Effects of *Stevia Rebaudiana* on Glucose Homeostasis, Blood Pressure and Inflammation: A Critical Review of Past and Current Research Evidence

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Abstract

The prevalence of obesity and its related comorbidities continues to rise in the United States and worldwide. Insulin resistance, increased inflammation and oxidative stress are the major pathogenic mechanisms involved in obesity-associated co-morbid conditions. Major efforts to curb the rising tide of obesity, including lifestyle modifications, anti-obesity medications and surgical interventions have shown minimal success. Therefore, introducing new methods to combat obesity, diabetes and associated disorders are desperately needed. *Stevia rebaudiana*, a natural, non-caloric sweetener has generated significant interest in the scientific community due to its effects on glucose homeostasis, blood pressure and inflammation, all known consequences of obesity. In this review, we assess the effects of Stevia on these parameters in humans as well as in animal models, highlighting its potential role as an effective intervention for the major cardiovascular risk factors associated with obesity.

Keywords

Stevia rebaudiana; Obesity; Natural sweetener; Diabetes; Glucose Homeostasis; Hypertension; Inflammation

Introduction

The pandemic of obesity continues to represent a major public health challenge in the United States and around the world, leading to increased rates of diabetes, hypertension, cardiovascular disease, obstructive sleep apnea and cancer, among other complications [1–8]. From 1960–2014, the prevalence of obesity in the U.S. increased from 11% to 35% in men, and from 16% to 40% in women, [9] and the prevalence of diabetes mellitus increased

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along with it, rising from 1% to 7% of the U.S. population. Today, 30.2 million people (12.2% of the U.S.) have diabetes mellitus, and 84.1 million (33.9% of the U.S.) have pre-diabetes [10]. Over the past decades, artificial (non-nutritive) sweeteners have increased in popularity as a promising way to decrease caloric intake when used to replace sucrose (table sugar). Use of artificial sweeteners may enable consumption of up to 380 fewer calories per day [11]. Currently available artificial sweeteners, such as aspartame, saccharin, sucralose and acesulfame have possible drawbacks, including a purported association with cancer, negative taste profile, increased risk of metabolic syndrome and obesity, alteration of gut microbiota, neurotransmitter impairment and negative pregnancy outcomes [12–18].

Due to the possible side effects of artificial sweeteners, steviol glycosides (Stevia) have gained popularity as a natural alternative. Stevia rebaudiana bertoni is a perennial shrub in the asteraceae family, native to southern Brazil and northern Paraguay. The plant's leaves contain diterpene steviol glycosides, the most abundant of which are stevioside and rebaudiosides A and C, which are 250–300 times sweeter than sucrose, [11,14,16,19–24] enabling their use as a potent sweetener. Commercial stevia mixtures are composed of roughly 80% stevioside, 8% rebaudioside A and 0.6% rebaudioside C. Upon consumption of stevia, the steviol glycosides are hydrolyzed to aglycone steviolin the colon by human intestinal microflora, specifically those from the bacteroidaceae family [25,26]. Some free steviol is excreted in feces, while some is absorbed into the bloodstream and metabolized by the liver into steviol glucuronide, which is excreted in urine [26,27].

For centuries, Stevia has been used by the endogenous people of South America for both sweetening and medicinal purposes; and for many years, it has been a popular sweetening agent in East Asian countries, with Japan currently consuming more Stevia than any other country [20]. Stevia was only more recently approved for commercial use in the United States (1995), European Union (2011) and Canada (2012). In contrast to artificial sweeteners, there are no reported negative health consequences of Stevia, such as toxicity, teratogenicity, mutagenicity or carcinogenicity. In contrast, the anti-hyperglycemic, antioxidant and antihypertensive properties of stevioside have been well documented, suggesting a potential medicinal use as an adjunctive treatment for several diseases.

