudioside A,  
Type 2 diabetes  
mellitus,  
Hypertension.

## Article Type:

Full Length Research Article

## ABSTRACT

Recent randomized controlled trials have demonstrated the utility of stevioside and related compounds, extracted and refined from stevia leaf plant, to improve blood glucose and blood pressure in patients with type 2 diabetes. However, up till date, no well-designed trials have shown that the dried leaves of the stevia plant have similar effects in medical use. A randomized, controlled, cross-over and double-blinded study was conducted to assess the effectiveness and safety of *Stevia rebaudiana* leaves to reduce blood glucose and mean arterial pressure in Sahrawi patients with type 2 diabetes. One hundred and fourteen patients (80 women and 34 men) participated in a cross-over study consisting of an intake of tea sweetened with dried leaves of *S. rebaudiana* (study group) or refined sugar (control group), after a wash out period of two days. Capillary blood glucose, hearth rate and blood pressure were then determined at minutes 0 (baseline), 30, 60, 120 and 240 after the ingestion. In all patients, blood glucose reduction of -35.8% (95%CI -27.0 to -44.6%) were observed in the *S. rebaudiana* group compared to an increase in the control group (+8.1%, 95%CI 3.1 to 13.2% [p<0.0001]). Only in patients with previous hypertension (n=58 [51.3%]), mean arterial pressure increased after control intake (+8.5%, 95%CI 3.8 to 13.6% [p<0.02]) while it decreased (-11.5%, 95%CI -6.0 to -16.1%; [p <0.01]) in the study group. Major hypoglycemia, arterial hypotension, or other adverse effects were not observed. According to study conditions, dried leaves of *S. rebaudiana*, in short-term use, reduced significantly postprandial blood glucose in all patients, and it decreased mean blood pressure in hypertensive patients without detectable adverse effects throughout 24 h after ingestion.

©2015 BluePen Journals Ltd. All rights reserved

## INTRODUCTION

*Stevia rebaudiana* dried leaves and steviol glycosides (stevioside, rebaudioside A) are currently used satisfactorily both as sweetener as food additives in several countries, such as Japan, China, Taiwan, South Korea, Malaysia and most of South American countries.

However, in Western countries continues the long controversy about their safety and the need to restrict it in human nutrition. This controversy began in 1980 when the Joint Expert Committee on Food Additives (JECFA), and later the United States Food and Drug Administration

(FDA) stated that the use of high purity steviol glycosides ( $\geq 95\%$ ) is safe for human consumption as a non-medical ingredient, with an acceptable daily intake (ADI) of up to 4 mg/kg of body weight/day (EFSA Panel 2010). In 2011, the European Commission authorized the use of high purity steviol glycosides ( $\geq 95\%$ ) in foods and beverages across the European Union but left to criterion of each nation to authorize the use of the plant leaves as a food additive. As a result, use of plant leaves is authorized in Germany while it is prohibited in other countries of the European Union.

Despite this regulatory controversy, the widespread use of dried leaves and their derivatives in the food industry justifies the increasing publication of studies, in animal and human models, designed in order to establish their impact on human health, especially in the control of hyperglycemia and hypertension. Unfortunately, up till date, there is no conclusive scientific evidence supporting the clinical efficacy for medical use both of the steviol glycosides compounds and of the dried leaves.

In relation to the effect of stevioside on hyperglycemia in humans, Gregersen et al. (2004) observed that, in 12 people with type 2 diabetes volunteers, stevioside in large doses (1 gram added to meals) reduced postprandial glucose levels in the blood in 18% but, on the contrary, other authors found no effects in short (Geuns et al., 2007) or long-term use (Barriocanal et al., 2008).

Regarding the effect of stevioside on hypertension, Hsieh et al. (2003) founded, after one week of treatment, a blood pressure lowering using 500 mg three times a day for 2 years while that recent studies have observed also no effect in blood pressure (Jeppesen et al., 2006; Ferri et al., 2006).

Concerning the clinical efficacy of dried leaves, most published clinical studies suggest, also, a beneficial effect in the control of hyperglycemia and hypertension. Schmeling et al. (1977), found a positive impact of leaves on glycemic control in animal diabetes. Oviedo et al. (1979) studied the stevia tea effect in type 2 diabetics, and found out a 35% reduction in fasting glucose. Curi et al. (1986) showed that oral intake of *S. rebaudiana* leaves for 3 days slightly suppressed plasma glucose during an oral glucose tolerance test in healthy subjects. Unfortunately, these studies are aged, and due to its observational design have failed to demonstrate conclusively a potential positive effect on glucose metabolism and haemodynamics.

Taking into account the discrepancy in these results, Ulbricht et al. (2010), in a recent evidence-based systematic review, indicated that it is essential to perform

well-designed studies conducted to clarify the efficacy of the dried leaves and steviol glycosides in clinical daily practice.

On the other hand, in the case of the dried leaves, the relevance of this research field is especially important due to its low cost (up to eight times lower than acesulfame, aspartame, neotame, saccharin, sucralose and even purified stevioside). This fact highlights the importance of exploring whether the dried leaves could have similar beneficial effects on hypertension and hyperglycemia to which some authors attribute to purified stevioside.

If it were possible to confirm this promising hypothesis in well-designed randomized clinical trials, their practical implications in health care could be very important, particularly, for the developing countries because in these regions are available only limited financial resources for public health services, and diabetes is now one of the most common metabolic diseases, and their worldwide prevalence is increasing at such a rapid that the World Health Organization (WHO) has identified it as an epidemic condition (Friedman, 2002).

