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

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1. Introduction

There is an intense search for low calorie sweeteners and high potency in order to provide an alternative to sugar for its use in food and drugs. Toxicological problems associated with more traditional sweeteners like cyclamate and saccharin have also stimulated this interest (Crammer and Ikan, 1977).

Stevioside, a steviol glycoside, is a diterpenoid glycoside contained in the leaves of Stevia rebaudiana Bertoni that has gained worldwide attention due to its potent sweetness (300 times sweeter than glucose according to the Merck Index). Stevia leaf extracts have apparently been used by native people as sweetener and traditional medicine before its description.

S. rebaudiana Bertoni or Ka’a He’e (as the natives named it) was discovered and botanically classified by Moisés Santiago Bertoni in 1899. Initially called Eupatorium rebaudianum, it was described in more detail and the name changed in 1905 to S. rebaudiana (Bertoni) Bertoni (Bertoni, 1899, 1905, 1918; Wood et al., 1955; Wood and Fletcher, 1956). The sweet principle was not isolated until 1909 by Dieterich (1909). Gradually, several substances have been isolated from the plant including stevioside and steviol.

In 1931, the extract was purified by Briedel and Lavieille (1931) to produce steviol. In 1952, the chemical structure of stevioside (dipertene glycoside) was established. Stevioside is described as a glycoside composed of three glucose molecules and an aglucon, steviol (Wood et al., 1955; Wood and Fletcher, 1956).

During the 1970s, other compounds were isolated, including rebaudioside A, also known as rebtose, with a sweet potency even higher than stevioside (Kohda et al., 1976). Steviol glycoside is currently used in several countries as a sweetener and it has been extensively tested to demonstrate that its use is safe for humans (Geuns and Buyse, 2004). In the year 2002, Stevia ranked second in sales of herbal supplements in USA. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2004), consumption of Stevia has been generally regarded as safe. However, JECFA requested additional information in order to change the temporary accepted daily intake (ADI) of 0–2 mg/kg/day for steviol glycoside, including the potential effects of low doses on blood glucose and blood pressure.
2. Aim

The aim of this study was to investigate the effect of steviol glycosides consumption in humans (both diabetics—Type 1 and Type 2—and non-diabetics with normal/low-normal blood pressure (BP)) in order to comply with the first part (the pharmacological effects of steviol glycosides in humans) of the Annex 2 of the 63rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

3. Patients and methods

3.1. Patient population

The study group consisted of 76 subjects (30 with Type 2 diabetes, 16 with Type 1 diabetes and 30 without diabetes and normal/low-normal BP levels (BP of <120/80 mmHg in at least 2 (two) measurements taken on different days). After a general examination, the volunteers were included according to the following inclusion criteria: with or without diabetes, normotensive or hypertensive under treatment, with a glycated hemoglobin (HbA1c) of less than 10%, a body mass index (BMI) between 20 and 35 kg/m², without established renal disease. For Group 2: Type 2 diabetes mellitus, male and female, 40–70 years old, diabetes onset at age greater than 30 years, diabetes duration of more than 1 year and less than 10 years, treated with diet and/or oral antidiabetic agents, normotensive or hypertensive under treatment, with a HbA1c of less than 10% and a BMI between 25 and 35 kg/m², without established renal disease. For Group 3: healthy subjects, male and female, 40–60 years old with normal or low-normal BP (≤120/80 mmHg) in at least 2 (two) measurements taken in different days and a BMI between 20 and 35 kg/m².

3.2. Study design

This was a 3-month, randomized, double-blind, placebo-controlled, parallel, long-term study.

During the study, treatment with insulin (Type 1 diabetics) and oral hypoglycemic drugs (Type 2 diabetics) continued the same. Those patients (Groups 1 and 2) on antihypertensive drugs also continued with the same treatment. Volunteers were randomly assigned to receive either steviol glycoside capsules 250 mg t.d.i. or matching placebo. Steviol glycoside was provided by Stevia, Rome, Italy. Volunteers at the investigation centre every 2 weeks during the 3-month study period for the determination of capillary blood glucose, BP and weight. At these visits, volunteers were asked about adverse events and the capsules were counted to check for compliance.