Anti-hyperglycemic Properties

Stevia glycosides have been widely shown to prevent weight gain and decrease serum glucose levels in animal and human models. As with any zero-calorie sweetener, stevia lacks calories and reduces serum glucose levels that typically rise about 1 hour after carbohydrate consumption, compared to sucrose. However, a 2010 study by Kujur et al. found that stevia significantly reduces mean serum glucose levels in subjects over a 1-month period. The study used Wister rats with diabetes induced by 5% alloxan monohydrate, and found that administering 50 mg/kg and 100 mg/kg of stevia daily resulted in significant time-dependent anti-hyperglycemic effects. When treated with 50 mg/kg of stevia in the aqueous, ether and methanolic extracts for 28 days, mean serum glucose levels fell from 220 to 161 mg/dL; 220 to 171 mg/dL; and 232to 163mg/dL, respectively. When treated with 100 mg/kg of the stevia extracts, mean serum glucose levels fell from 220 to 137 mg/dL; 209 to 168 mg/dL; and 218 to 181 mg/dL, respectively. There were non-significant differences in serum glucose levels

between the 50mg/kg and 100mg/kg doses. Rats administered glyburide, a known oral diabetes medication that was used as a positive control, hada reduction in mean serum glucose levels from 211 to 101 mg/dL after 28 days. Thus, the administration of stevia to diabetic rats lowered serum glucose levels to a slightly less degree compared to treatment with glyburide [20].

Steviosides were found to have similar anti-hyperglycemic effects in human subjects. A 2004 study by Gregersen et al. enrolled subjects with type 2 diabetes in a paired-crossover study. On two occasions, subjects were given a standard test meal of 412 kcal (1,725 kJ) along with 1g of stevioside or 1g of maize (control), and their serum glucose, insulin, glucagon, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) levels were measured at 30-minute intervals, starting 30 minutes before the study and ending 240 minutes after the test meal was given. The investigators found that 240 minutes after administration of stevioside, mean postprandial blood glucose levels were significantly reduced compared to controls, a difference of 18%. There was also a significant 40% increase in the insulinogenic index ($AUC_{i,insulin}/AUC_{i,glucose}$), a marker of beta cell function, in patients administered stevioside compared to controls. Investigators found no significant differences in circulating insulin, glucagon, GLP-1, GIP, triglyceride or free fatty acid levels [21].

Several groups have investigated the mechanisms by which stevia extracts decrease serum glucose levels. An early study by Jeppesen et al. examined the effects of steviol and stevioside on insulin secretion from incubated mouse islet cells and the beta-cell line, INS-1. Cells extracted from mouse pancreases were incubated with 16.7 mmol/L glucose and either stevioside or steviol, in concentrations varying from 1 nmol/L to 1 mmol/L. The investigators found that both stevioside and steviol increased insulin secretion from the cells, with maximal effects at 1mmol/L for stevioside and 1 umol/L for steviol. Of note, significant increases in insulin secretion due to stevia occurred only when glucose serum levels were 8.3 mmol/L or higher. Non-significant increases in insulin secretion occurred when glucose serum levels were between 3.3 mmol/L and 8.3 mmol/L, and no change in insulin secretion due to stevia was detected when glucose levels were 3.3 mmol/L or lower [22]. Hypoglycemia, a dangerous and potentially fatal outcome of some anti-hyperglycemic agents, is generally believed to become symptomatic when serum glucose levels are at or below 3.9 mmol/L (70 mg/dl), although this can vary per individual. Symptoms, which are neurogenic and neuroglycopenic in nature, include palpitations, tremor, sweating, confusion, seizure, coma and death [23]. Based on results from the Jeppesen study, stevia extracts cause glucose-mediated insulin secretion without the risk of hypoglycemia.

The specific molecular mechanisms by which stevia extracts increase insulin secretion was the subject of a 2017 study by Philippaert et al. This team investigated the effects of stevioside on transient receptor potential cation channel subfamily melastatin 5 (TRPM5), a monovalent Ca^{2+}-activated cation channel found in type II taste receptor cells and pancreatic beta cells. TRPM5 is involved in the perception of sweet, bitter and umami tastes and in insulin secretion from the pancreas. Using a whole-cell patch-clamp technique, the investigators measured the current through the TRPM5 channel and found that in the presence of steviol and stevioside, there was an increased frequency of Ca^{2+} oscillations

inside the cells and delayed inactivation of Ca^{2+}-activated TRPM5 current. This effect was seen with an effector concentration for half-maximum response of 690nM, in glucose concentrations exceeding 3 mM. The investigators also found that the presence of stevioglycosides increases the frequency of glucose-stimulated action potentials and shifts voltage-dependent activation of TRPM5 towards more negative membrane potentials, from a $V_{1/2}$ of +145 mV to +42.8 mV. A more negative resting membrane potential causes cell depolarization and subsequent signal potentiation to occur with less electrical input. Collectively, these findings suggest that stevioside directly interacts with TRPM5 channels, prolongs their activation and increases the release of insulin.