Actually, one of the regions where the prevalence of type 2 diabetes has increased more is the south of Morocco, especially among people of Sahrawi ethnicity. Rguibi and Belahsen (2004) observed that this disease affected 13% of this population, and was even more common among obese women with hypertension or a family history of diabetes.

The hypothesis of this study was that the dried leaves of *S. rebaudiana* used as tea sweetener are, in the short-term (24 h), clinically effective and safe in order to induce a favourable decrease on postprandial blood glucose and mean arterial pressure in Sahrawi patients with type 2 diabetes.

## MATERIALS AND METHODS

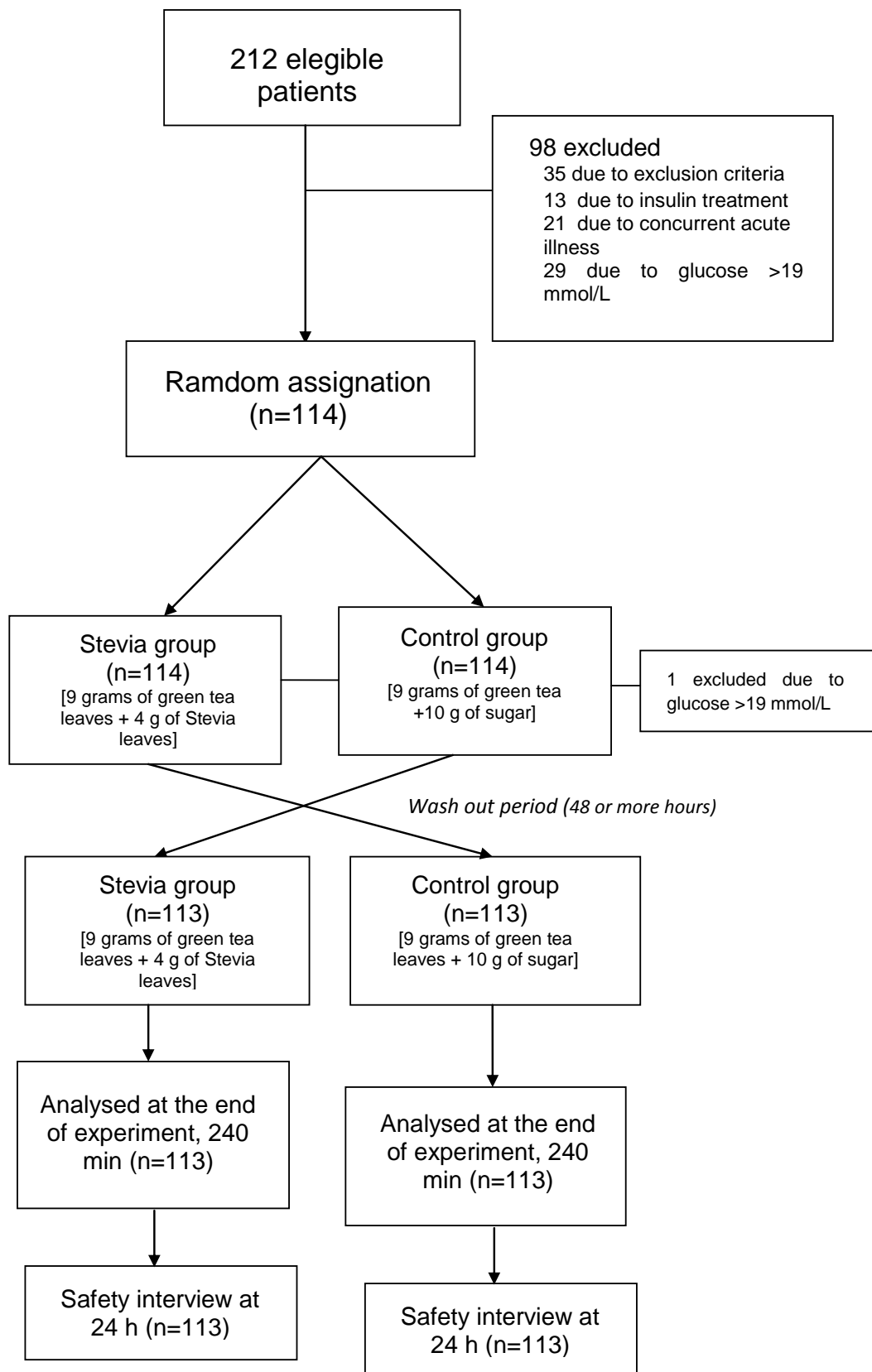
### Study design

This was a randomized, controlled, cross-over, and double-blind study conducted from January 5, 2013 to June 14, 2014 (Figure 1). Patients were recruited from the outpatient clinic at the Radjem Health Center (Laâyoune, Morocco).

### Ethics

There are two independent Ethics Committees which approved the protocol: Ethics Committee and Institutional Review Board of Radjem International in Barcelona (Spain) and Ethics Committee, and Institutional Review Board of Radjem Health Center in Laâyoune (Morocco). Informed consent was obtained from all individual

\*Corresponding author. E-mail: angelapallares@telefonica.net. Tel: + 932533127.



**Figure 1.** Cross over design of *Stevia rebaudiana* dried leaf versus sugar study.

participants included in the study. Participants provided informed consent orally (66%) or writing (34%). The study was conducted in accordance with the Declaration of Helsinki and Tokyo for humans.

After a general examination, the patients were included according to the following inclusion criteria:

- type 2 diabetes treated with diet and/or oral agents;
- development of diabetes in an age greater than 30 years;
- diabetes duration greater than 1 year; and
- body mass index between 25 and 42 kg/m<sup>2</sup>.

