UNIVERSIDADE ESTADUAL DE MARINGÁ - UEM
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LETÍCIA DE ARAUJO FUNARI FERRI

INVESTIGAÇÃO DO EFEITO ANTI-HIPERTENSIVO DO ESTEVIOSÍDEO EM PACIENTES HIPERTENSOS

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas (Área de Concentração – Produtos Naturais Biologicamente Ativos), da Universidade Estadual de Maringá, para a obtenção do grau de Mestre em Ciências Farmacêuticas.

Orientador: Prof. Dr. Roberto Barbosa Bazotte

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Este trabalho está de acordo com as normas definidas pela Coordenação do Programa de Pós-Graduação em Ciências Farmacêuticas, esta dissertação de Mestrado foi redigida na forma de um artigo científico, conforme as normas da revista à qual o artigo foi encaminhado.

RESUMO

O esteviosídeo é o principal componente doce presente nas folhas de Stevia rebaudiana (Bert) Bertoni e tem sido utilizado comercialmente como edulcorante. Estudos prévios demonstram efeito hipotensor do esteviosídeo. Neste estudo foi investigado o efeito anti-hipertensivo do esteviosídeo obtido das folhas da Stevia rebaudiana (Bert) Bertoni (Compositae). Indivíduos com diagnóstico de hipertensão arterial sistêmica não tratada foram submetidos a uma etapa inicial unicega onde todos receberam placebo (4 semanas). Os voluntários selecionados nesta fase participaram de um ensaio clínico randomizado, duplo cego e placebo controlado. Para este estudo foram randomizados em dois grupos: o primeiro grupo recebeu cápsulas de placebo durante 24 semanas e o segundo, esteviosídeo 3.75 mg/kg/dia (7 semanas), 7.5 mg/kg/dia (11 semanas) e 15.0 mg/kg/dia (6 semanas). As cápsulas deveriam ser ingeridas duas vezes ao dia antes das refeições (almoço e jantar). Após a fase placebo e após cada fase, foi realizado controle laboratorial (urinálise, hematologia, hormônios e bioquímica), cálculo do índice de massa corpórea e eletrocardiograma. Durante o estudo, a pressão arterial foi aferida quinzenalmente. O esteviosídeo não mostrou nenhum efeito adverso clinicamente relevante. Os pacientes não relataram nenhum efeito adverso grave. Assim, podemos concluir que o esteviosídeo nas doses utilizadas neste estudo não apresentou efeito antihipertensivo.

ABSTRACT

Stevioside is the main sweet component in the leaves of Stevia rebaudiana (Bert) Bertoni and has been used as a commercial sweetening agent. Previous studies demonstrated the hypotensive effect of stevioside. In this study, the antihypertensive effect of stevioside obtained from leaves of Stevia rebaudiana (Bert) Bertoni (Compositae) were investigated. Eligible patients with untreated mild essential hypertension were submitted to a single blind phase (4 weeks) in which all patients received placebo. The volunteers selected in this phase were submitted to a randomized placebo controlled double blind study. For this purpose they were randomized in two groups: the first group received capsules containing placebo during 24 weeks and the second group received 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 ingested twice-daily, i.e., before lunch and before dinner. After the placebo phase and after each dose of stevioside, body mass index, electrocardiogram and laboratory tests (urinalysis, hematology, hormones, biochemistry) were performed. During the investigation systolic and diastolic blood pressure were measured biweekly. Stevioside did not show any clinical effect in all parameters investigated. Moreover the patients did not report serious adverse effect. Thus, we can conclude that the stevioside in the doses employed in this study did not show antihypertensive effect.

SUMÁRIO

Investigation of the antihypertensive effect of stevioside in patients with mild essential hypertension

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Abstract

Stevioside is the main sweet component in the leaves of *Stevia rebaudiana (Bert) Bertoni* and has been used as a commercial sweetening agent. In this study, the antihypertensive effect of stevioside obtained from leaves of *Stevia rebaudiana (Bert) Bertoni* (Compositae) was investigated. Eligible patients with untreated mild essential hypertension were submitted to a single blind phase (4 weeks) in which all patients received placebo. The volunteers selected in this phase were submitted to a randomized placebo controlled double blind study. For this purpose they were randomized in two groups: the first group received capsules containing placebo during 24 weeks and the second group received 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 ingested twice daily, i.e., before lunch and before dinner. After the placebo phase and after each dose of stevioside, body mass index, electrocardiogram and laboratory tests (urinalysis, hematology, hormones, biochemistry) were performed. During the investigation systolic and diastolic blood pressure were measured biweekly. Stevioside did not show any clinical effect in all parameters investigated. Moreover the patients did not report serious adverse effect. Thus, we can conclude that the stevioside until 15.0 mg/kg/day did not show antihypertensive effect.