The investigators linked their findings *in vitro* to the prevention of diabetes with *in vivo* studies on wild type and TRPM5 knock out (TRPM5−/−) mice. After placing mice on a 20-week high-fat diet, they found that wild type mice were protected against the development of diabetes when co-administered 25 mg/kg/day stevioside. After a 2-hour glucose challenge, the wild type mice had plasma glucose levels peaking at 300 mg/dl, while the wild type mice treated with stevioside had significantly lower plasma glucose levels, 220 mg/dl. In contrast, the TRPM5−/− mice saw no significant difference in mean glucose levels after 20 weeks of a high-fat diet, regardless of treatment with 25 mg/kg/day stevioside. Taken together, these data illustrate that stevioglycosides potentiate the TRPM5-mediated glucose-induced depolarizing current in pancreatic beta cells, enhancing insulin secretion and resulting in lower long-term serum glucose levels [28].

Stevioside has also been found to increase the translocation of Glut4to the plasma membrane. Glut4, an insulin-regulated glucose channel present primarily in adipose tissue and striated muscle, is the channel through which glucose enters cells from the bloodstream. In the presence of insulin, Glut4 is transported from cytosolic storage vesicles to the plasma membrane [29]. In their 2017 study, Prata et al. examined the relationship between Glut4 translocation and stevioside exposure in neonatal rat cardiac fibroblasts. Using Glut4-specific antibody targeting and confocal microscopy, the investigators demonstrated that Glut4 translocation occurs after 1-hour incubation with steviol glycosides 1 mg/mL, with similar efficiency to when the cells are incubated with100 nM insulin. The investigators correlated these findings to rates of glucose uptake into the cells. After incubation with insulin, glucose uptake into cells was 139% of the control (cells which were neither incubated with insulin or stevioside); after incubation with stevioside mixtures, glucose uptake was 117% of control for mixture 1 (>97% rebaudioside A and <3% other steviol glycols), 126% of control for mixture 2 (63.4% rebaudioside A, 22.85% stevioside, and 8.2% rebaudioside C), 135% of control for mixture 3 (>50% rebaudioside A and >25% stevioside), and 120% of control for mixture 4 (>50% rebaudioside A, 25% stevioside and 20% other steviol glycosides). In summary, regardless of the relative content of the stevioside mixture, incubation with steviol glycosides significantly enhanced Glut4 translocation and glucose uptake, similar in efficacy to incubation with insulin.

The authors investigated the mechanism by which stevioside increases Glut4 translocation and found it to be due to activation of PI3K/Akt pathway, a known intracellular signaling sequence triggered when insulin binds to insulin receptors. Elevated phosphorylation of

molecules in this pathway, IGF-1R, PI3K and Akt, when treated with either insulin or the stevioside mixtures indicated a common pathway for insulin and stevioside signaling [30].

Finally, stevioglycosides were also found to decrease serum glucose levels by tampering the process of gluconeogenesis. A 2005 study by Chen et al. demonstrated stevioside's inhibitory effect on the activity of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme required for gluconeogenesis. The investigators administered stevioside to diabetic mice twice daily for 15 days in concentrations of 0.5 mg/kg, 1.0 mg/kg and 5.0 mg/kg; at the end of the 15 days, they compared PEPCK mRNA and protein levels to diabetic mice administered physiological saline as a control. The researchers found that mean levels of both PEPCK mRNA and protein were significantly and dose-dependently reduced in subjects given stevioside. For the physiological saline and increasing stevioside doses of 0.5 mg/kg, 1.0 mg/kg and 5.0 mg/kg, PEPCK mRNA levels were 1.18, 0.81, 0.56 and 0.35, and PEPCK protein levels were 0.99, 0.75, 0.57 and 0.52, respectively [31]. From these data, the researchers concluded that in addition to enhancing insulin secretion, stevioside prevents the synthesis of new glucose by inhibiting PEPCK gene expression.