Exclusion criteria were:

- insulin therapy within the last 6 months;
- concomitant cardiovascular diseases, psychological disorders, neurological problems, kidney or other endocrine disease;
- drug abuse;
- any intercurrent acute illness;
- fasting glucose levels below 4 or above 19 mmol/L on the day of the experiment; and
- treatment with glucocorticoids.

The sweetened tea was served at 9 AM in random order on two non-consecutive days (cross-over) at least 2 days apart (wash-out period). The patients were instructed to fast for 10 h and to abstain from smoking. The subjects were sedentary during the experimental period of 24 h, before/after; respectively.

The patients treated with oral antidiabetic medication did not take these tablets prior to the experiments. Height and weight of the patients were measured. Capillary blood glucose in minutes 0 (baseline), 30, 60, 120 and 240 were analyzed, in relation to the intake. During the same points of the study the heart rate and blood pressure were measured using an aneroid sphygmomanometer Omron HEM-4030 (Global Osrom, Kyoto, Japan) recently calibrated with a measurement error of  $\pm 3$  mmHg, according to data provided by the manufacturer.

A computer including Yarrow algorithm generated a sequence of random numbers corresponding to treatment A (sugar) or B (stevia). Teapots were labelled according to the randomization. Both researchers and participants were unaware of treatment administered (A or B). Patients who received treatment were cited for the other treatment after 48 hours. The team that performed the statistical analysis ignored also the identity of the treatments until the end of the calculation.

The authors also want to emphasize that this is a short-term study (240 min with a safety interview at 24 h). The safety of doses above or longer term safety is outside the objectives of work.

### Sugar as control

The choice of sugar as control was a restriction of the Moroccan ethics committee (there are two ethics committee: Moroccan and Spanish) involving representatives of the volunteers. Members considered that highly sweetened tea with sugar is the national drink of Morocco and the cause of the dramatic increase of type 2 diabetes in this country (daily consumption of tea with large amounts of sugar in southern Morocco can exceed six liters per person and week).

According to its opinion, using sugar as a control would have two advantages. First, if the sweetening stevia was accepted by the volunteers, traditionally addicted to sugar, this would reduce sugar intake and contribute to epidemiological control of type 2 diabetes in this region. Second, the sugar would be possibly a double-blind design, given that the use of placebo would be detected by the participants and the use of artificial sweeteners was considered unsafe by the committee members.

### Quality of stevia batches; stevioside and rebaudioside content

All plants were cultivated according to the organic agriculture method (not involve employing of chemicals like fertilizers or pesticides). For the chemical analysis of different batches of *S. rebaudiana* plant, a sample of leaves was collected and dried under shade at room temperature. Dried leaves were packed in polyethylene bags and stored in deep freezer until used. Dried leaves were used without further manipulation. There was no extraction process.

The content of stevioside was determined using the method of Kolb et al. (2001) of high performance liquid chromatography (Laboratory for Agrobiotechnology, Public University of Navarra, Pamplona, Spain). Batches with compounds not approved by European regulations or with less than 6% of stevioside or less than 6% of rebaudioside were rejected.

### Quality of green tea

Green tea samples were analysed by a high-performance liquid chromatography-mass spectrometry according to the method of Del Rio et al. (2004). This method ensured that green tea used in this study was free from the commonly-used pesticides and other impurities.

### Quality of refined sugar

Sugar samples were analysed by a simple solvent extraction followed by selective analysis using a gas

chromatography–mass spectrometric according to method of Sinha et al. (2011). This method ensured that refined sugar used in this study was free from the commonly-used pesticides and other impurities.

### Ingestion

Each volunteer received two treatments blindly separated by an interval of 48 or more hours. They were given an infusion of 9 g of green tea leaves (*Camellia sinensis*, brand The Caravan, 6-111-175 certified by the Office National du Sucre et du The, Casablanca, Morocco), that joined 250 ml of boiling water for 2 min. In the study group, 4 g of *S. rebaudiana* dried leaves (Zhenhe Hebei Industrial Co. Limited, Shijiazhuang, China), was added and in the control group 10 g of refined sugar (99% sucrose) was added.

According to the European Food Safety Authority, in all study patients the daily intake for steviol glycosides, expressed as steviol equivalents, was lower than 4 mg/kg bodyweight.

The calculation of stevia tea in milliliters per patient was performed using the following formula:

$$\text{Stevia tea (ml/patient)} = \frac{\text{Concentration of stevioside in each stevia batch} \times 9 \text{ g}}{250 \text{ ml of drink}}$$

Patient's bodyweight in kilograms multiplied by 3.5 mg meant the exact dose. The amount in milliliters of the beverage to be administered to each patient was calculated based on these data.

The total energy content of the test beverage was 42 kcal in the study group and 76 kcal in the control group.

### Determination of blood glucose

Capillary glucose determinations were made with strips Glucocard™ G sensor (Menarini, Italy) and glucometers Glucocard™ G meter, GT-1810 (Menarini, Italy) with a calibrated measuring range of 0.5-33.5 mmol/L.

Four glucometers whose correlation measures between them were 0.98 was used. The accuracy of test strips showed a coefficient of variation of 3.8-5.2%, according to data provided by the manufacturer.

To confirm the experimental usefulness of this method, a blind, cross-over pilot study comparing two samples with 18 subjects each; was previously conducted. Capillary glycemia by strip reflectometry and venopuncture with Beckman analyzer with glucose oxidase-peroxidase was measured. The following results  $r=0.976$  ( $_{95\%} \text{ CI } 0.939-0.992$ ) was obtained in the pilot sample, which were considered by Review Board as sufficiently accurate for experimental purposes in

agreement with published studies (Carrera et al., 2000).