Keywords: *Stevia rebaudiana (Bert) Bertoni* – Compositae – stevioside - blood pressure - clinical investigation – placebo effect.

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Introduction

Stevia rebaudiana (Bert) Bertoni is a perennial native shrub from northeastern Paraguay and southern Brazil. Although it is estimated to exist about 200 native species in South America, the sweetening properties have only been described in *Stevia rebaudiana (Bert) Bertoni.*

Leaves of *Stevia rebaudiana (Bert) Bertoni* have been used by the Guarani Indians from Paraguay as sweetener and this empirical knowledge was passed by oral tradition for many centuries.

Today, it is well established that stevioside is the main sweet component in the leaves of *Stevia rebaudiana (Bert) Bertoni*.

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

Moreover, there are also two clinical trials about the effects of stevioside from China on human hypertension. In both reports (Chan et al., 2000; Hsieh et al., 2003), the toxicological profile were 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 stevioside obtained from leaves of *Stevia rebaudiana (Bert) Bertoni* cultivated in northeastern Paraguay and southern Brazil was evaluated in a double blind,

placebo controlled clinical trial, as well as the toxicological profile was expanded by assessing metabolic and hormonal parameters that were not evaluated by these studies (Chan et al., 2000; Hsieh et al., 2003).

Patients and Methods

Stevioside

The plant *Stevia rebaudiana (Bert) Bertoni* (Compositae) *was* identified and a voucher was deposited in the herbarium of our university (Maringá, PR, Brazil) during our first clinical (Curi et al., 1986) and toxicological (Bazotte et al., 1986) study.

Standardized stevioside was obtained from dried leaves by a method described in a previous paper (Alvarez and Kusumoto, 1987), which produces a mixture of stevioside (70%) and rebaudioside (20%). The impurity refers mainly to others rebaudiosides (2%), mucilage and pigments. The technical specification of stevioside obtained by this method and employed in this study is presented in Table 1.

Patients selection

Because the doses of stevioside employed in this study overcome the acceptable daily intake (ADI) for this sweetener, i.e., 5.5mg/Kg/day we decided to employ a limited number of patients.

Therefore, to compensate the limited number of patients we chose to study a population with homogenous baseline characteristics: age, nutritional habits and lifestyle. We enrolled 75 workers of the experimental farm of the State University of Maringá (Maringá, Paraná State, Brazil).

Inclusion criteria for the trial were age (18-65 years), untreated essential hypertension with mean systolic blood pressure (SBP) 120 -159 mmHg and diastolic blood pressure (DBP) 80- 99 mmHg (average of three readings).

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, angina pectoris) and medications which could interfere in the blood pressure. These exclusion criteria and adverse effects reports were revised at each follow-up clinical visit.

Eligible patients (18 subjects) for placebo run-in period (placebo phase) were men and women with newly diagnosed mild essential hypertension (pre hypertension and stage 1) and untreated hypertension, 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).

All volunteers enrolled for the study were given a written informed consent to participate in the study.

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).

Then information about each eligible patient was obtained using a questionnaire. In addition, they were instructed to maintain their life style during the clinical investigation; except for the fact that all patients were reminded to refrain from smoking or caffeine ingestion for 30 min before each clinical visit.

SBP and DBP were measured biweekly by the same physician with large experience in measuring blood pressure (BP). BP was measured to the nearest 2 mmHg, using a standard mercury sphygmomanometer, after the subject had been sitting for 15 min rest, 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 is over the palpitated brachial artery below the cuff at the antecubital fossae. The systolic pressure is determined by Phase I of Korotkoff sounds (onset of at least 2 consecutive beats) and the diastolic pressure is determined at the cessation of the Korotkoff sounds (Phase V). BP was measured consecutively three times at 5-min intervals and the mean of these measurements was used for analysis. None of the 3 consecutive BP readings could be >2 mmHg from the calculated average of the 3 readings. Additional readings had to be done until this was achieved.