Anti-inflammatory and Antioxidant Properties

Obesity is considered a pro-inflammatory state with chronic, low-grade inflammation of adipose tissue. Dysregulation of immune T cells and B cells and elevation of inflammatory markers, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in obese individuals are known to contribute to the development of diabetes mellitus [32,33]. Recent evidence has found that stevioside prevents the pro-inflammatory state of obesity and thus is protective against the development of insulin resistance. A 2012 study by Wang et al. examined how the addition of stevioside to diets prevents the production of inflammatory markers in mice. In this study, subjects were fed a normal or high-fat diet for four months and in the fourth month either 10 mg/kg/day of stevioside or a control vector was added to their diets. Following this, mRNA levels of inflammatory cytokines TNF-α, IL-6, IL-10, IL1-β, MIP-1α, KC, CD11b and CD14 found in adipose tissue were measured by quantitative reverse transcriptase PCR. The investigators found that after three months, the mice that were fed the high fat diet had significantly elevated levels of all inflammatory markers in their adipose tissue compared to mice that were fed a normal diet, suggesting the pro-inflammatory state of obesity had been achieved. Using immunofluorescence staining against macrophage markers F4/80, CD11b and MIP-1α, they also found significant macrophage infiltration in the adipose tissue of mice fed the high fat diet compared to those fed the normal diet. With the addition of daily stevioside to the high fat diet in the fourth month, levels of all inflammatory markers were significantly reduced compared to the addition of the control vector. The relative levels of the inflammatory markers in the stevioside to control groups were TNF-α: 3 to 4.8, IL-6: 4 to 8, IL-10: 1 to 7.2, IL1-β: 1 to 4, MIP-1α: 2.2 to 6.2, KC: 3.8 to 13, CD11b: 2 to 2.2, and CD14: 1 to 3. Similarly, addition of stevioside in the fourth month significantly reduced macrophage infiltration in adipose tissue of mice fed the high fat diet.

To determine the mechanism by which stevioside down-regulates inflammatory cytokine levels in adipose tissue, the investigators examined the effect of stevioside on the NF-kB

pathway. NF-kB is a transcription factor and ubiquitous regulator of immune responses. Normally activated in response to stressors such as infectious pathogens, UV damage and free radicals, it is over-activated in many autoimmune diseases and inflammatory conditions, including obesity. To measure NF-kB activity, Wang et al. performed western blots to measure levels of phosphorylated-IKKβ and phosphorylated-IKBα, markers of NF-kB pathway activation. They found significant upregulation of both markers in the high fat diet group after 3 months, and significantly reduced levels in the high fat diet group following the month of stevioside administration compared to control vector [34]. Taken together, this study's data suggest a role for stevia in reducing the inflammatory state associated with obesity and insulin resistance, at least in part by reducing NF-kB pathway activity.

A 2019 study by Casas-Grajales et al. examined the anti-inflammatory properties of steviosides in the liver. The investigators induced liver injury using thioacetamide (TAA), a hepatotoxin that induces fibrosis and cirrhosis, in Wistar rats. Rats were administered 200 mg/kg of TAA thrice daily for 8 weeks, with or without coadministration of twice daily intraperitoneal stevioside 20 mg/kg. The rats who were given TAA alone exhibited altered liver morphology and disrupted liver parenchyma, resulting in the formation of necrotic cells and hepatocyte nodules as seen by microscopy. The rats who were co-administered stevia with TAA experienced significantly fewer morphological and histological changes. No hepatic changes were seen with administration of 20 mg/kg stevioside to the control rats (no TAA treatment). A principal function of the liver is energy storage in the form of glycogen, so liver function in the Wistar rats was assessed by measuring glycogen levels in the liver samples. In control rats, glycogen levels were 7.12 g per 100 g of liver; in the rats given TAA, glycogen levels were significantly reduced to 1.36 g; and in the rats co-administered TAA and stevioside, glycogen levels were 3.76 g, a significant increase from glycogen levels in the TAA alone group ($p < 0.05$). Thus, the administration of steviosides was able salvage liver function to a significant degree.

To probe the mechanisms by which stevioside can reduce or prevent hepatic damage, these authors also investigated the NF-kB pathway. They used immunohistochemistry staining to examine p65 expression in the livers of the mice treated with TAA or co-treated with TAA and stevioside. In the TAA-treated group, there was significant elevation of p65 expression, 0.125% of the stained liver section, compared to in the control group, 0.025% of the stained section; and in the TAA-stevioside group, there was significantly less p65 expression than in the TAA alone group, 0.091% of the section. These data were supported by qRT-PCR and western blot analyses, which showed p53 mRNA and protein levels elevated in the TAA-treated rats, with significant recovery by co-administration with stevioside. mRNA and protein levels of pro-inflammatory cytokines IL-17a, IL1β, TNF-α, IL-6 and IL-10 were also found to be elevated due to TAA, which was prevented by stevioside co-treatment. Compared to cytokine protein levels in control mice, relative levels when treated with TAA and TAA + STV were: IL-17a 4.1 and 2.9; IL1β 3.0 and 1.25; TNF-α 3.2 and 0.95; IL-6 7.0 and 5.2; and IL-10 3.8 and 2.4. These data show that expression of all cytokines was significantly elevated by TAA-induced injury, and significant recovery was seen by co-administration with stevioside [35].

