Indian Journal of Drugs, 2015, 3(4), 102-108 ISSN: 2348-1684

Review Article

ANTIHYPERTENSIVE (BLOOD PRESSURE LOWERING) EFFECTS OF STEVIOSIDE, FROM *STEVIA REBAUDIANA* BERTONI, ON RATS, DOGS AND HUMANS – A SHORT REVIEW

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Received: 01.08.2015
Accepted: 22.12.2015

Access this article online

Website:
www.drugresearch.in

Quick Response Code:

ABSTRACT

Here, we have reviewed antihypertensive (blood pressure lowering) effects of a natural, zero-calorie sweetener–'stevioside' (from the leaves of *Stevia rebaudiana* Bertoni) on rats, dogs and humans. The review of the medicative effects of stevioside on different animals (rats and dogs) and humans revealed that stevioside is a future plant drug (natural product) for treatment of hypertension.

KEY WORDS: *Stevia rebaudiana Bertoni, stevioside, natural zero-calorie sweetener, antihypertensive effects, blood pressure reducing effects.*

INTRODUCTION

Plants have always been an exemplary source of drugs and many of the currently available medicines have been directly or indirectly derived from plants. Therefore, the research and development for the use of natural products as therapeutic agents, especially those derived from plants, have been increasing in recent years. A great deal of attention has focused on the naturally occurring antispasmodic phytochemicals as potential drugs for the treatment of cardiovascular diseases. Arterial hypertension is a common and progressive disorder that poses a major risk for cardiovascular and renal diseases (Tirapelli et al., 2010). Hypertension most commonly referred as high blood pressure is a medical condition in which the blood pressure is chronically elevated. In recent years, it has become an increasingly important medical and public health problem worldwide. Overall, 26.4% of the adult population in year 2000 had hypertension and approximately 29.2% were projected to develop this condition by year 2025. The estimated total number of adults with hypertension in year 2000 was 972 million; of which 333 million in developed countries and 639 million in developing countries. The number of adults with hypertension in year 2025 has been predicted to increase by about 60% to a total of 1.56 billion populations (Verma et al., 2010).

Stevioside, a natural zero-calorie sweetener, is a diterpenoid glycoside, comprising an aglycone (steviol) and three molecules of glucose. This substance is a sweet glycoside extracted from Stevia rebaudiana Bertoni. This plant is a small shrub originally grown in South America, particularly in Brazil and Paraguay where it is known as Stevia or honey leaf. The major components of the leaf are stevioside (5-10% of total dry weight), rebaudioside A (2-4%), rebaudioside C (1-2%) and dulcoside A (0.4-0.7%). In addition to their sweetness, Stevia extract and stevioside have been used as a traditional medicine by local people in South America for hundreds of years (Kinghorn et al., 2002; Tirapelli et al., 2010). Currently, stevioside (Jeppesen et al., 2003) and medium-polar extract containing stevioside and other stevia glycosides (Misra et al., 2011) have shown good anti-diabetic effects. Accordingly, the effects of stevioside and extracts prepared from the leaves of Stevia on cardiovascular parameters are well demonstrated. The first experiment aiming to investigate the cardiovascular effects of this compound in rats was conducted in 1977 (Humboldt et al., 1977; Tirapelli et al., 2010). In this paper, we have collected studies performed over rats, dogs and humans to review those studies and to make a conclusion, whether stevioside can be used as an alternative molecule in treatment to combat hypertension (blood pressure-lowering) in a natural way.