Placebo phase (Phase 0)

The patients previously selected (18 subjects) were submitted to a single-blind phase (4 weeks) in which they received two capsules containing talcum (placebo), one before lunch and one before dinner.

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

At the last day of 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), 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 withdrawn 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 attention in the internal medicine ambulatory of the State University of Maringá.

Active treatment phases: 1, 2, and 3.

The fourteen patients (12 men, 2 women) selected from the placebo phase (Phase 0) were randomized in two groups: the first received capsules containing placebo (6 men and 1 woman, 43.3 ± 5.64 years) and the second one capsules containing stevioside (6 men and 1 woman, 46.3 ± 8.08 years) during 24 weeks (phases 1, 2 and 3). During this period two patients were withdrawn.

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

It should be pointed out that all procedures adopted in the placebo phase were repeated in phase 1 (stevioside 3.75 mg/kg/day versus placebo during 7 weeks), phase 2 (stevioside 7.5 mg/kg/day versus placebo during 11 weeks) and phase 3 (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 stevioside (phase 1) was based on a previous placebo controlled double blind study where

it was observed absence of toxicity in 25 subjects that received stevioside capsules during 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. Additionally, a similar procedure was adopted before phases 2 and 3.

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, 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 (Labtest®).

Urinalysis

Urine from overnight fasted patients was collected at the end of each phase of study for microscopic analysis of sediment (RBCs, WBCs, cylinders), glucose and evaluation of microalbuminuria. Another collection of 24 h was done for analysis of sodium.

Other parameters

At the end of each phase of study the body mass index (BMI = body weight /height²) and the homeostasis model assessment (HOMA-IR), an accepted index for insulin resistance (Turner et al., 1979) was calculated by the formula:

HOMA IR = Fasting insulin (µU/ml) x fasting glucose (mmol/l)/ 22.5

In addition, the compliance was evaluated by counting the remnant capsules at the end of each phase.

Statistical Analysis

A computer generated randomization program was employed to assign patients to receive either 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 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 are reported as mean ± standard deviation of mean (SD).

Results and Discussion

Considering that there was no difference between placebo and stevioside group during the treatment, the data obtained from phases 1 and 2 (except SBP and DBP) are not shown.

SBP and DBP were measured biweekly (Table 2) and the values obtained in the last day of each phase were indicated in the Table 2. SBP and DBP decreased ($p < 0.05$) during the treatment with stevioside, but a similar effect was observed in the placebo group. Thus, it can be concluded that the effect of stevioside decreasing SBP and DBP could be some type of adaptation by the presence of the physician during the clinical visits, an opposite effect of the white coat hypertension. However, in disagreement with these data (Table 2), several investigations (Humboldt and Boech, 1978; Chan et al.,1998; Hsu et al.,2002; Lee et al.,2001; Melis, 1992) showed that stevioside decreased the BP in normal and hypertensive rats. The antihypertensive effect of stevioside was related to an inhibition of extracellular calcium influx (Lee et al., 2001; Melis, 1992). However, it must be emphasized that in these reports the stevioside was injected.

Additionally, 3 studies using oral stevioside, the usual way of consumption of this sweetener were published (Liu et al., 2003; Chan et al., 2000; Hsieh et al., 2003). The antihypertensive effects of oral stevioside in humans were obtained in two placebo-double blind reports in which 15 mg/kg/day (Chan et al.,2000) and 30 mg/kg/day (Hsieh et al., 2003) were employed with a reduction of 10 mmHg in systolic BP and 6 mmHg in diastolic BP. The reduction of the 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).

Since stevioside is degraded by intestinal micro flora of rats (Koyama et al.,2003), pigs (Geuns et al., 2003) and human (Gardana et al., 2003) to the diterpenoid aglycone steviol, the antihypertensive effect of orally administered

stevioside could be mediated by steviol. In agreement with these observations is the finding of an antihypertensive effect of intraperitoneally injected isosteviol in hypertensive rats (Liu et al., 2001).

We have used in the phase 3 the same dose (15 mg/kg/day) employed by Chan et al (Chan et al., 2000), however we have not find hypotensive effect. These different results could be explained by higher values of SBP and DBP of the Chan's patients (Chan et al., 2000), our smaller number of patients enrolled, our different proportion men/ women, race (Brazil and China) and BMI (higher in our study) as well as the frequency of daily ingestion of the capsules (twice a day in our study and thrice in Chan's study). Another possibility not informed in details by Chan et al (Chan et al., 2000) could be differences in the composition of stevioside.

