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Investigation of the Antihypertensive Effect of Oral Crude Stevioside in Patients with Mild Essential Hypertension

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The antihypertensive effect of crude stevioside obtained from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (Compositae) on previously untreated mild hypertensive patients was examined. Patients with essential hypertension were submitted to a placebo phase for 4 weeks. The volunteers selected in this phase were randomly assigned to receive either capsules containing placebo during 24 weeks or crude stevioside 3.75 mg/kg/day (7 weeks), 7.5 mg/kg/day (11 weeks) and 15.0 mg/kg/day (6 weeks). All capsules were prescribed twice a daily (b.i.d.), i.e. before lunch and before dinner. After the placebo phase and after each dose of crude stevioside, body mass index, electrocardiogram and laboratory tests were performed. During the investigation blood pressure (BP) was measured biweekly and the remaining data were collected at the end of each stevioside dose step. All adverse events were prospectively recorded but no major adverse clinical effects were observed during the trial. Systolic and diastolic BP decreased ($p < 0.05$) during the treatment with crude stevioside, but a similar effect was observed in the placebo group. Therefore, crude stevioside up to 15.0 mg/kg/day did not show an antihypertensive effect. Moreover, the results suggest that oral crude stevioside is safe and supports the well-established tolerability during long term use as a sweetener in Brazil. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: Stevia rebaudiana (Bertoni) Bertoni; stevioside; blood pressure; clinical trial; placebo effect.

INTRODUCTION

Stevia rebaudiana (Bertoni) Bertoni (Compositae) is a perennial native shrub from north-eastern Paraguay and southern Brazil (Geuns, 2002).

The leaves of *Stevia rebaudiana* (Bertoni) Bertoni have been used by Guarani Indians from Paraguay to treat diabetes and this empirical knowledge was passed by oral tradition for many centuries (Bazotte *et al.*, 1986). This empirical knowledge led to several investigations on the antidiabetic properties of this plant including previous studies which confirmed that treatment with *Stevia rebaudiana* (Bertoni) Bertoni leaves could reduce fasting glycaemia in rats (Ueda *et al*., 1983) and in humans (Curi *et al*., 1986).

In Brazil, the reports on *Stevia rebaudiana* (Bertoni) Bertoni started with Boech, who first demonstrated the hypotensive effect of crude stevioside in rats (Humbold and Boech, 1978). Since this study, several publications confirmed the antihypertensive properties of crude stevioside in rats (Melis, 1992a,b,c; Chan *et al*., 1998; Melis, 1999; Lee *et al*., 2001; Hsu *et al*., 2002) and in dogs (Liu *et al*., 2003). The antihypertensive effect of stevioside was attributed to an inhibition of extracellular calcium influx (Melis, 1992a; Lee *et al*., 2001).

Furthermore, two clinical trials investigated the effect of crude stevioside on systolic (SBP) and diastolic (DBP) blood pressure (Chan *et al*., 2000; Hsieh *et al*., 2003). In both reports, the toxicological profile was restricted to hematology and serum evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK) and electrolytes.

In the present investigation the antihypertensive potential of oral crude stevioside obtained from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni cultivated in northeastern Paraguay and southern Brazil was evaluated in a prospective, randomized, double-blind, placebo-controlled, single-center clinical trial. Moreover, the toxicological profile was expanded by assessing metabolic and hormonal parameters that were not evaluated by those studies (Chan *et al*., 2000; Hsieh *et al*., 2003).

METHODOLOGY

Standardized crude stevioside. *Stevia rebaudiana* (Bertoni) Bertoni leaves, identified by Dr Antonio Barioni Gusman, University of São Paulo, were

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collected on a farm in north-eastern of Paraná state (southern Brazil), prepared and stored for reference at the State University of Maringá Herbarium under the code 9131. Standardized crude stevioside was obtained from dried leaves by the method described by Alvarez and Kusumoto (1987), which results in a mixture of crude stevioside and rebaudioside A.