The ability of stevioside to counteract free radicals and reduce oxidative damage has been well-documented [36,37], but the mechanism of action was only recently elucidated. Several studies found that stevioside enhances the endogenous pathway of nuclear factor erythroid 2 related factor 2 (Nrf2) [35,38], a transcription factor that regulates expression of enzymes to counteract oxidative stresses [39]. In one such study, Ramos-Tavor et al. [38] demonstrated the ability of stevia extract to prevent carbon tetrachloride (CCl_4)-induced cirrhosis in rats. The investigators injected CCl_4 400 mg/kg three times per week into the peritoneum of experimental rats for 12 weeks to induce cirrhosis. These experimental rats were compared to subjects administered the same regimen of CCl_4 plusoral stevia extract 50 mg/1 mL water once daily, and to control subjects, who were administered 1 mL water daily. In the CCl_4 only subjects, the investigators found a significant decrease in Nrf2 levels compared control subjects: 0.35 compared to 1. The diminished Nrf2 levels were seen alongside significant macro-and microscopic alterations to the rat livers: steatosis, disrupted liver parenchyma, hyperchromatic hepatocytes and atypical and pleomorphic nuclei. Conversely, in the stevia + CCl_4 group, there was no significant difference in Nrf2 levels compared to controls: 0.95 compared to 1. In these subjects, there was no change in liver morphology or histology. Using glycogen stores as a measure of hepatic function, the investigators measured amounts of glycogen per 100 g of liver and found 4.8 g of glycogenin the control subjects, 0.76 g of glycogen in the CCl_4-treated subjects and 4.8 g of glycogenin the CCl_4+ stevia subjects. Thus, there was complete recovery of hepatic function with co-administration of stevioside. Collectively, the investigators concluded that stevia extractspreventCCl$_4$-induced hepatic damage and dysfunction by bolstering the Nrf2 antioxidant response [40].

Satiety and Weight Loss Properties

Several studies have investigated the relationship between stevia consumption, satiety and weight loss. In a 2016 study, Abo Elnaga et al. administered stevia to rats in 25 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg daily doses for 12 weeks and found a positive correlation between stevia consumption, decreased food intake and weight loss, compared to mice administered water and sucrose as negative and positive controls, respectively. Mice who received the highest dosage of stevia, 1000 mg/kg/day, consumed the least amount of food, 7.86 g/day, followed by the mice who received stevia doses of 500 mg/kg/day, 250 mg/kg/day, and 25 mg/kg/day, who consumed 8.5 g/day, 12.8 g/day and 13.8 g/day of food, respectively. There was also a positive correlation between stevia dose and weight loss. For the 1000 mg/kg/day, 500 mg/kg/day, 250 mg/kg/day, and 25 mg/kg/day stevia groups, weight loss was −48.29%, −44.98%, −41.38% and −40.29%, respectively, compared to weight gain in both the negative and positive control groups of +25.12% and +27.88%, respectively. The authors suggest poor palatability of stevia may be the cause of reduced appetite and increased weight loss, as measured by decreasing feed efficiency ratios as stevia doses increase: −2.91, −3.22, −5.21 and −6.14 for the 25 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg per day groups. There were no significant differences in organ weight (liver, heart, kidney, lung, pancreas, spleen and brain) between stevia and control groups, but in agreement with aforementioned studies, plasma glucose concentrations were significantly reduced after 12 weeks of stevia treatment in a dose-dependent manner [41]. Similar results

were found in other studies, in which rats fed stevia were compared to control subjects who were fed sucrose or water [42,43].