Thus, up to our knowledge, this was the first controlled placebo double blind clinical study in which blood levels of LDL-C, VLDL-C, insulin, glycated hemoglobin, fructosamine (Table 3); GGT, urea, testosterone, estradiol, free and total PSA (Table 4); urine level of sodium (Table 5) and microalbuminuria (not shown) were measured. We did not find any modification in the ECG, urinalysis, hematological profile (not shown), BMI (Table 3), blood levels of HDL-C, HbA_{1c}, fructosamine (Table 3), AST, ALT, GGT, CPK, creatinine, urea (Table 4) sodium, potassium, chloride and urine sodium (Table 5).

In contrast with previous reports, which demonstrated that *Stevia rebaudiana (Bert) Bertoni* affected the reproductive system (Melis, 1999; Oliveira-Filho et al., 1989; Mazzei-Planas and Kuc, 1968), the blood levels of testosterone, estradiol, free and total PSA (Table 4) were not modified during the treatment with stevioside. It must be noted that these studies (Melis, 1999;

Oliveira-Filho et al., 1989; Mazzei-Planas and Kuc, 1968) employed leaves of *Stevia rebaudiana (Bert) Bertoni,* which contain not only steviol glycosides but also thousands of compounds. Therefore, the conclusions obtained from the toxicological potential of the leaves *Stevia rebaudiana (Bert) Bertoni*, must not be extrapolated to isolated stevioside.

Furthermore, information about the biological effects of stevioside must be analyzed carefully. For instance, in contrast with our results (Table 5) stevioside has been described as increasing sodium (Melis, 1992_a; Melis, 1992_c) and potassium excretion (Melis and Sainati, 1991) in rats. Nevertheless, in these reports the stevioside was intravenously administered (Melis, 1992_a; Melis, 1992_c, Melis and Sainati, 1991).

As shown in Table 3, there were 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 (Table 3) and LDL-C/HDL-C ratio (not shown) values were decreased (phase 3 versus phase 0). Taken together, these findings suggested an improvement of insulin action during the treatment not only with stevioside but also with placebo. Thus, in spite of the fact that all patients were instructed to maintain their life style during the trial, these results were compatible with modifications in the lifestyle during the study.

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

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Table 1. Technical specifications of stevioside

Description		White Crystalline water-soluble compound.
Chemical name		19-O-ß-glucopiranosil-13-O[ß-glucopiranosil (1,2)-ß-glucopiranosil]-steviol
Elemental composition		$C_{38}H_{60}O_{18}$
Molecular weight		804.88 KD
Assay		
	Stevioside	70% minimal
	Rebaudioside A	20% minimal
	Rebaudiosides	2% maximal
	Melting Point	198 ºC
	Optical rotation	$[a]_{25,D}$ -39.3º
pH		5.0 - 7.0 (1%)
Humidity		3% (maximal)
Ash		1% (maximal)
Density		0.390 a 0.420 g/ml
Heavy metals**:** arsenic (< 1.000 ppm), lead (< 3.600 ppm), mercury (< 0.005 ppm), chromium (< 0.007 ppm), cadmium (< 0.300 ppm).		
Nutritional information (per g): carbohydrate (90%), protein (0%), total fat (0%), saturated fat (0%), cholesterol (0%), fiber (0%)		

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

	Phase	0	1	2	3
Stevioside	SBP	140 ± 13	134 ±14	126 ± 8[a]	123 ± 12
	DBP	94 ± 8	85 ± 5[a]	84 ± 5	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 x Placebo group)

[a]$p < 0.05$ as compared with phase 0

Table 3. Effect of stevioside (15 mg/kg/day) and placebo on body mass index (BMI) and blood levels of `total cholesterol, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), triacylglycerol, glucose, insulin, glycated hemoglobin and fructosamine and insulin resistance assessed by homeostasis model assessment (HOMA-IR)