Patient selection. Since the doses of crude stevioside overcame the acceptable daily intake (ADI), i.e. 5.5 mg/kg/day (Geuns, 2002), a limited number of patients was used. To compensate this shortcoming a population was studied with homogenous baseline characteristics: demographical, nutritional and lifestyle. Seventy five workers at the experimental farm of the State University of Maringá (Maringá, Paraná State, Brazil) were enrolled.

Inclusion criteria were untreated mild essential hypertension (pre hypertension and stage 1), as defined in the seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (Chobanian *et al*., 2003) on three consecutive measurements, age >18 years and signing informed consent.

Exclusion criteria were pregnancy or childbearing potential, hepatic and or renal dysfunction, diabetes mellitus, malignancy, secondary hypertension of any etiology, organ damage caused by hypertension, cardiovascular diseases (stroke, myocardial infarction and angina pectoris) and medication with an effect on blood pressure (BP). Exclusion criteria and adverse effects reports were revised at each follow-up clinical visit.

The clinical investigation was performed in agreement with the ethical guidelines of the Declaration of Helsinki and was approved by the Human Ethics Committee of the State University of Maringá (approval number 021/2002-COPEP). A written informed consent was obtained from all subjects prior to enrolment in the study.

Information on the eligible patients' medical history and life style was collected with a structured clinical report form. In addition, the patients were instructed to maintain their lifestyle during the clinical trial; except for the fact that all patients were reminded to refrain from smoking or caffeine ingestion 30 min before measuring BP. SBP and DBP were measured biweekly by the same physician with great experience in measuring BP.

Blood pressure was measured to the nearest 2 mmHg, using a standard mercury sphygmomanometer, after the subject had been sitting for at least 15 min and with the arm resting at heart level. The brachial artery was located along the inner upper arm by palpation and the bladder was centered on it. The level of maximum inflation was determined by observing the pressure at which the radial pulse was no longer palpable as the cuff was inflated (palpated systolic) by adding 30 mmHg. The stethoscope position was over the palpitated brachial artery below the cuff at the antecubital fossae. SBP was determined by phase I of Korotkoff sounds (onset of at least two consecutive beats) and the DBP was determined at the cessation of the Korotkoff sounds (phase V). BP was measured three times consecutively at 5 min intervals and the mean was used for analysis. None of the three consecutive BP readings could be >2 mmHg from the calculated average of the three readings. Additional readings had to be done until this was achieved.

Phase 0 (placebo phase). Eighteen patients previously selected (DBP: 80–99 mmHg and SBP: 120–159 mmHg) were submitted to a 4 week single-blind phase in which they received capsules containing talcum twice a day, i.e. before lunch and dinner.

Clinical follow-up visits were scheduled in the morning to ensure that BP measurement occurred before the first daily intake of the capsule.

On the last day of the run-in phase, the patients were instructed to bring the remaining capsules (to check compliance) and a sample of the first morning urine. Immediately after arriving in the farm the body mass index (BMI = body weight/height^2), SBP and DBP were evaluated followed by the collection of blood samples. After these procedures all patients received a breakfast and after the meal an electrocardiogram (ECG) was done.

During the placebo phase three patients were excluded because of high values of BP (DBP >110 mmHg and/or SBP >160 mmHg) and one was excluded because an arrhythmia was detected. All excluded patients were advised to seek medical care.

Phases 1, 2, and 3 (active treatment). Fourteen patients (12 men, 2 women) selected from the placebo phase (phase 0) were randomly assigned: the first group was given capsules containing placebo (6 men and 1 woman, 43.3 ± 5.64 years) and the second group received capsules containing crude stevioside (6 men and 1 woman, 46.3 ± 8.08 years) during 24 weeks (phases 1, 2 and 3).

The capsules and flasks (coded package) with placebo or crude stevioside were indistinguishable by appearance.