ANTIHYPERTENSIVE EFFECTS OF STEVIOSIDE IN RATS

Stevioside is a sweet-tasting glycoside, composed of a diterpenic carboxylic alcohol with three glucose molecules, mainly used as a substitute for non-alcoholic sweetener. Since, it has previously been shown to reduce blood pressure in studies in animals and human, therefore, Chan et al. (1998) investigated the effect of intravenous stevioside on the blood pressure in spontaneously hypertensive rats (SHR). The hypotensive effect on both systolic and diastolic blood pressure was dose-dependent for intravenous doses of 50, 100 and 200 mg kg^{-1} in conscious SHR. The maximum reductions in systolic and diastolic blood pressure were 31.4±4.2% and 40.8±5.6% (mean ± SEM) respectively and the hypotensive effect lasted for more than 60 min with a dose of 200 mg kg^{-1}. Serum dopamine, norepinephrine and epinephrine levels were not changed significantly 60 min after intravenous injection of stevioside 100 mg kg^{-1} in anesthetized SHR. The data showed that stevioside given intravenously to conscious SHR was effective in blood pressure reduction and there was no change in serum catecholamines in anaesthetized animals with this natural compound (Chan et al., 1998). A study of Lee et al. (2001) shows that intraperitoneal injection of stevioside 25 mg kg^{-1} also has antihypertensive effect in spontaneously hypertensive rats (SHRs). In isolated aortic rings from normal rats, stevioside could dose-dependently relax the vasopressin-induced vasoconstriction in both the presence and absence of endothelium. However, stevioside had no effect on phenylephrine- and KCl-induced phasic vasoconstriction. In addition, stevioside lost its influence on vasopressin-induced vasoconstriction in Ca^{2+} free medium. The results indicate that stevioside caused vasorelaxation via an inhibition of Ca^{2+} influx into the blood vessel. This phenomenon was further confirmed in cultured aortic smooth muscle cells (A7r5). Using 10^{-5} M methylene blue for 15 min, stevioside could still relax 10^{-8} M vasopressin-induced vasoconstriction in isolated rat aortic rings, showing that this vasorelaxation effect was not related to nitric oxide. The data showed that the vasorelexation effect of stevioside was mediated mainly through Ca^{2+} influx inhibition (Lee et al. 2001). Hsu et al. (2002) had undertaken a study to evaluate the antihypertensive effect of stevioside in different strains of hypertensive rats and to observe whether there is difference in blood pressure lowering effect. Noninvasive tail-cuff method was employed to measure blood pressure. Stevioside at the concentrations of 50, 100 and 200 mg kg^{-1} were administered intraperitoneally (i.p.) to normotensive Wistar-Kyoto rats (NTR), SHR, deoxycorticosterone acetate-salt (DOCA-NaCl) sensitive hypertensive rats (DHR) and renal hypertensive rats (RHR). Significant hypotensive effect of stevioside administered i.p. was noted in different strains of rats at the dose of 50 mg kg^{-1}. When stevioside was

increased to the concentrations of 100 and 200 mg kg^{-1}, i.p., it also caused slow and persistent lowering of blood pressure in SHR and NTR. Data also showed that stevioside given at the concentrations of 100, 200 and 400 mg kg^{-1} i.p. resulted in lowering of blood pressure in SHR dose-dependently. Blood pressure returned to previous levels after the drug was discontinued for 2-3 days. Drinking of 0.1% stevioside solution in mature SHR could have antihypertensive effect and also prevented hypertension in immature SHR. This study reconfirmed stevioside has hypotensive effect and the effect is more prominent in hypertensive rats (Hsu et al. 2002). Jeppesen et al. (2003) have examined potential antihyperglycemic and blood pressure-lowering effects of stevioside, in a long-term study in the type 2 diabetic Goto-Kakizaki (GK) rats. Rats were fed 0.025 g Kg^{-1} day^{-1} of stevioside (purity > 99.6) for 6 weeks. An intra-arterial catheter was inserted into the rats after 5 weeks, and conscious rats were subjected to arterial glucose tolerance test (2.0 g Kg^{-1}) during week 6. Stevioside had an antihyperglycemic effect (incremental area under the glucose response curve [IAUC]): 985±20 (stevioside) versus 1,575±21 (control) mmol/L × 30 minutes, ($P < 0.05$), it enhanced the first-phase insulin response (IAUC: 343 ± 33 [stevioside] v 136 ± 24 [control] µU/mL insulin x 30 minutes, $P < 0.05$) and concomitantly suppressed the glucagon levels (total AUC: 2,026 ± 234 [stevioside] v 3,535 ± 282 [control] pg mL^{-1} × 180 minutes, $P < 0.05$). In addition, stevioside caused a pronounced suppression of both the systolic (135 ± 2 v 153 ± 5 mm Hg; $P < 0.001$) and the diastolic blood pressure (74 ± 1 v 83 ± 1 mm Hg; $P < 0.001$). Bolus injections of stevioside (0.025 g kg^{-1}) did not induce hypoglycemia. Stevioside augmented the insulin content in the beta-cell line, INS-1. Stevioside may increase the insulin secretion, in part, by induction of genes involved in glycolysis. It may also improve the nutrient-sensing mechanisms, increase cytosolic long-chain fatty acyl-coenzyme A (CoA), and downregulate phosphodiesterase 1 (PDE1) estimated by the microarray gene chip technology. In conclusion, stevioside enjoys a dual positive effect by acting as an antihyperglycemic and a blood pressure-lowering substance; effects that may have therapeutic potential in the treatment of type 2 diabetes and the metabolic syndrome (Jeppesen et al. 2003).