### Safety interview

All products used in the study (green tea, stevia, and sugar) were analyzed according to high-performance liquid chromatography method. The presence of pesticides or other toxic compounds were discarded. In addition, to ensure patient's safety in all they underwent after 24 h, a clinical examination and extraction of venous blood samples for analyses of biochemical, haematological, and toxicological parameters was carried out.

### Statistical analyses

The sample size was calculated by Altman diagram from previous studies that established as a clinically significant decrease in endpoints of over 10%. Assuming a loss of 30%, it was established that there was a need for this cross-over trial of more than 89 patients. The study power, in order to detect a 5% difference between treatments, was 95% confidence limit.

Student-t-test or Mann-Whitney test for continuous variables and Fisher exact test for categorical variables were used. Data are presented as mean  $\pm$  standard deviation values with a confidence interval of 95% ( $_{95\%} \text{ CI}$ ).  $P < 0.05$  was considered statistically significant.

## RESULTS

### Socio-demographic variables and health status

Two hundred and twelve patients were invited to participate in the study. Of these, 35(16.5%) did not accept their inclusion, 13(6.1%) had been treated with insulin, 21(9.9%) had a concurrent acute illness, and 29(13.5%) had a higher glucose 19 mmol/L on the day of the experiment.

One hundred and fourteen patients (80 women and 34 men) formed the study population. During the control phase, one patient had severe hyperglycemia and was also excluded. The final analysis included 113 cases. The socio-demographic and baseline health status of sample are presented in Table 1.

### Stevioside content

The average content of stevioside in *S. rebaudiana* leaves (batches approved;  $n=18$ ) was  $8.5 \pm 2.3$  g ( $_{95\%} \text{ CI } 7.2-9.3$  g) per 100 g of dry leaf with a range of different batches between 8.1 and 9.8 g. The rebaudioside content was  $8.2 \pm 1.6$  g ( $_{95\%} \text{ CI } 7.4-8.9$  g) per 100 g of dry leaf

**Table 1.** Socio-demographic and health status.

Age, years (range) [95%CI ]	52.3±11.6 (38.2-75.3) [95%CI 50.1-54.3]
<b>Genre</b>	<b>n(%; 95 %CI range %)</b>
Feminine	79(71.5; 63.2-79.7)
Masculine	34(28.5; 20.2-36.7)
<b>Education level</b>	<b>n(%; 95%CI range %)</b>
Illiterate	60(53.5; 44.3-62.6)
Primary studies	42(36.8; 27.9-45.6)
Secondary studies	9(7.8; 2.8-12.7)
University studies	2(1.9; 0.6-4.4)
Average annual income per capita, euros, X±DS (range) [95%CI]	422.3±205.6 (120.2-1.050.3) [95%CI 384.3-459.6]
<b>Daily walking minutes</b>	<b>X±DS[95%CI range %]</b>
Men	36.3±24.2[31.8-40.7]
Women	11.1±9.5[9.3-12.8]
BMI, X±DS kg/m <sup>2</sup> (range) [95%CI ]	33.5±8.2 (30-42) [33.9-35.0]
Time since diagnosis of diabetes, years, X±DS (range) [95%CI]	7.2±5.6 (1-12) [6.1-8.2]
<b>Treatment</b>	<b>n(%; 95%CI range %)</b>
Sulfonylureas	2(1.7; 0.1-4.0)
Metformin	9(8; 3.0-12.9)
Diet, exercise, oral antidiabetic medication	18(16; 9.2-22.7)
Diet and exercise	1(0.8; 0.8-2.8)
None	83(73.4; 65.4-81.6)
<b>Artificial sweetener consumption</b>	<b>n(%; 95%CI range %)</b>
Men	5(14.7; 8.2-21.2)
Women	6(7.5; 2.6-12.3)

n=113

**Table 2.** Blood glucose, hearth rate and blood pressure at baseline (n=113).

Variable	Control X±DS (95%CI range)	Stevia group* X±DS (95%CI range)	p
Capillary glycemia (mmol/L)	10.3±43.4 (9.5-11.0)	10.4±4.0 (9.2-10.7)	0.09
Heart rate (beats per min)	94.6±18.5 (72.3-110.4)	92.2±16.4 (71.6-108.4)	0.10
Systolic blood pressure (mmHg)	140.2±32.3 (134.2-146.1)	143.5±40.6 (136.0-150.9)	0.12
Diastolic blood pressure (mmHg)	85.8±3.9 (85.0-86.5)	87.8±4.0 (87.0-88.5)	0.10
Mean blood pressure (mmHg)	103.3±13.3 (100.6-105.4)	105.6±16.3 (102.0 -107.9)	0.09

\* Statistical significance level was p&lt;0.05.