It should be pointed out that all procedures adopted in the placebo phase (phase 0) were repeated in phase 1 (crude stevioside 3.75 mg/kg/day versus placebo during 7 weeks), phase 2 (crude stevioside 7.5 mg/kg/day versus placebo during 11 weeks) and phase 3 (crude stevioside 15.0 mg/kg/day versus placebo during 6 weeks).

The decision to use 3.75 mg/kg/day as the initial dose of crude stevioside (phase 1) was based on a previous placebo-controlled double-blind study in which an absence of toxicity was observed in 25 subjects receiving stevioside capsules for 90 days (Silva *et al*., 2004). In addition, the decision to change the daily doses from 3.75 mg/kg/day (phase 1) to 7.5 mg/kg/day (phase 2) occurred only after a careful review of all indicatives of toxicity and adverse effects report chart. Accordingly, a similar procedure was adopted before phases 2 and 3.

Two patients were excluded during phase 3: one from the placebo group (no compliance) and one from the stevioside group (epigastralgia).

Blood analysis. Venous blood was collected after an overnight fasting at the end of each phase for hematology (red blood cells count, hemoglobin, hematocrit, white blood cells blood counts – total and differential, platelet count) and serum determination of ALT, AST, CPK, creatinine, urea, sodium, potassium, chloride, glucose, insulin, glycated hemoglobin, fructosamine, gamma-glutamyltransferase (GGT), total cholesterol,

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high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerides, free and total prostatic specific antigen (PSA), testosterone and estradiol. All these parameters were measured using commercially available kits.

Urinalysis. Urine was collected at the end of each phase for microscopic analysis of sediment (red and white blood cells, cylinders), evaluation of glucose and of microalbuminuria. Another collection of 24 h was done for sodium analysis.

Other parameters. At the end of each phase the BMI and the homeostasis model assessment (HOMA-IR), a validated index for insulin resistance (Turner *et al*., 1979) was calculated by the formula: HOMA IR = Fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.

Statistical analysis. A computer generated randomization program was employed to assign patients to receive either crude stevioside or placebo. This program ensured that both groups had similar baseline values.

Considering the limited final number of patients (6 in each group) appropriated no parametrical statistical methods were employed, i.e. Mann-Whitney test for comparison between groups and Friedman-Wilcoxon test for comparison within group.

The software Stattistica 6.0 was used for all statistical procedures. A 95% level of confidence ($p < 0.05$) was accepted for all comparisons. Results were reported as mean ± standard deviation of mean (SD).

RESULTS AND DISCUSSION

During the treatment DBP ($p < 0.05$) decreased not only in the patients who received crude stevioside but also in the placebo group (Table 1). It could be inferred that this effect is a response to the presence of a physician during the clinical visits, i.e. an opposite effect of white coat hypertension. However, several investigations (Humboldt and Boech, 1978; Melis, 1992a,b,c; Chan *et al*., 1998; Lee *et al*., 2001; Liu *et al*., 2001; Hsu *et al*., 2002) showed that stevioside decreased BP in rats. But it must be emphasized that in those reports parenteral administration of stevioside was employed. In addition, a discrete reduction of BP was also obtained by nasogastric administration of stevioside in dogs, but the dose was very high, i.e. 200 mg/kg (Liu *et al*., 2003).

On the other hand, the antihypertensive effect of oral crude stevioside was obtained in two placebo double-blind clinical trials (Chan *et al*., 2000; Hsieh *et al*., 2003). Since stevioside is degraded by intestinal microflora of rats (Koyama *et al*., 2003b), pigs (Geuns *et al*., 2003) and humans (Gardana *et al*., 2003) to the diterpenoid aglycone steviol, the antihypertensive effect of orally administered stevioside could be mediated by steviol. Nonetheless, the presence of steviol in the blood after oral ingestion of stevioside was not confirmed (Geuns, 2003).