3.3. Study assessments

Blood pressure was measured in the clinic using a 90217 Ultralite ABP continuous blood pressure measurement monitor from Spacelabs Healthcare Inc., Issaquah, Washington, USA. At the beginning and the end of the study period, 24-h blood pressure was measured using the same equipment. Venous blood for laboratory tests was drawn between 07:00 and 09:00 AM after an overnight fast. Blood was collected at the beginning and the end of the study period for the determination of glucose, insulin (for groups 2 and 3), total cholesterol, HDL and LDL cholesterol, triglycerides, creatinine, electrolyte and creatin phosphokinase concentrations, renal function (creatinine) and hepatic function (ALT, AST, γGT). Glucose, creatinine, triglycerides, ALT, AST, γGT, total cholesterol and triglycerides were measured enzymatically using available kits. HDL cholesterol was determined by precipitation, and LDL cholesterol concentration was calculated using the Friedewald approximation.

Anthropometrical determinations measured at the beginning and at the end of the study period, included: weight, height, BMI, waist circumference. Volunteers attended the investigation centre every 2 weeks during the 3-month study period for determination of capillary blood glucose, BP and weight. At these visits, volunteers were asked about adverse events and the capsules were counted to check for compliance.

3.4. Statistical analysis

Data are reported as mean ± SD. Significance was set at P < 0.05 for differences between the active treatment and placebo groups at baseline, and between post and pre-treatments within groups. Student’s t-test was used to compare treatment groups at baseline and the paired-samples t-test was used to compare the means of the post and pre-treatment levels within groups. Power analyses were also conducted to determine whether the samples were large enough to allow for the detection of a clinically significant change between baseline and post treatment levels within the control and treatment groups. A clinically significant difference was defined based on the range of “normal” values for each of the parameters considered. The normal ranges for systolic BP, diastolic BP, glucose, and HbA1c were (90–120 mmHg), (60–80 mmHg) [Mancia et al., 2007], (60–99 mg/dl) [Teuscher and Richterich, 1971] and (4–5.9%) [Nuttal, 1998], respectively. Clinically significant differences were therefore defined as 20 mmHg, 50 mg/dl, and 1%, respectively.

4. Results

Eighty-six volunteers (45 women, 41 men) were enrolled in the study and 76 completed it. Ten volunteers (4 in Group 1, 3 in Group 2 and 3 in Group 3) decided to discontinue the study for no specific reason, but not due to side effects. The results of the study are shown in Tables 1–3. Mean age was 25.4 years for Group 1, 58.2 years for Group 2 and 28.1 years for Group 3. Volunteers’ baseline clinical and biochemical characteristics were similar at randomization, except for the Type 1 Diabetics group, where a significant difference between the placebo and steviol glycosides group was observed for systolic BP and triglycerides.

No significant changes from baseline were detected within the steviol glycoside treatment group for BP (systolic and diastolic), glucose, and HbA1c. Specifically, mean diastolic BP levels changed from 72.6 to 68.9 mmHg (Type 1 diabetics), and from 77.3 to 75.7 mmHg (Type 2 diabetics), and from 69.9 to 69.8 mmHg (non-diabetics), while mean systolic BP levels changed from 117.1 to 115.9 mmHg (Type 1 diabetics), and from 124.3 to 120.8 mmHg (Type 2 diabetics), and from 111.0 to 113.3 mmHg (non-diabetics). For glucose, mean levels changed from 144.9 to 155.2 mg/dl (Type 1 diabetics), and from 151.2 to 133.8 mg/dl (Type 2 diabetics), and from 82.5 to 82.9 mg/dl (non-diabetics), while mean HbA1c levels changed from 7.1% to 7.3% (Type 1 diabetics), and from 6.8% to 6.6% (Type 2 diabetics), and from 5.3% to 5.6% (non-diabetics).

Similarly, no significant changes from baseline were detected within the placebo group for BP (systolic and diastolic), glucose, and HbA1c, except for the Type 1 Diabetics group in the case of mean systolic BP and glucose. Specifically, mean diastolic BP levels changed from 70.7 to 69.7 mmHg (Type 1 diabetics), and from 76.7 to 77.4 mmHg (Type 2 diabetics), and from 68.8 to 69.9 mmHg (non-diabetics), while mean systolic BP levels changed from 108.3 to 105.7 mmHg (Type 1 diabetics, paired t-test p-value = 0.002), and from 124.9 to 125.4 mmHg (Type 2 diabetics), and from 111.7 to 112.2 mmHg (non-diabetics). For glucose, mean levels changed from 219.3 to 298.3 mg/dl (Type 1 diabetics, paired t-test value = 0.043), and from 131.3 to 118.9 mg/dl (Type 2 diabetics), and from 82.9 to 83.9 mg/dl (non-diabetics), while mean HbA1c levels changed from 8.2% to 8.3% (Type 1 diabetics), and from 6.8% to 6.7% (Type 2 diabetics), and from 5.3% to 5.4% (non-diabetics).