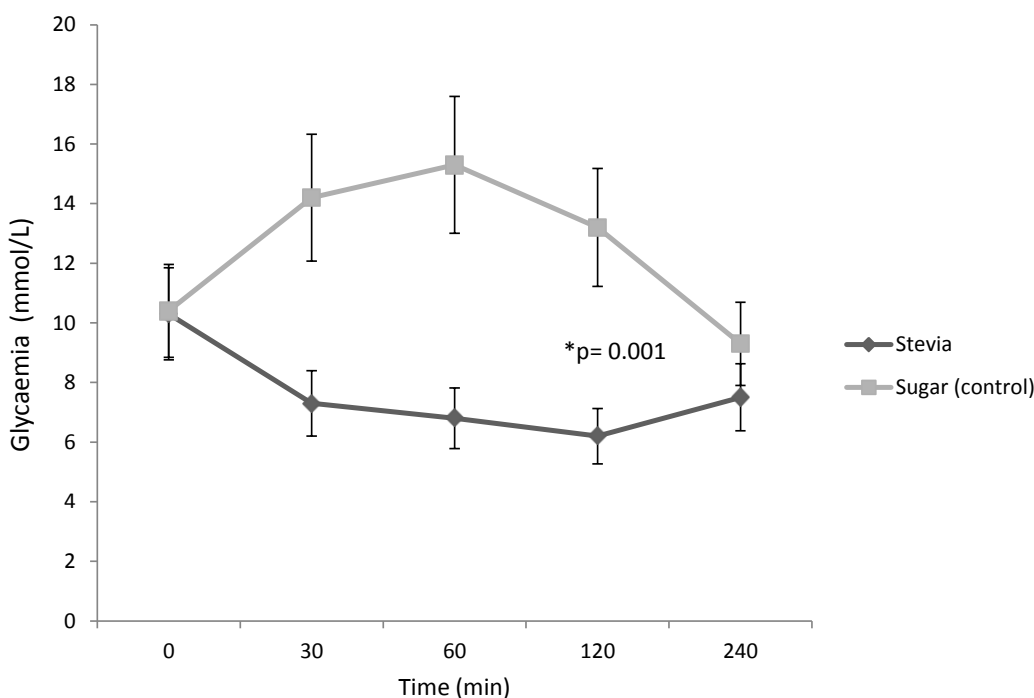
with a range of different batches between 8.3 and 9.6 g.

### Intake for steviol glycosides

Throughout the phase of treatment with dried leaves, patients received a mean of 305.2±102.3 g (95%CI 306.1-403.8) of steviol equivalents.

### Baseline variables

The baseline variables were similar in both groups. The 78.0% (95%CI 71.0-85.6%) of participants had elevated levels (more than 5.5 mmol/L) in basal glucose levels, and 51.3% (95%CI 42.0-60.6%) had systolic blood pressure (more than 140 mmHg) and/or diastolic blood pressure (90 mmHg or more) at the time of starting the



**Figure 2.** Effect of *Stevia rebaudiana* on capillary glucose in type 2 diabetics (n=113)\*. \*Statistical significance level was p<0.05.

**Table 3.** Comparison of blood glucose and blood pressure at 120 min (n=113).

Variable	Control X±DS (95%CI range)	Stevia group* X±DS (95%CI range)	p
Capillary glucose (mmol/L) in all patients (n=113)	11.2±3.6 (10.5-11.9)	6.7±1.8 (6.4-7.1)	
Systolic blood pressure (mmHg) in hypertensive patients (n=58)	152.2±41.3 (144.6-159.7)	121.5±41.1 (113.9-129.0)	0.003
Diastolic blood pressure (mmHg) in hypertensive patients (n=58)	92.5±5.8 (91.4-93.5)	77.8±5.2 (76.8-78.7)	0.002
Mean blood pressure (mmHg) in hypertensive patients (n=58)	112.3±18.1 (107.3-116.6)	91.6±17.2 (86.6-95.38)	0.001

\* Statistical significance level was p <0.05.

experiment. Table 2 illustrates these results.

**Effects on blood glucose**

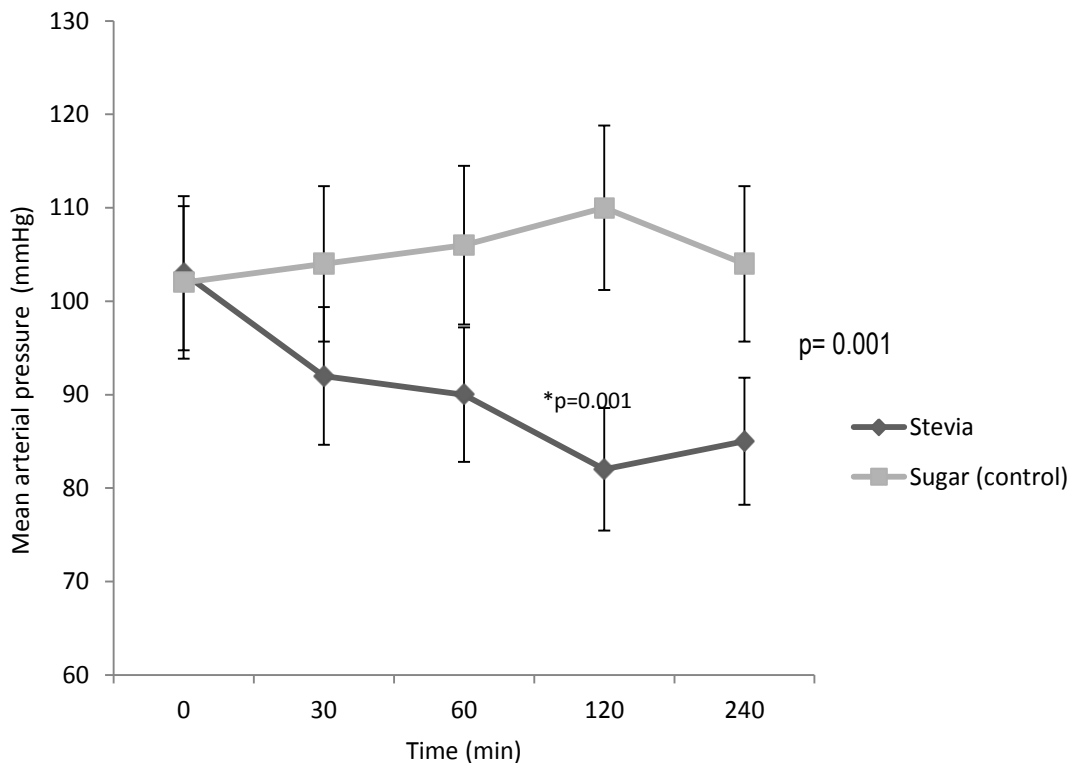
At 120 min after consumption, we observed a significant decrease in capillary blood glucose (-35.8%, 95%CI -27.0-44.6%) in the *S. rebaudiana* group and an increase also significantly in the control group (+8.1%, 95%CI 3.1-13.2% [p <0.0001]) as viewed in Table 3.