Since we used the same dose employed by Chan *et al*. (2000), the absence of a hypotensive effect could be a consequence of our lower basal values of BP (pre hypertension and stage I); the small number of patients; higher BMI; race and/or frequency of daily ingestion of the capsules (twice a day vs thrice a day in Chan's study). Another possibility not reported by Chan *et al*. (2000) could be the composition of crude stevioside.

The blood levels of testosterone, estradiol, free and total PSA (Table 3) were not modified by crude stevioside treatment. In contrast, previous reports demonstrated that leaves of *Stevia rebaudiana* (Bertoni) Bertoni, which contain not only steviol glycosides but also thousands of compounds affected the reproductive system (Mazzei-Planas and Kuc, 1968; Oliveira-Filho *et al*., 1989; Melis, 1999). Therefore, the conclusions obtained from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni, cannot be expanded to crude stevioside.

In contrast to our data (Table 2), stevioside´s potential for sodium excretion in rats has been described. Nevertheless, in these reports the stevioside was administered intravenously (Melis and Sainati, 1991; Melis, 1992a,c).

Table 2 also shows decreased ($p < 0.05$) blood levels of total cholesterol, LDL-C, VLDL-C, triacylglycerol, glucose and insulin (phase 3 versus phase 0). In agreement with these results HOMA IR (Table 2) and LDL-C/HDL-C ratio (not shown) were decreased ($p < 0.05$) (phase 3 versus phase 0). Taken together, these findings suggested an improvement of insulin action during the treatment not only with crude stevioside but also with placebo. Thus, in spite of the fact that all patients were instructed to maintain their lifestyle during the trial, the results were compatible with modifications in the lifestyle during the study.

Finally, changes in the ECG, blood analysis, urinalysis (not shown), glycated haemoglobin, fructosamine, BMI (Table 2); blood levels of AST, ALT, GGT,

Table 1. Systolic (SBP) and diastolic blood pressure (DBP) before (phase 0) and after treatment with crude stevioside 3.75 mg/kg/day (phase 1), 7.5 mg/kg/day (phase 2) and 15.0 mg/kg/day (phase 3)

	Phase	0	1	2	3
Stevioside	SBP	140 ± 13	134 ± 14	126 ± 8[a]	123 ± 12
	DBP	94 ± 8	85 ± 5[a]	84 ± 5[a]	84 ± 8[a]
Placebo	SBP	133 ± 12	128 ± 5	132 ± 6	124 ± 6
	DBP	94 ± 8	86 ± 3[a]	83 ± 5[a]	82 ± 4[a]

Values (mmHg) are mean ± SD ($n = 6$).
$p > 0.05$ for all comparisons (Stevioside group vs Placebo group).
[a] $p < 0.05$ compared with phase 0.

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Table 2. Effect of crude stevioside on body mass index (BMI), homeostasis model assessment (HOMA-IR), urine sodium (Na^+) and blood levels of cholesterol, high (HDL-C), low (LDL-C) and very low density lipoprotein (VLDL-C), triacylglycerol, glucose, insulin, glycated hemoglobin and fructosamine

