An Evidence-Based Systematic Review of Stevia by the Natural Standard Research Collaboration

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Abstract: The objective of this study was to evaluate the scientific evidence on stevia, including expert opinion, folkloric precedent, history, pharmacology, kinetics/dynamics, interactions, adverse effects, toxicology, and dosing. This review serves as a clinical support tool. Electronic searches were conducted in 10 databases, 20 additional journals (not indexed in common databases), and bibliographies from 50 selected secondary references. No restrictions were placed on the language or quality of the publications. All literature collected pertained to efficacy in humans, dosing, precautions, adverse effects, use in pregnancy and lactation, interactions, alteration of laboratory assays, and mechanisms of action. Standardized inclusion and exclusion criteria were used for selection. Grades were assigned using an evidence-based grading rationale. Based on the availability of scientific data, two indications are discussed in this review: hypertension and hyperglycemia. Evaluation of two long-term studies (1 and 2 years in length, respectively) indicates that stevia may be effective in lowering blood pressure in hypertensive patients, although data from shorter studies (1-3 months) did not support these findings. A pair of small studies also report positive results with respect to glucose tolerance and response, although the relatively low methodological rigor of these experiments limits the strength of these findings. Further investigation is warranted in both indications.

Keywords: Adverse effects, stevia (*Stevia rebaudiana*), dosing, evidence-based, interactions, pharmacodynamics, pharmacology, pharmacokinetics, systematic review. Systematic Aggregation, Analysis, and Review of the Literature.

SEARCH STRATEGY

To prepare this Natural Standard review, electronic searches were conducted in nine databases, including AMED, CANCERLIT, CINAHL, CISCOM, the Cochrane Library, EMBASE, HerbMed, International Pharmaceutical Abstracts, Medline, and NAPRALERT. Search terms included the common name(s), scientific name(s), and all listed synonyms. Hand searches were conducted of 20 additional journals (not indexed in common databases), and of bibliographies from 50 selected secondary references. No restrictions were placed on language or quality of publications. Researchers in the field of complementary and alternative medicine (CAM) were consulted for access to additional references or ongoing research.

SELECTION CRITERIA

All literature was collected pertaining to efficacy in humans (regardless of study design, quality, or language), dosing, precautions, adverse effects, use in pregnancy lactation, interactions, alteration of laboratory assays, and mechanismof action (*in vitro*, animal research, human data). Standardized inclusion/exclusion criteria were utilized for selection.

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DATA ANALYSIS

Data extraction and analysis were performed by healthcare professionals conducting clinical work and/or research at academic centers, using standardized instruments that pertained to each review section (defining inclusion/ exclusion criteria and analytic techniques, including validated measures of study quality). Data were verified by a second reviewer.

REVIEW PROCESS

A blinded review was conducted by multidisciplinary research-clinical faculty at major academic centers with expertise in epidemiology and biostatistics, pharmacology, toxicology, complementary and alternative medicine (CAM) research, and clinical practice. In cases of editorial disagreement, a three-member panel of the Editorial Board addressed conflicts, and consulted experts when applicable. Authors of studies were contacted when clarification was required.

CLINICAL BOTTOM LINE/EFFECTIVENESS

Brief Background

Extracts of leaves from Stevia rebaudiana Bertoni (SrB) have been used for many years in traditional treatment of diabetes in South America [1]. Paraguay's rural and indige-

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nous populations have used Stevia rebaudiana for the control of fertility [2].

Rebaudioside A and stevioside are steviol glycosides extracted from the plant Stevia rebaudiana Bertoni, used as natural sweeteners or dietary supplements. These compounds possess up to 250 times the sweetness intensity of sucrose, and they are noncaloric and noncariogenic sweeteners [3]. Stevioside, a natural plant glycoside isolated from the plant Stevia rebaudiana, has been commercialized as a noncaloric sweetener in Japan for more than 20 years.