Concerning the evolution of this variable, as has been mentioned and it is shown in Figure 2, the greatest effect

on capillary glucose of *S. rebaudiana* leaves was observed at 120 min after administration and persisted during 4 h of monitoring.

**Effects on hemodynamic variables**

We detected a significant rise in systolic and/or diastolic blood pressures (+8.5%, 95%CI 3.8-13.6% [p <0.02]) in control group compared with a marked decrease (-11.5%, 95%CI 6.0 to 16.1% [p <0.01]) in study group but only in patients previously diagnosed hypertension (n=51) or



**Figure 3.** Effect of *Stevia rebaudiana* on mean arterial pressure in type 2 diabetics with hypertension (n=58)\*. \*Statistical significance level was  $p < 0.05$ .

who were diagnosed at the clinical examination before the study (n=7).

In contrast, in patients with blood pressures within normal range, successive controls were not significantly different from baseline measurements. Regarding the evolution of mean arterial pressure, Figure 3 shows how the intake of sweetened tea in the hypertensive patients of control group caused an increase on mean blood pressure throughout 240 min whereas the maximum decrease in the study group was observed at 120 min. Heart rate did not change either after the intake.

### Adverse effects

Relating to side effects, no hypoglycemia (capillary glucose less than 3.3 mmol/L) was detected during the experiments and one day after the experiments. Safety interview at 24 h recorded no adverse effects or complications attributable to the treatment (neither clinical nor analytical).

### DISCUSSION

This is, in our knowledge, the first study carried out

according to a randomized controlled clinical trial methodology. The main findings of the present study were that, dry leaf of plant led to significant improvements in short term control of hyperglycemia and hypertension in a population affected by type 2 diabetes and poor health status.

A critical point in the study design was to ensure the quality of stevia leaves in relation to their content of active compounds. Typically, qualitative analyses show that among the identified 88 compounds, the majority are mono and sesquiterpenes (50 types identified). Taking into consideration that some terpenes may be different according to the origin of plant, it is reasonable that *S. rebaudiana* grown in different areas may possess certain specific characteristics that can be ascribed to cultivation on a different plantation (Marković et al., 2008). It is believed that a high content of stevioside glycosides and related compounds could be the key to reproduce the study since the use of stevia leaf with concentration less than 6.0% of stevioside and 6.0% of rebaudioside could produce lower impact on metabolic and hemodynamic variables.

In relation to the results obtained, the socio-demographic and health status data of the participants revealed and reflected the precarious health situation of the sample studied.



As is well known, numerous studies have addressed animal model to elucidate the mechanism of stevioside for glucose reduction. Chen et al. (2005) demonstrated that stevioside was able to regulate blood glucose levels by enhancing not only insulin secretion, but also insulin utilization in insulin-deficient rats. However the exact mechanism of stevia leaves in humans remain in discussion.

Treatment with dry leaf of *S. rebaudiana* was associated with a decrease in postprandial blood glucose which reached an average of -35.8%. This finding is inconsistent with the trial of Gregersen et al. (2004) whom estimated it in a -18%. The reason for the discrepancy may be due to their different experimental design. Gregersen et al. evaluated the efficacy of high doses of purified stevioside added to the meal (one gram of stevioside encapsulated in gelatin) while we used four times less of dried leaves. Since in the Gregersen et al. study the stevioside was administered orally as 91% pure stevioside while we did it as dried leaves, the possibility that other substances in the leaves may be responsible for the observed effects cannot be excluded.

Corroborating previous animal studies involving intravenous stevioside (Chan et al., 1998), our results show an acute decrease in blood pressure by -11.5% only in hypertensive patients. Contrarily, all previous studies, involving oral use of stevioside in humans have demonstrated that the stevia refined compounds only affect the blood pressure after more than one week of treatment (Hsieh et al., 2003; Ulbricht et al., 2010). On the other hand, in animal model, Liu et al. (2003) demonstrated that after nasogastric administration of stevioside powder (200 mg/kg), the blood pressure of anesthetized dogs began to significantly decrease at 60 min and returned to baseline level at 180 min. Stevioside compounds also showed significant hypotensive effects in renal hypertensive dogs, in a dose-dependent manner. In cultured rat aortic smooth muscle cells (A7r5 cell line), stevioside can dose-dependently inhibit the stimulatory effects of vasopressin and phenylephrine on intracellular  $Ca^{2+}$  in a calcium-containing medium. It was concluded that its hypotensive mechanism may be probably due to inhibition of the  $Ca^{2+}$  influx. The differences between the results in animal model using stevia powder and in humans using stevioside could be due to the synergistic effect of other compounds present in the dried plant and not in the purified stevioside. However, clinical significance of these differences remains uncertain.