	Stevioside		Placebo	
Parameter	Before treatment	After treatment	Before treatment	After treatment
BMI (kg/m^2)	27.3 ± 2.6	27.4 ± 2.6	25.9 ± 2.8	25.8 ± 2.8
HOMA-IR	2.7 ± 1.7	1.2 ± 0.6[a]	2.4 ± 1.0	1.2 ± 1.1[a]
Urine Na^+ (mEq/24 h)	246.5 ± 89.8	190.7 ± 70.6	227.2 ± 49.9	228.8 ± 39.2
Cholesterol (mg/dL)	239.5 ± 26.6	205.7 ± 17.6[a]	235.5 ± 39.3	213.8 ± 29.2
HDL-C (mg/dL)	45.5 ± 5.6	45.0 ± 7.1	52.3 ± 6.3	50.3 ± 4.7
LDL-C (mg/dL)	161.3 ± 25.7	133.3 ± 13.9[a]	152.7 ± 30.5	143.5 ± 25.2
VLDL-C (mg/dL)	32.7 ±11.9	27.3 ±10.4	30.5 ± 7.4	20.0 ± 2.4[a]
Triacylglycerol (mg/dL)	163.2 ± 60.2	136.3 ± 52.8	152.8 ± 36.5	100.5 ± 11.3[a]
Glucose (mg/dL)	91.8 ± 7.8	80.7 ± 6.7[a]	88.5 ± 6.2	79.3 ± 5.0[a]
Insulin (μU/L)	12.4 ± 5.9	7.4 ± 4.4[a]	9.9 ± 2.4	4.7 ± 2.1
Glycated hemoglobin	6.6 ± 0.3	6.6 ± 0.2	6.7 ± 0.2	6.6 ± 0.4
Fructosamine (mmol/L)	2.6 ± 0.2	2.6 ± 0.2	2.4 ± 0.3	2.4 ± 0.2

Values are mean ± SD ($n = 6$). $p > 0.05$ for all comparisons (Stevioside group vs Placebo group).
[a] $p < 0.05$ compared before and after treatment.

Table 3. Effect of crude stevioside on blood levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), creatine phosphokinase (CPK), creatinine, urea, testosterone, free and total PSA, estradiol, sodium (Na^+), potassium (K^+) and chloride (Cl^-)

	Stevioside		Placebo	
Parameter	Before treatment	After treatment	Before treatment	After treatment
AST (U/L)	7.7 ± 3.0	12.8 ± 3.5	6.5 ± 2.0	11.5 ± 1.4
ALT (U/L)	5.7 ± 1.8	9.2 ± 2.6	6.0 ± 1.3	9.5 ± 3.6
GGT (U/L)	16.0 ± 4.0	18.3 ± 3.0	19.5 ± 2.4	20.9 ± 3.2
CPK (U/L)	157.9 ± 62.5	99.8 ± 43.9	125.9 ± 68.9	104.9 ± 31.7
Creatinine (mg/dL)	1.02 ± 0.2	0.98 ± 0.1	0.95 ± 0.2	0.93 ± 0.1
Urea (mg/dL)	37.2 ± 4.5	43.3 ± 11.2	38.5 ± 1.9	41.0 ± 5.2
Testosterone (ng/mL)	484.8 ± 92.9	479.2 ± 159.4	542.2 ± 92.0	591.7 ± 116.8
Free PSA (ng/mL)	0.18 ± 0.1	0.22 ± 0.1	0.34 ± 0.2	0.34 ± 0.1
Total PSA (ng/mL)	0.94 ± 0.9	0.81 ± 0.6	2.07 ± 1.8	1.94 ± 1.6
Estradiol (pg/mL)	36.0 ± 6.0	33.1 ± 5.5	36.1 ± 9.4	37.1 ± 4.5
Blood K^+ (mEq/L)	3.8 ± 0.3	3.6 ± 0.3	4.0 ± 0.3	3.8 ± 0.2
Blood Cl^- (mEq/L)	99.8 ± 2.1	102.5 ± 1.6	98.5 ± 1.0	103.3 ± 1.5
Blood Na^+ (mEq/IL)	138.2 ± 2.2	139.3 ± 5.4	137.0 ± 1.3	139.7 ± 2.0

Values are mean ± SD ($n = 5$–6). $p > 0.05$ for all comparisons (Stevioside group vs Placebo group).

CPK, creatinine, urea, chloride, potassium and sodium (Table 3) were not found. In addition, no major adverse clinical effects were observed during the study.

In conclusion, the results suggest that oral crude stevioside is safe and supports the well-established tolerability during long term use as a sweetener, particularly in Brazil. However, in contrast to previous clinical trials (Chan *et al*., 2000; Hsieh *et al*., 2003) crude stevioside administered orally did not show an antihypertensive effect.

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