4.1. Power analyses

No statistically significantly changes were observed between baseline and post treatment in systolic BP, diastolic BP, glucose, or HbA1c except for the placebo Type 1 diabetics group. Hence analyses were conducted to estimate the power to detect a medically significant change in post treatment levels as compared to baseline levels, where a medically significant difference was defined as 20 mmHg for systolic and diastolic BP, 50 mg/dl for glucose, and 1% for HbA1c. The power to detect these differences as statistically significant (α = 0.05), given the sample sizes and observed variability in both the placebo and steviol glycoside groups.
blood glucose samples are required per patient to represent typical glucose levels in diabetic subjects. However, as pointed out earlier, the degree of compliance was similar in both groups (steviol glycoside and placebo). The types and incidence of side effects were similar between the steviol glycoside and placebo groups. Shortly after the initiation of treatment, volunteers in both groups (3 in the steviol glycoside group and 5 in the placebo group) experienced adverse effects (abdominal fullness, headache, dizziness).

### 4.2. Compliance

All volunteers who completed the study followed the prescribed treatment schedule throughout the 3-month period, and the degree of compliance was similar in both groups (steviol glycoside and placebo).

### 4.3. Tolerability

Steviol glycoside was well tolerated. The types and incidence of side effects were similar between the steviol glycoside and placebo groups. Shortly after the initiation of treatment, volunteers in both groups (3 in the steviol glycoside group and 5 in the placebo group) experienced adverse effects (abdominal fullness, headache, dizziness).

### Table 1

Characteristics of the steviol glycosides and placebo groups at baseline and post-treatment: Group 1 (Type 1 diabetes)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Steviol glycosides (n = 8)</th>
<th>Placebo (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 (3.3)</td>
<td>23.1 (3.1)</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>117.1 (6.6)</td>
<td>115.9 (8.6)</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>72.6 (6.5)</td>
<td>68.9 (7.2)</td>
</tr>
<tr>
<td><strong>Post-treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 (3.1)</td>
<td>22.4 (1.0)</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>115.9 (8.6)</td>
<td>108.3 (3.0)</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>68.9 (7.2)</td>
<td>70.7 (4.4)</td>
</tr>
</tbody>
</table>

### Laboratory

- **Glucose**: 144.9 (95.1) vs. 155.3 (78.3) vs. 219.3 (74.1) vs. 298.3 (58.8)
- **HbA1c (%)**: 7.1 (1.6) vs. 7.3 (1.1) vs. 8.2 (1.4) vs. 8.3 (1.6)
- **Total cholesterol (mg/dl)**: 148.9 (25.9) vs. 159.5 (36.7) vs. 144.6 (16.4) vs. 150.6 (10.0)
- **LDL-C (mg/dl)**: 46.3 (6.3) vs. 44.6 (8.0) vs. 53.0 (7.7) vs. 48.7 (11.4)
- **Triglycerides (mg/dl)**: 86.8 (33.6) vs. 86.8 (41.5) vs. 51.3 (11.5) vs. 67.3 (15.1)
- **Creatinine (mg/dl)**: 0.9 (0.1) vs. 0.9 (0.1) vs. 0.8 (0.2) vs. 0.9 (0.1)
- **CPK (U/L)**: 34.4 (4.2) vs. 33.3 (13.5) vs. 37.6 (5.8) vs. 40.8 (13.2)
- **AST (U/L)**: 21.4 (8.1) vs. 18.6 (9.4) vs. 19.3 (7.4) vs. 23.3 (3.0)
- **ALT (U/L)**: 18.6 (7.2) vs. 16.5 (6.9) vs. 16.3 (5.8) vs. 19.3 (6.2)
- **ALT (U/L)**: 22.0 (12.3) vs. 29.3 (20.3) vs. 26.3 (5.3) vs. 30.6 (7.6)
- **Sodium (mEq/L)**: 138.8 (3.3) vs. 140.4 (3.3) vs. 138.1 (1.7) vs. 138.3 (1.6)
- **Potassium (mEq/L)**: 4.2 (0.4) vs. 4.5 (0.5) vs. 4.5 (0.5) vs. 4.9 (0.3)
- **Chloride (mEq/L)**: 100.0 (2.0) vs. 100.9 (2.4) vs. 99.3 (1.0) vs. 99.8 (1.6)

### Values

- All values are mean (SD).
- Values are mean (SD).
- p < 0.05 versus placebo baseline.
- **p < 0.05 versus steviol glycosides at baseline**.