ANTIHYPERTENSIVE EFFECTS OF STEVIOSIDE IN DOGS

Previous studies have shown that it lowered blood pressure in spontaneously hypertensive rats by intravenous injection. This study was designed to evaluate the hypotensive effect of stevioside in dogs and to define the underlying mechanism. After nasogastric administration of stevioside powder (200 mg kg^{-1}), the blood pressure of healthy mongrel dogs began to significantly decrease at 60 min and returned to baseline level at 180 min. The reduction of blood pressure was more rapid (at 5-10 min) and effective after intravenous injection. However, no significant change of blood pressure was noted after injection through left vertebral artery, implicating that the hypotensive effect is not related to the central nervous system. Stevioside 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. However, no intracellular Ca^{2+} inhibitory effect was observed in calcium-free medium, implicating that stevioside may inhibit the Ca^{2+} influx from extracellular fluid. Our present data show that stevioside did not influence the calcium ionophore (A23187) induced Ca^{2+} influx, indicating that the antagonistic effect was through Ca^{2+} channels. This study confirmed that stevioside is an effective antihypertensive natural product, and its hypotensive mechanism may be probably due to inhibition of the Ca^{2+} influx (Liu et al., 2003).

ANTIHYPERTENSIVE EFFECTS OF STEVIOSIDE ON HUMANS

Chan et al. (2000) evaluated the effect of stevioside in human hypertension. A multicentre, randomized, double-blind, placebo-controlled study was undertaken. This study group consisted of 106 Chinese hypertensive subjects with diastolic blood pressure between 95 and 110 mmHg and ages

ranging from 28 to 75 years with 60 subjects (men 34, women 26; mean ± s.d., 54.1 ± 3.8 years) allocated to active treatment and 46 (men 19, women 27; mean ± s.d., 53.7 ± 4.1 years) to placebo treatment. Each subject was given capsules containing stevioside (250 mg) or placebo thrice daily and followed-up at monthly intervals for 1 year. After 3 months, the systolic and diastolic blood pressure of the stevioside group decreased significantly (systolic: 166.0 ± 9.4–152.6 ± 6.8 mmHg; diastolic: 104.7 ± 5.2–90.3 ± 3.6 mmHg, $P < 0.05$), and the effect persisted during the whole year. Blood biochemistry parameters including lipid and glucose showed no significant changes. No significant adverse effect was observed and quality of life assessment showed no deterioration. This study shows that oral stevioside is a well-tolerated and effective modality that may be considered as an alternative or supplementary therapy for patients with hypertension (Chan et al. 2000).