We hypothesized that the presence of other active compounds in the leaves of stevia could determine synergistic effects as a more rapid absorption and effect (minutes) on vasorelaxation by inhibition of  $Ca^{2+}$  influx into the blood vessels.

However, a large multicenter trial in human using stevia leaves and including other metabolic parameters (blood insulin and glucagon) is required to elucidate these

findings.

### Strengths and limitations

The results of this study corroborate the findings of previous old observational studies (Schmeling et al., 1977; Oviedo et al., 1979; Curi et al., 1986). However, interpretation of the results and the assessment of their external validity and applicability requires three prior considerations about the limitations of our fieldwork.

The first one is the possible sampling bias that could limit the external validity of the experiment. Radjem Health Center annually provides assistance to 2,000 patients with type 2 diabetes of which 70% are obese women. In this study, 65% of the subjects fitted in that profile. Obviously, the patients differ greatly from those attended in Western countries.

The second limitation is related to the dietary habits of participants hardly comparable with those of the European settings. Since the stevia leaves and stevia compounds were approved in Morocco as a sweetener and dietary supplement, their consumption is increasing progressively. However, sugar is still used in large quantities for serving sweet tea. Green tea heavily sugared is the national drink consumed throughout the day (Benjelloun, 2002). Out of study, consumption of this beverage, reported by the participants, was over 6 liters a week and the sugar intake reached 590 grams during the same period. The choice of sweet tea as a test meal to perform our study was due to these Sahrawi cultural characteristics because this beverage is the most important component of traditional breakfast. We believe that this cultural factors typify the sample with too specific characteristics that will require further studies to confirm these results in other ethnic groups and different cultural settings.

Thirdly, due to the inability to assess the impact that *S. rebaudiana* intake might have in reducing the cost of drug treatment for type 2 diabetes. This approach is very interesting and would have improved the cost-benefit analysis of *S. rebaudiana* but had to be excluded from the aims of the study.

It is necessary to explain the reason for blood pressure increase in the control group. Bogdanski et al. (2012) observed that after 3 months of green tea compounds, both systolic and diastolic blood pressures were significantly decreased in the tea group as compared with the placebo group. On the other hand, several studies suggested also that anxiety is an independently variable associated with an increased risk for high blood pressure (Paterniti et al., 1999). We believe that the experiment produced in the volunteers of this study, great anxiety and it caused an increase in blood pressure and heart rate. Stevia could correct these factors.

An interesting observation was the persistence of the

effect of *S. rebaudiana* leaves beyond 240 min, much longer duration than that reported by other works (Chan et al., 1998). Also, in routine follow-up of included patients, we observed that the effect is often maintained from breakfast to lunch. The reason for this observation was not known and deserves further specific studies with a rigorous methodology.

Taken together, the results obtained confirm the popular reputation of *S. rebaudiana* leaves to reduce glycemia and blood pressure, but the possibility of long-term toxic effects must be considered in further studies.

In view of the fact that *S. rebaudiana* leaves contain dipertene glycosides (stevioside, rebaudiosides A, B, C, D, steviobioside, etc) and a thousand of other compounds, including non-glycoside dipertenes, triterpenoids, flavonoids, coumarins, caffeic acid, chlorogenic acid and a variety of volatile oils (Darise et al., 1983), the identification of other hypoglycemic molecule and/or fraction and the demonstration of the absence of long-term toxicological potential will be necessary to open the possibility for widespread use (Ferreira et al., 2006).

## Conclusion

According to study conditions, the dry leaf of *S. rebaudiana* is a favorable alternative sweetener as well as a short-term (24 h) beneficial adjunctive treatment in Sahrawi patients with type 2 diabetes because it reduces postprandial blood glucose and blood pressure in hypertensive patients without hypoglycemia, arterial hypotension or other side effects.

However, before recommending its widespread use, future randomized and double-blinded studies, involving other populations and different socioeconomic and cultural contexts, designed to determinate the effectiveness and safety of *S. rebaudiana* leaves are required.

## CONFLICT OF INTEREST

The authors declare that there is no duality of interest associated with this article.

## ACKNOWLEDGEMENTS

This study was financed by the grant number 221011 of URV Solidària-SEGEU, a non-profit institution of the Rovira i Virgili University, Tarragona, Spain. The authors declare that there is no duality of interest associated with this article.

Registered at Pan African Clinical Trial Registry (PACTR201410000894447). The protocol complies with

all regulations of the European Food Safety Authority (Annex II to Regulation [EC] No 1333/2008 of the European Parliament and of the Council with regard to steviol glycosides).

The dried leaves of stevia are approved without legal restrictions for use as a food additive in Morocco (Harmonized System Code 1901.90.1010).

The authors wish to thank the Honorable Mayor of El Aaiún, Mr. Hamdi Ould Errachid his invaluable help in carrying out the project. We gratefully acknowledge the South Hispanophone Association in the figure of volunteer supervisor, Mrs. Mahjouba Bougharioun. We thank also Dr. Youssef Nava from the Alter Forum International (a nonprofit organization) for his assistance in the last phase of the project.

We also acknowledge the support of following organizations which have donated altruistically the consumables for fieldwork: Teixé Foundation, Dolça Revolució, and Menarini Diagnostic Laboratories.