### Table 2

Characteristics of the steviol glycosides and placebo groups at baseline and post-treatment: Group 2 (Type 2 diabetes)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Steviol glycosides (n = 15)</th>
<th>Placebo (n = 15)</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.7 (3.4)</td>
<td>29.2 (2.9)</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>127.3 (15.1)</td>
<td>124.3 (13.5)</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>77.3 (9.1)</td>
<td>74.7 (8.3)</td>
</tr>
<tr>
<td><strong>Post-treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.2 (2.9)</td>
<td>30.1 (3.3)</td>
</tr>
<tr>
<td>24-h SBP (mmHg)</td>
<td>124.3 (13.5)</td>
<td>127.9 (13.7)</td>
</tr>
<tr>
<td>24-h DBP (mmHg)</td>
<td>74.7 (8.3)</td>
<td>76.7 (5.6)</td>
</tr>
</tbody>
</table>

### Laboratory

- **Glucose (mg/dl)**: 151.2 (54) vs. 133.8 (34.5) vs. 131.3 (46.2) vs. 118.9 (34.0)
- **HbA1c (%)**: 13.3 (15.3) vs. 11.6 (11.1) vs. 14.7 (10.2) vs. 15.3 (9.6)
- **Total cholesterol (mg/dl)**: 186.9 (23.3) vs. 171.5 (21.6) vs. 172.7 (31.6) vs. 173.8 (23.0)
- **LDL-C (mg/dl)**: 44.6 (8.7) vs. 42.3 (6.7) vs. 44.6 (10.9) vs. 40.8 (6.2)
- **ALT (U/L)**: 111.4 (23.8) vs. 107.8 (22.2) vs. 93 (31.8) vs. 110.2 (18.6)
- **Triglycerides (mg/dl)**: 43.2 (51.8) vs. 153.7 (71.3) vs. 133.8 (53.3) vs. 128.1 (39.2)
- **Creatinine (mg/dl)**: 0.9 (0.1) vs. 0.9 (0.1) vs. 0.9 (0.2) vs. 0.8 (0.2)
- **CPK (U/L)**: 33.1 (10.3) vs. 34.8 (14.8) vs. 48.5 (27.0) vs. 38.8 (31.6)
- **AST (U/L)**: 19.8 (5.3) vs. 19.3 (3.2) vs. 22.0 (5.0) vs. 22.4 (5.5)
- **ALT (U/L)**: 21.3 (7.9) vs. 22.8 (8.9) vs. 21.1 (5.1) vs. 21.6 (5.3)
- **γGT (U/L)**: 25.6 (10.4) vs. 27.9 (15.8) vs. 23.4 (12.0) vs. 27.2 (19.2)
- **Sodium (mEq/L)**: 138.5 (2.0) vs. 141.5 (3.2) vs. 139.5 (1.6) vs. 140.9 (2.1)
- **Potassium (mEq/L)**: 4.2 (0.2) vs. 4.6 (0.4) vs. 4.4 (0.2) vs. 4.4 (0.3)
- **Chloride (mEq/L)**: 100.2 (1.9) vs. 101.9 (2.2) vs. 100.4 (1.2) vs. 101.5 (1.5)

### Values

- All values are mean (SD).
- BMI, body mass index; 24-h SBP, 24-h systolic blood pressure; 24-h DBP, 24-h diastolic blood pressure; CPK, creatinine phosphokinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, gamma glutaryltransferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein.

### Range

Glucose levels in poorly controlled diabetic patients are expected to be extremely variable; hence, it is not surprising that the study did not have enough power to detect a significant difference in glucose levels in diabetic subjects. However, as pointed out earlier, the study did have enough power to detect a medically significant difference in HbA1c levels, the more clinically relevant parameter, since as pointed out by McCarter et al., 2006, a large number of blood glucose samples are required per patient to represent typical diurnal glucose patterns, while one HbA1c measurement may be sufficient and representative of the mean blood glucose level obtained from multiple blood glucose samples drawn over the preceding weeks and months.

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ness, nausea and asthenia), but symptoms disappeared after 1 week of treatment. No drop-outs were due to side effects.

No significant changes in body weight or biochemical parameters were observed in the two treatment groups.