Hsieh et al. (2003) investigated the long-term (2-year) efficacy and tolerability of stevioside in patients with mild essential hypertension. In addition to it, effects of stevioside on left ventricular mass index (LVMI) and quality of life (QOL) were also determined. This was a multicenter, randomized, double-blind, placebo-controlled trial in Chinese men and women aged between 20 and 75 years with mild essential hypertension (systolic blood pressure [SBP] 140-159 mm Hg and diastolic blood pressure [DBP] 90-99 mm Hg). Patients took capsules containing 500 mg stevioside powder or placebo 3 times daily for 2 years. Blood pressure was measured at monthly clinic visits; patients were also encouraged to monitor blood pressure at home using an automated device. LVMI was determined by 2-dimensional echocardiography at baseline and after 1 and 2 years of treatment. QOL was assessed using the Medical Outcomes Study 36-Item Short-Form Health Survey. Electrocardiographic, laboratory, and QOL parameters were assessed at the beginning of treatment, and at 6 months, 1 year, and 2 years. One hundred seventy-four patients (87 men, 87 women) were enrolled in the study, and 168 completed it: 82 (42 men, 40 women; mean [SD] age, 52 [7] years) in the stevioside group and 86 (44 women, 42 men; mean age, 53 [7] years) in the placebo group. After 2 years, the stevioside group had significant decreases in mean (SD) SBP and DBP compared with baseline (SBP, from 150 [7.3] to 140 [6.8] mm Hg; DBP, from 95 [4.2] to 89 [3.2] mm Hg; $P < 0.05$) and compared with placebo ($P < 0.05$). Based on patient's records of self-monitored blood pressure, these effects were noted beginning approximately 1 week after the start of treatment and persisted throughout the study. There were no significant changes in body mass index or blood biochemistry, and the results of laboratory tests were similar in the 2 groups throughout the study. No significant difference in the incidence of adverse effects was noted between groups, and QOL scores were significantly improved overall with stevioside compared with placebo ($P < 0.001$). Neither group had a significant change in mean LVMI. However, after 2 years, 6 of 52 patients (11.5%) in the stevioside group had left ventricular hypertrophy (LVH), compared with 17 of 50 patients (34.0%) in the placebo group ($P < 0.001$). Of those who did not have LVH at baseline, 3 of 46 patients (6.5%) in the stevioside group had developed LVH after 2 years, compared with 9 of 37 patients (24.3%) in the placebo group ($P < 0.001$). In this 2-year study in Chinese patients with mild hypertension, oral stevioside significantly decreased SBP and DBP compared with placebo. QOL was improved, and no significant adverse effects were noted (Hsieh et al. 2003).

The antihypertensive effect of crude stevioside obtained from the leaves of *S. rebaudiana* Bertoni 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^{-1} day^{-1} (7 weeks), 7.5 mg kg^{-1} day^{-1} (11 weeks) and 15.0 mg kg^{-1} day^{-1} (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^{-1} day^{-1} 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 (Ferri et al. 2006).