## REFERENCES

- Barriocanal L. A., Palacios M., Benitez G., Benitez S., Jimenez J. T., Jimenez N. & Rojas V. (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. *Reg. Toxicol. Pharmacol.* 51:37-41.
- Benjelloun S. (2002). Nutrition transition in Morocco. *Public Health Nutr.* 5(1A):135-140.
- Bogdanski P., Suliburska J., Szulinska M., Stepień M., Pupek-Musialik D. & Jablecka A. (2012). Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res.* 32(6):421-427.
- Carrera T., Bonamusa L., Travé J. B., Almirall L. & Navarro J. M. (2000). Evaluación del analizador glucocard memory 2 para medir la concentración de glucosa en sangre capilar. *Aten Primaria.* 26(5):327-330.
- Chan P., Xu D. Y., Liu J. C., Chen Y. J., Tomlinson B., Huang W. P. & Cheng J. T. (1998). The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. *Life Sci.* 63:1679-1684.
- Chen T. H., Chen S. C., Chan P., Chu Y. L., Yang H. Y. & Cheng J. T. (2005). Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. *Planta med.* 71:108-113.
- Curi R., Alzarez M. & Bazotte R. B. (1986). Effect of *Stevia rebaudiana* on glucose tolerance in normal adult humans. *Braz. J. Biol. Res.* 19:771-774.
- Darise M., Kohda H., Mizutani K., Kasai R. & Tanaka O. (1983). Chemical constituents of flowers of *Stevia rebaudiana*. *Agric. Biol. Chem.* 47:133-135.
- Del Rio D., Stewart A. J., Mullen W., Burns J., Lean M. E., Brightenti F. & Crozier A. (2004). HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *J. Agric Food Chem.* 52(10):2807-2815.
- EFSA Panel on Food Additives and Nutrient Sources added to food (ANS). (2010). Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive. *EFSA J.* 8:1537-1621.
- Ferreira E. B., de Assis Rocha Neves F., da Costa M. A., do Prado W. A., de Araújo F., Ferri L. & Bazotte R. B. (2006). Comparative effects of *Stevia rebaudiana* leaves and stevioside on glycaemia and hepatic gluconeogenesis. *Planta Med.* 72:691-696.

- Ferri L. A., Alves-Do-Prado W., Yamada S. S., Gazola S., Batista M. R. & Bazotte R. B. (2006). Investigation of the antihypertensive effect of oral crude stevioside in patients with mild essential hypertension. *Phytother. Res.* 20:732-736.
- Friedman E. A. (2002). Diabetic nephropathy: Improving prognosis. *Saudi J. Kidney Dis. Transpl.* 13(3):281-310.
- Geuns J. M., Buyse J., Vankeirsbilck A. & Temme E. H. (2007). Metabolism of stevioside by healthy subjects. *Exp. Biol. Med.* 232:164-173.
- Gregersen S., Jeppesen P. B., Holst J. J. & Hermansen K. (2004). Antihyperglycemic effects of stevioside in Type 2 person with diabetes subjects. *Metabolism* 53:73-76.
- Hsieh M. H., Chan P., Sue Y. M., Liu J., Liang T. H., Huang T. Y., Tomlinson B., Chow M. S., Kao P. F. & Chen Y. J. (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo controlled study. *Clin. Ther.* 25:2797-2808.
- Jeppesen P. B., Barriocanal L., Meyer M. T., Palacios M., Cañete F., Benitez S., Logwin S., Schupmann Y., Benitez G. & Jimenez J. T. (2006). Efficacy and tolerability of oral stevioside in patients with type 2 diabetes: a long-term, randomized, double-blinded, placebo-controlled study. *Diabetol.* 49(Suppl.1), A0843.
- Kolb N., Herrera J. L., Ferreyra D. J. & Uliana R. F. (2001). Analysis of sweet diterpene glycosides from *Stevia rebaudiana*: Improved HPLC method. *J. Agric. Food Chem.* 49:4538-4541.
- Liu J. C., Kao P. K., Chan P., Hsu Y. H., Hou C. C., Lien G. S., Hsieh Y. J. & Cheng J. T. (2003). Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. *Pharmacology* 67:14-20.
- Marković I. S., Đarmati Z. A. & Abramović B. F. (2008). Chemical composition of leaf extracts of *Stevia rebaudiana* grown experimentally in Vojvodina. *J. Serbian Chem. Soc.* 73:283-297.
- Oviedo C. A., Franciani G. & Moreno R. (1979). Acción hipoglucemiante de la *Stevia rebaudiana* (Kaa-he-e). *Excerpt. Med.* 209:92-99.
- Paterniti S., Alperovitch A., Ducimetière P., Dealberto M. J., Lépine J. P. & Bisslerbe J. C. (1999). Anxiety but not depression is associated with elevated blood pressure in a community group of French elderly. *Psychosom Med.* 61(1):77-83.
- Rguibi M. & Belahsen R. (2004). Overweight and obesity among urban Sahraoui women of South Morocco. *Ethn Dis.* 14(4):542-547.
- Schmeling G. A., Carvalho F. V. & Espinosa A. D. (1977). *Stevia rebaudiana*. Avaliação do efeito hipoglicemiante em coelhos aloxanizados. *Ciencia Cultura* 29:599-601.
- Sinha S. N., Bhatnagar V. K., Doctor P., Toteja G. S., Agnihotri N. P. & Kalra R. L. (2011). A novel method for pesticide analysis in refined sugar samples using a gas chromatography–mass spectrometer (GC–MS/MS) and simple solvent extraction method. *Food Chem.* 126:379-386.
- Ulbricht C., Isaac R., Milkin T., Poole E. A., Rusie E., Grimes Serrano J. M., Weissner W., Windsor R. C. & Woods J. (2010). An evidence-based systematic review of stevia by the natural standard research collaboration. *Cardiovasc Hematol Agents Med Chem.* 8(2):113-127.