A 2010 study conducted by Anton et al. examined the effect of stevia consumption on satiety in human subjects, using lean (BMI= 19.5–24.9 kg/m^2) and obese (BMI= 30–39.9 kg/m^2) individuals. Subjects attended three study days, in which they were given 469 kcal breakfasts and buffet style lunches and dinners from which they could eat as much as they wanted. 20 minutes before lunch and dinner, participants were blindly given snacks containing stevia, aspartame or sucrose to see if sweetener consumption affected the amount of food consumed in the meals. When the number of calories consumed from the lunch and dinner buffets was calculated and subjective satiety levels were tallied, investigators did not find significant differences in either parameter between the three groups. They only found significantly lower total daily calorie intake in the stevia (2257 kcal/day) and aspartame (2248 kcal/day) groups compared to the sucrose group (2557 kcal/day) due to the zero-calorie nature of the sweeteners [44]. Their data overall demonstrate no change in appetite and food consumption with artificial sweetener use, however long-term conclusions cannot be drawn as food intake was only measured over single study days. A long-term, blinded interventional study would be necessary for drawing any conclusions about stevia's effect on appetite and weight loss in people.

Protective Properties Against Cardiovascular Disease

One of the most common complications of diabetes mellitus is cardiovascular disease, and recent studies have suggested that stevia and its metabolites stevioside, rebaudioside A and steviol promote cardiovascular health and reduce hypertension. A 2010 study by Geeraert et al. examined the effects of stevia on cardiovascular parameters in double LDL and leptin receptor knock-out (DKO) mice, subjects chosen because they exhibit many properties of metabolic syndrome. The DKO mice were given 10 mg/kg stevia or saline solution daily for 12 weeks, after which investigators measured a variety of parameters, including levels of adiponectin, IL-6, TNF-α and autoantibodies against MDA-modified low-density lipoprotein, representative of oxidized low-density lipoprotein (ox-LDL). The investigators found significantly higher levels of adiponectin, a hormone that promotes lipolysis, in the stevia-treated group (6,517 mg/ml) compared to controls (3,296 mg/ml). They also found significantly lower levels of total cholesterol and ox-LDL, 10.71mmol/L and 3.45, respectively, in the stevia-treated group compared to control-group, 13.51 mmol/L, and 9.20, respectively. They did not find significant differences in weight, triglycerides, IL-6 or TNF-α between the two groups. The investigators measured atherosclerosis by immunohistochemically staining smooth muscle and plaque components in harvested aortic tissue from the mice. There was decreased atherosclerosis in the DKO mice treated with steviosides, which was attributed to decreased infiltration of macrophages, lipids and ox-LDL [45].

Multiple clinical studies have investigated the purported antihypertensive effects of stevia compounds. Chan et al. designed a multi-center, double-blind, placebo-controlled study using 106 adults with mild to moderate hypertension (baseline diastolic blood pressures ranging from 95–110 mmHg), who were otherwise healthy. The subjects were instructed to

take capsules containing 250 mg of stevioside or placebo three times daily and were followed up monthly for 1 year. The investigators found that after only 3 months, mean systolic blood pressure decreased significantly from 166.5 mmHg to 152.6 mmHg, and mean diastolic blood pressure decreased significantly from 102.1 mmHg to 90.3 mmHg. These blood pressure changes were maintained for the duration of the 1-year study period. In contrast, there were no significant changes in the control group, in which systolic and diastolic blood pressures decreased from 166.0 mmHg to 164.8 mmHg, and 104.7 mmHg to 103.8 mmHg, respectively [46].

Similar results were seen in a 2-year multicenter, double-blind placebo-controlled trial conducted by Hsieh et al., which examined patients with primary hypertension. The patients, all of whom had blood pressure 140–159/90–99 mmHg at baseline, were given 500 mg stevioside powder or placebo three times a day and monitored monthly for 24 months. Those treated with stevioside had significant reductions in blood pressure: mean systolic blood pressure decreased from 150 mmHg to 140 mmHg, and mean diastolic blood pressure decreased from 95 mmHg to 89 mmHg. In contrast, the placebo group had non-significant changes: mean systolic blood pressure increased from 149 mmHg to 150 mmHg and mean diastolic blood pressure decreased from 96 mmHg to 95 mmHg [47].

The specific mechanism by which stevioside causes vasorelaxation was explored by Lee et al. in their 2001 study. The investigators incubated aortic rings constricted by vasopressin or phenylephrine, in medium containing stevioside with or without calcium. In the Ca^{2+}-containing medium, stevioside 10–5 M relaxed the constricted aortic rings by $54.9 \pm 6.5\%$ while in the Ca^{2+}-free medium, no vasorelaxation was seen. In the Ca^{2+}-containing medium, the presence of stevioside decreased the intracellular calcium concentration significantly from 339.6 ± 18.4 to 102.2 ± 13.2 nM, and from 651.0 ± 13.4 to 229.5 ± 19.2 nM, when vasoconstriction was induced by vasopressin or phenylephrine, respectively. Conversely, in the Ca^{2+}-free medium, the concentration of intracellular calcium did not change from 428.8 ± 47.7 nM in the presence of stevioside. Knowing that vasopressin causes vasoconstriction by increasing the influx of calcium into cells, the investigators concluded that stevioside induces vasorelaxation by inhibiting the influx of calcium into cells from the extracellular space [48].