Stevia may be imported only if "explicitly labeled as a dietary supplement or for use as a dietary ingredient in a dietary supplement." Although stevia may be marketed as a dietary supplement or an ingredient of a dietary supplement under the Dietary Supplement Health and Education Act (DSHEA), products that are labeled as using stevia plant parts or extracts as flavoring agents, sweeteners, or other food additive purposes are deemed as "unsafe" because "available toxicological information on stevia is inadequate to demonstrate its safety," according to the U.S. Food and Drug Administration (FDA). Regulatory agencies in Canada and Europe have not approved use of stevia as a food additive. The FDA, however, recently granted the steviol glycoside rebaudioside A (reb-A) GRAS (generally recognized as safe) status as a general-purpose sweetener for food and drink, not just as a supplement. Coca-Cola and PepsiCo have both announced their intentions to use reb-A in beverages as a zero-calorie sweetener.

Available research is promising for use in hypertension, although more studies are warranted to support the effectiveness in different populations. Based on animal study and preliminary human study, stevia may aid in the prevention or treatment of type 2 diabetes [4], but more well-designed clinical trials are needed to confirm this use.

Scientific Evidence for Common/Studied Uses:

Hypertension	B
Hyperglycemia	C

*See APPENDIX A for Natural Standard evidence-based validated grading rationale™.

Historical or Theoretical Uses Which Lack Sufficient Evidence

Alcohol abuse [5], antibacterial [6], anti-inflammatory [7, 8], antimicrobial [9], antimutagenic [10], antineoplastic (antitumor) [8], antiviral (human rotavirus activity) [11], contraceptive [2], diarrhea [8], digestive aid, diuretic [8], food additive, immunomodulation [7, 8], obesity [12].

Expert Opinion and Historic Precedent

Extracts of leaves of the plant Stevia rebaudiana Bertoni (SrB) have been used for many years in traditional treatment of diabetes in South America [1] and commercialized as a sweetener in Japan and Brazil for more than 20 years.

Stevia rebaudiana has been traditionally used by Paraguay's rural and indigenous populations for the control of fertility [2].

Although stevia is not currently listed on the FDA Generally Regarded as Safe (GRAS) or Everything Added to Food in the United States (EAFUS) lists, the FDA has recognized it as a dietary supplement. Rebaudioside A (reb-A), a steviol glycoside that is extracted from stevia, however, obtained FDA GRAS status as of December 2008.

Brief Safety Summary

Likely Safe: When taken orally (250-500mg stevioside) thrice daily for up to two years in adult Chinese hypertensive patients [13, 14]. Available clinical trials have been conducted in Chinese adults; the effects are unknown in other populations.

Possibly Safe: When stevioside (250-500mg) taken orally thrice daily for up to two years is used in adult hypertensive patients, who are not Chinese [13, 14]. Available clinical trials were conducted in Chinese adults; the effects are unknown in other populations.

Possibly Unsafe: When taken in children, or pregnant or lactating women or for periods longer than two years, due to insufficient available evidence. When used in patients with hypotension [13-15], hypocalcemia [1], hypoglycemia [4, 13, 15-17], or impaired kidney function [18-20].

Likely Unsafe: When used in patients with known allergy/hypersensitivity to the daisy family (Asteraceae/ Compositae). Other members of the daisy family include ragweed, chrysanthemums, marigolds, and many other herbs. According to a review article, however, allergic reactions to stevia are lacking in the available literature [12].

DOSING/TOXICOLOGY

General

Recommended doses are based on those most commonly used in available trials, or on historical practice. However, with natural products it is often not clear what the optimal doses are to balance efficacy and safety. Preparation of products may vary from manufacturer to manufacturer, and from batch to batch within one manufacturer. Because it is often not clear what the active components of a product may be, standardization may not be possible, and the clinical effects of different brands may not be comparable.

Standardization

Based on available clinical trials, there is no well-known standardization for stevia.