Parameters	Stevioside		Placebo	
	Before Treatment	After Treatment	Before Treatment	After Treatment
BMI (Kg/m^2)	27.3 ± 2.60	27.4 ± 2.57	25.9 ± 2.76	25.8 ± 2.76
Cholesterol (mg/dl)	239.5 ± 26.58	205.7±17.65[a]	235.5 ± 39.26	213.8 ± 29.19
HDL-C (mg/dl)	45.5 ± 5.62	45.0 ± 7.12	52.3 ± 6.26	50.3 ± 4.68
LDL-C (mg/dl)	161.3 ± 25.66	133.3±13.90[a]	152.7 ± 30.55	143.5 ± 25.21
VLDL-C (mg/dl)	32.7 ±11.91	27.3 ±10.42	30.5 ± 7.37	20.0 ± 2.38[a]
Triacylglycerol (mg/dl)	163.2 ± 60.16	136.3± 52.76	152.8 ± 36.47	100.5±11.31[a]
Glucose (mg/dl)	91.8 ± 7.78	80.7 ± 6.75[a]	88.5 ± 6.21	79.3 ± 5.02[a]
Insulin (µU/l)	12.4 ± 5.92	7.4 ± 4.39[a]	9.9 ± 2.36	4.7 ± 2.10
Glycated hemoglobin	6.6 ± 0.35	6.6 ± 0.25	6.7 ± 0.20	6.6 ± 0.36
Fructosamine (mmol/l)	2.6 ± 0.23	2.6 ± 0.21	2.4 ± 0.30	2.4 ± 0.24
HOMA IR	2.74 ± 1.69	1.19 ± 0.64[a]	2.37 ± 1.00	1.24 ± 1.10[a]

Values are mean ± SD (n = 6)

$p > 0.05$ for all comparisons (Stevioside group x Placebo group)

[a]$p < 0.05$ as compared before and after treatment

Table 4. Effect of stevioside (15 mg/kg/day) and placebo on blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), creatine phosphokinase (CPK), creatinine, urea, testosterone, free and total PSA, estradiol

Parameters	Stevioside		Placebo	
	Before Treatment	After Treatment	Before Treatment	After Treatment
AST (U/l)	7.7 ± 2.98	12.8 ± 3.53	6.5 ± 1.98	11.5 ± 1.38
ALT (U/l)	5.7 ± 1.80	9.2 ± 2.61	6.0 ± 1.29	9.5 ± 3.59
GGT (U/l)	16.0 ± 3.96	18.3 ± 3.05	19.5 ± 2.43	20.9 ± 3.19
CPK (U/l)	157.9±62.46	99.8 ± 43.94	125.9±68.88	104.9±31.72
Creatinine (mg/dl)	1.02 ± 0.17	0.98 ± 0.11	0.95 ± 0.17	0.93 ± 0.15
Urea (mg/dl)	37.2 ± 4.49	43.3 ± 11.25	38.5 ± 1.93	41.0 ± 5.21
Testosterone (ng/ml)	484.8±92.87	479.2±159.38	542.2±90.96	591.7±116.85
Free PSA (ng/ml)	0.18 ± 0.10	0.22 ± 0.11	0.34 ± 0.17	0.34 ± 0.14
Total PSA (ng/ml)	0.94 ± 0.90	0.81 ± 0.61	2.07 ± 1.85	1.94 ± 1.64
Estradiol (pg/ml)	36.0 ± 5.98	33.1 ± 5.52	36.1 ± 9.42	37.1 ± 4.51

Values are mean ± SD

n = 6, except for testosterone, free and total PSA in which n = 5

$p > 0.05$ for all comparisons (Stevioside group x Placebo group)

Table 5. Effect of stevioside (15 mg/kg/day) and placebo on sodium (Na^+), potassium (K^+) and chloride ($Cl^?$) levels

Parameters	Stevioside		Placebo	
	Before Treatment	**After Treatment**	**Before Treatment**	**After Treatment**
Blood K^+ (mEq/24 h)	3.8 ± 0.28	3.6 ± 0.26	4.0 ± 0.29	3.8 ± 0.22
Blood $Cl^?$ (mEq/24 h)	99.8 ± 2.11	102.5 ± 1.61	98.5 ± 0.96	103.3 ± 1.49
Blood Na^+ (mEq/l)	138.2 ± 2.19	139.3 ± 5.37	137.0 ± 1.29	139.7 ± 1.97
Urine Na^+ (mEq/24 h)	246.5 ± 89.8	190.7 ± 70.58	227.2 ± 49.92	228.8 ± 39.20

Values are mean ± SD (n = 6)

$p > 0.05$ for all comparisons (Stevioside group x Placebo group)

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