In contrast to studies involving hypertensive subjects, data involving normotensive subjects show no effects of stevia on blood pressure. A 2008 study by Maki et al. found no significant differences in systolic or diastolic blood pressures after 4 weeks of rebaudioside A 1000 mg/kg/day administration in healthy individuals with normal or low-normal blood pressures (systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg). In the treatment group, mean systolic blood pressures, diastolic blood pressures and mean arterial pressure (MAP) each decreased by 1.3 mmHg, while the respective categories decreased by 0.4 mmHg, 0.7 mmHg and 0.6 mmHg in the control group [49]. A 2008 study by Barriocanal et al. supported these findings, as they also found no significant differences in blood pressure changes between groups given 250 mg stevia 3 times daily or a placebo for 3 months. This study used subjects with type 1 diabetes, type 2 diabetes, or no diabetes with normal or low-normal blood pressures. The only significant finding was a decrease in systolic blood pressure in the type 1 diabetic group: mean 24-hour SBP fell from 117.1

mmHg to 115.9 mmHg in the stevia treated group and from 108.3 mmHg to 105.7 mmHg in the placebo group [50]. Stevioside exhibits promising therapeutic activity in individuals with hypertension, without the potential for causing hypotension, demonstrating its clinical utility for treatment of cardiovascular patients.

Safety Profile

Steviosides are relatively new zero-calorie sweeteners to be approved for use in western countries, therefore studies have been conducted to elucidate their safety profiles and the non-toxic doses at which they can be administrated. In a 2011 study by Awney et al. using young male rats, low doses of stevioside (15 mg/kg/day) caused no toxicological effects on body weight, relative organ weight, hematological and biochemical parameters or enzyme activity. In the same study, high doses of stevioside (1,500 mg/kg/day) were reported to cause changes in some of those biological parameters, with unclear effects on morbidity and mortality [51]. In contrast, a 2010 study in mice by Kujur et al. found that doses up to 5,000 mg/kg/day of *S. rebaudiana* extracts do not produce significant changes in appearance, alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma or death [20]. Finally, in a study cited by the Food and Drug Administration (FDA), rats administered stevia for 90 days in doses of 0, 500, 1,000, or 2,000 mg/kg/day showed no signs of toxicity and no changes in hematology, coagulation, serum chemistry, urinalysis, and gross pathological or histopathologic exams. The only recorded changes were decreases in body weight, significant in male rats and non-significant female rats, which were attributed to decreased caloric intake [52]. No hypersensitivities or allergies have been reported due to stevia consumption since 2008, and the few that were reported prior to that year were attributed to improperly filtered stevia extracts. Thus, stevia is considered to have little to no allergic potential, [53] and the FDA has approved stevia consumption in doses up to 4 mg/kg body weight/day [54].

Conclusion

In this review, we presented past and current evidence from cell culture, animal and human studies that provide compelling evidence to the beneficial effects of stevia on glucose homeostasis, markers of inflammation, lipid profiles and blood pressure. Stevia has been shown to have favorable effects on glucose homeostasis by increasing glucose-mediated insulin secretion while decreasing gluconeogenesis, without causing hypoglycemia. Administration of steviosides can potentially induce weight loss and is associated with decreased inflammatory markers such as IL-6 and TNF-α, as well as oxidized-LDL and decreased atherosclerosis. This is together with a favorable effect on blood pressure in hypertensive subjects without causing hypotension. These findings collectively, without significant adverse effects, present stevia as a viable addition to the armamentarium against the epidemic of obesity. The medicinal use of stevia for treating hyperglycemia, hyperlipidemia and hypertension is an exciting new avenue worthy of exploration by the medical community. Further studies in the form of randomized controlled trials are needed to confirm the beneficial effects of stevia and introduce it formally as a viable therapeutic agent for obesity, metabolic syndrome and cardiovascular disease.

Acknowledgement

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