5. Discussion

Steviol glycosides compounds appear to be safe as sweeteners, and have been used for decades in several countries such as Japan, China, Taiwan, South Korea, Malaysia and most of South American countries without reported adverse effects. In addition, many studies have been conducted in order to understand the absorption, digestion, metabolism and excretion (ADME) of steviol glycosides in animals and humans. The animal studies have included in vitro studies as well as in vivo studies. The in vivo studies have involved different species, different testing protocols, and diabetic and non-diabetic animals. Likewise the human studies have involved subjects from different geographic regions and with different health status (i.e., normal, hyper and hypotensive, diabetic, etc.). Varied testing protocols have been followed for each of the human studies including differences in dosing regimens and time frames for collecting samples for analysis. The differences between studies make comparisons more difficult but may have the benefit of providing a broader understanding of the mechanisms involved and general assurances that adverse effects are not being observed. The objectives of the present research were to evaluate the impact of consuming steviol glycosides (250 mg 3 times/day for 3 months) on blood glucose control and on blood pressure in normal and hypertensive individuals and in Type 1 and Type 2 diabetics.

5.1. Effects of steviol glycosides on blood glucose and insulin

The results of other research suggest that effects on blood glucose levels may be different depending on the status of the subjects and that there are differences in diabetic and non-diabetic animals and humans. Previous perfusion studies in animals have shown a glucose-dependent insulin release to stevioside (Jeppesen et al., 2000) with fading of the insulinotropic effect of stevioside in the presence of normal to low glucose (Jeppesen et al., 2000; Usami et al., 1980).

In vivo studies in diabetic rats have demonstrated a decrease in blood glucose after stevioside administration (25 mg/kg/day stevioside for 6 weeks) (Jeppesen et al., 2003). However, these effects of oral stevioside or steviol glycosides are not seen in non-diabetic animals. In a long-term chronic toxicity study, Xili et al. (1992) found no change in blood glucose in rats fed a diet containing 1.2% stevioside for 24 months, and Suanarunsawat and Chaiyabutr (1997) found no change in blood glucose in an acute oral study.

Similarly, studies in diabetic humans have demonstrated a decrease in blood glucose after acute stevioside administration (1 g with a meal) (Gregersen et al., 2004; Jeppesen et al., 2005). Chan et al. (2006) found an increase in fasting blood glucose in diabetic patients receiving placebo but not those receiving 500 mg stevioside 3 times daily for 3 months. Two studies with Stevia extract in normal humans have demonstrated an apparent dose-related decrease in blood glucose; Curi et al. (1986) gave extracts of 5 g of Stevia leaves 3 times daily for 3 days while Haebisch (1992) gave 110 mg or 27 mg stevioside equivalents acutely. In contrast, Chan et al. (2000) found no effect of much larger doses of stevioside (250 mg 3 times daily for 1 year) on fasting blood glucose in hypertensive, non-diabetic subjects. Similarly, Geuns et al. (2007) found no effect on blood glucose in healthy human subjects after treatment with 250 mg 3 times daily for 30 days. The fact that neither Geuns et al. (2007) nor Chan et al. (2000) found a reduction in blood glucose at relatively high doses of stevioside suggests that perhaps the effect seen at lower doses was due to some other mechanism. Food Standards Australia and New Zealand Food (FSANZ) in its review of these studies concluded that the apparent differences in effects may be due to the differences in testing protocols (FSANZ, 2007). FSANZ also noted that the results “suggest that effects only occur when blood glucose concentrations are elevated, as in the diabetic state and that there is a relatively low risk of hypoglycemia in normal subjects from consumption of dietary concentrations of stevioside (FSANZ, 2007).” The present results support these conclusions, as no statistically significant change was observed in the post-treatment mean glucose and HbA1c levels in the steviol glycoside group.