DISCUSSION

Study of Chan et al. (1998) for intravenous (i.v.) delivery effect of stevioside (50, 100 and 200 mg Kg^{-1}) on blood pressure in SHR revealed that hypotensive effect was dose-dependent and maximum reductions were 31.4±4.2% and 40.8±5.6% for systolic and diastolic blood pressure, respectively and the hypotensive lasted for 60 minutes with a dose of 200 mg Kg^{-1}. Serum constitution was not changed significantly. Similar results were obtained in the study of Lee et al. (2001) [8] for intraperitoneal (i.p.) delivery of stevioside (at 25 mg Kg^{-1}). Results revealed that stevioside could relax the vasopressin-induced vasoconstriction in both the presence and absence of endothelium, in a dose-dependent manner. The data also revealed that the vasorelexation effect was mediated mainly through inhibition of Ca^{2+} influx. Similar mechanism was demonstrated by Liu et al. (2003) in healthy mongrel dogs and Liu et al. (2003) further revealed that no intracellular Ca^{2+} inhibitory effects was observed in calcium-free medium. Hsu et al. (2002) had evaluated effects of stevioside (50 mg Kg^{-1}) in different strains of hypertensive rats and to observe whether there is strain-based difference in blood pressure lowering effect; results showed that significant hypotensive effect was noted in different strains of rats. When stevioside was increased to the concentration of 100 and 200 mg Kg^{-1}, i.p., it also caused slow but persistent lowering of blood pressure in SHR & NTR. Data revealed that lowering of blood pressure in SHR was in dose-dependent manner. Blood pressure returned to previous levels after the drug was discontinued for 2-3 days. Study of Jeppesen et al. (2003) revealed antihypertensive and antihyperglycemic effects of stevioside in type 2 diabetic GK rats, where rats were fed 0.025 g Kg^{-1} day^{-1} of stevioside (purity > 99.6) for 6 weeks. An intra-arterial catheter was inserted into the rats after 5 weeks, and conscious rats were subjected to arterial glucose tolerance test (2.0 g Kg^{-1}) during week 6. Stevioside had an antihyperglycemic and antihyperglycemic effects, which caused a pronounced suppression of both the systolic (135 ± 2 v 153 ± 5 mm Hg; P <0.001) and the diastolic blood pressure (74 ± 1 v 83 ± 1 mm Hg; P <0.001). Bolus injections of stevioside (0.025 g kg^{-1}) did not induced condition of hypoglycemia, which was further confirmed in one of our study (Misra et al., 2011). Stevioside augmented the insulin content in the beta-cell line, INS-1. Stevioside may increase the insulin secretion, in part, by induction of genes involved in glycolysis. It may also improve the nutrient-sensing mechanisms, increase cytosolic long-chain fatty acyl-coenzyme A (CoA), and downregulate phosphodiesterase 1 (PDE1) (Jeppesen et al., 2003). During human study, Chan et al. (2000) performed a multi-centre, randomized, double blind, and placebo-controlled study to evaluate the effect of stevioside in human hypertension. This study was performed on 106 Chinese hypertensive subjects (men and women) with diastolic blood pressure between 95 and 110 mmHg and ages were ranging from 28 to 75 years. After three months of study, the systolic and diastolic blood pressure decreased significantly and the effect persisted during the whole year. Blood biochemistry parameters including lipid and glucose showed insignificant changes. Additionally, no significant adverse effect was observed and quality of life assessment showed no deterioration. Similarly, Hsieh et al. (2003) also investigated the long-term (2years) efficacy and tolerability of

stevioside in 168 human patients with mild essential hypertension. In addition to above, effects of stevioside on left ventricular mass index (LVMI) and quality of life (QOL) were also determined. Patients took 500mg stevioside capsules or placebo 3 times daily for 2 years. The results revealed that there were no significant changes in body mass index or blood biochemistry, and oral stevioside significantly decreased SBP and DBP compared with placebo and QOL was improved and no significant adverse effects were noted. Ferri et al (2006) carried out human study in which antihypertensive effect of crude stevioside on previously untreated mild hypertensive patients was examined with low dosage of crude stevioside (3.75 to 15 mg Kg^{-1} day^{-1}) twice a daily (b.i.d.), i.e. before lunch and before dinner. All adverse events were prospectively recorded but no major adverse clinical effects were observed. SBP and DBP decreased ($p < 0.05$) with crude stevioside intake, but a similar effect was observed in the placebo group, therefore, crude stevioside up to 15.0 mg kg^{-1} day^{-1} 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.

CONCLUSION

Stevioside enjoys a dual positive effect by acting as an antihyperglycemic and a blood pressure-lowering substance; effects that may have therapeutic potential in the treatment of type 2 diabetes and the metabolic syndrome. Based on above studies performed on rats of same and different strains, dogs and humans, pure stevioside is seems to be effective for the treatment of mild essential hypertension, while crude stevioside was ineffective up to the dose of 15 mg Kg^{-1} Day^{-1}. Further, all the studies indicated stevioside to be safe and support the well-established tolerability during long term use as a sweetener.

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