| Table 3 Characteristics of the steviol glycosides and placebo groups at baseline and post-treatment: Group 3 (non-diabetics with normal/low-normal BP) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | Steviol glycosides (n = 13)     | Placebo (n = 17)                |                                |
|                                | Baseline                        | Post-treatment                  | Baseline                        | Post-treatment                  |
| BMI (kg/m²)                    | 22.9 (2.7)                      | 23.2 (2.7)                      | 24.4 (3.8)                      | 24.5 (3.5)                      |
| 24-h SBP (mmHg)                | 110.8 (8.9)                     | 113.3 (10.8)                    | 111.7 (10.4)                    | 112.2 (11.9)                    |
| 24-h DBP (mmHg)                | 69.9 (7.2)                      | 69.8 (7.1)                      | 68.8 (5.5)                      | 69.8 (8.1)                      |
| Glucose (mg/dl)                | 82.5 (6.6)                      | 82.9 (7.8)                      | 82.9 (10.2)                     | 83.9 (7.0)                      |
| Insulin (µU/ml)                | 4.2 (1.8)                       | 4.9 (2.4)                       | 8.4 (8.1)                       | 8.7 (8.1)                       |
| HbA1c (%)                      | 5.3 (0.4)                       | 5.6 (0.6)                       | 5.3 (0.6)                       | 5.4 (0.7)                       |
| Total cholesterol (mg/dl)      | 164.7 (30.8)                    | 173.7 (27)                      | 164.1 (32.5)                    | 173.5 (29.9)                    |
| LDL-C (mg/dl)                  | 51.7 (9.1)                      | 50.1 (10.5)                     | 53.2 (8.1)                      | 52.8 (9.7)                      |
| Triglycerides (mg/dl)          | 96.5 (44.2)                     | 96.6 (33.2)                     | 91.8 (62.6)                     | 108.6 (100.5)                   |
| Creatinine (mg/dl)             | 0.9 (0.2)                       | 0.9 (0.1)                       | 0.9 (0.1)                       | 0.8 (0.1)                       |
| CPK (U/L)                      | 34.8 (13.5)                     | 31.5 (7.1)                      | 30.6 (18.1)                     | 40.6 (35.5)                     |
| AST (U/L)                      | 20.1 (5.5)                      | 23.2 (7.1)                      | 23.1 (5.5)                      | 19.6 (3.8)                      |
| ALT (U/L)                      | 18.8 (10.4)                     | 19.5 (10.1)                     | 18.0 (5.3)                      | 16.4 (4.8)                      |
| γ-GT (U/L)                     | 29.5 (22.5)                     | 22.5 (20.1)                     | 20.9 (14.5)                     | 19.6 (12.2)                     |
| Sodium (mEq/L)                 | 140.2 (21.1)                    | 140.5 (32.3)                    | 139.7 (2.6)                     | 138.8 (14.0)                    |
| Total cholesterol (mg/dl)      | 100.6 (1.7)                     | 101.2 (2)                       | 100.6 (1.6)                     | 99.9 (0.9)                      |

Values are mean (SD). BMI, body mass index; 24-h SBP, 24-h systolic blood pressure; 24-h DBP, 24-h systolic blood pressure; CPK, creatinine phosphokinase; AST, aspartateaminotransferase; ALT, alanine aminotransferase; γ-GT, gamma glutaryltransferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein.
5.2. Effects of steviol glycosides on blood pressure

A number of studies have examined the effect of oral steviol glycosides on normal and hypertensive humans and animals. In normal and hypertensive rats and normal dogs, a small but significant reduction in blood pressure (either mean arterial pressure or systolic and diastolic pressure) has been observed (Melil, 1995, 1996; Jeppesen et al., 2003; Liu et al., 2003). A blood pressure lowering effect has been previously shown in chronic studies in humans using 250 mg 3 times daily (the same dose used in our study) when given to patients with high blood pressure for 1 year (Chan et al., 2000) or 500 mg 3 times daily for 2 years (Hsieh et al., 2003). However, (and in accordance to the results of our study), in a more recent study in Type 2 diabetic patients, no effect on blood pressure was found when stevioside was given at much higher doses (500 mg 3 times daily for 3 months) (Jeppesen et al., 2006); similarly in non-hypertensive subjects, Geuns et al. (2007) (250 mg 3 times daily for 3 days) found no effect on blood pressure. Ferri et al. (2006) did not find any effect on blood pressure at doses up to 15 mg/kg bw/day in mildly hypertensive humans. In reviewing these findings, FSANZ (FSANZ, 2007) considered the effects would be occurring in patients with already elevated blood pressure. FSANZ further concluded that there would be no effect on blood pressure at dietary levels of stevioside (FSANZ, 2007). The present results support these conclusions, as no statistically significant change was observed in the post-treatment mean systolic and diastolic BP levels in the steviol glycoside group.

6. Conclusions

We conclude that consumption of steviol glycosides in humans as a sweetener by normal and diabetic subjects, (including those with normal/low-normal blood pressure) is safe and does not produce either hypoglycemia or hypotension. Although previous studies have shown glucose-dependent and blood pressure-dependent effects of stevioside, these were effects at doses that are very much higher than the doses that would be encountered due to the use of stevioside as a sweetener. Although higher doses of stevioside have the ability of lowering both glycaemia and blood pressure, according to a review by the Australian/New Zealand authorities (FSANZ, 2007) this is anticipated only when these parameters are abnormally elevated.

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References