DOSING

Adult (age ≥18)

Oral

Hyperglycemia: Stevioside 1g with meals exhibited antihyperglycemic effects in patients with type 2 diabetes [4]. Aqueous extracts of 5g of leaves administered to volunteers at regular six-hour intervals for three days increased glucose tolerance and decreased plasma glucose levels during the test and after overnight fasting in all healthy volunteers in one study [17].

Hypertension: Stevioside (250mg) capsules given thrice daily decreased systolic and diastolic blood pressure after three months of therapy in one study [13]. Capsules containing 500mg stevioside powder taken three times daily for two years decreased systolic and diastolic blood pressure in patients in another study [14]. However, a study using crude steviosides up to 15mg/kg twice daily for 24 months did not demonstrate any antihypertensive effects when compared to placebo [21]. Another study administered 250mg stevioside capsules for three months without apparent effect on blood pressure or glucose level [22].

Pediatric (age <18)

Insufficient available evidence.

Toxicology

The Scientific and Regulatory Affairs division of The Coca-Cola Company funded a report in 2008 concluding that high-purity rebaudioside A (rebiana) produced to food-grade specifications and according to Good Manufacturing Practices (GMP) is safe for human consumption under its intended conditions of use as a general-purpose sweetener [23].

The Joint Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives (JECFA) conducted several reviews of stevioside and steviol glycosides, indicating that stevia poses no major toxicity risks.

Data on an eight-week treatment suggest that Stevia rebaudiana extract does not adversely affect male body and testicular weights or cauda epididymal sperm counts [24]. No notable changes in sperm morphology and motility were observed. There were no abnormal changes in the number of implantation sites, the number of viable fetuses, and the number of dead fetuses in females mated with plant extract-treated males relative to controls. Based on these results, it could be concluded that Stevia rebaudiana extract does not have a toxic effect on male rat reproduction and progeny outcome. In contrast, another animal study conducted in rats indicated that chronic administration (60 days) of a Stevia rebaudiana aqueous extract may decrease male fertility [20]. At a dose as high as 2.5g/kg in hamsters, stevioside had no effect on growth and fertility of both sexes [25].

Other acute and subacute toxicity studies have revealed a very low toxicity of stevia and stevioside [12].

The genetic toxicities of stevioside and its aglycone, steviol, were examined with mutagenicity tests using bacteria (reverse mutation assay, forward mutation assay, umu test, and rec assay), cultured mammalian cells (chromosomal aberration test and gene mutation assay), and mice (micronucleus test). Stevioside was not mutagenic in any of the assays examined. The aglycone, steviol, however, produced dose-related positive responses in some mutagenicity tests [26].

One study indicates that stevioside and steviol are neither mutagenic nor clastogenic *in vitro* at limited doses [27].

In a 2008 review, Brusick reported that stevioside and steviol have been subjected to extensive genetic testing, and the majority of the findings show no evidence of genotoxic activity [28]. The genotoxicity of steviol, a metabolite of stevia extract, was evaluated for its genotoxic potential using the comet assay [29]. After oral treatment, stomach, colon, liver, kidney, and testis DNA were not damaged in either the *in vitro* or the *in vivo* studies. Since all studies showed negative responses, stevia extract and steviol were concluded to not have DNA-damaging activity in cultured cells and mouse organs.

Pezzuto *et al*. demonstrated that steviol shows a dose-dependent positive response in forward mutation assay using Salmonella typhimurium TM677 in the presence of a metabolic activation system [6, 30]. Studies were carried out to identify the genuine mutagenic active substance from among eight steviol derivatives. 15-Oxo-steviol was found to be mutagenic at one-tenth the level of steviol itself under the presence of S9 mixture. Interestingly, specific mutagenicity of the lactone derivative under the presence of S9 mixture was ten times lower than that of the lactone derivative without the addition of S9 mixture [31].

In an *in vitro* study, isosteviol, a constituent of stevia, prevented the growth of human cancer cells, with LD50 values of 84-167mcM, and 500mcg of the compound caused a marked reduction in TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation (inhibitory effect, 53.0%) [32].

PRECAUTIONS/CONTRAINDICATIONS

Allergy

According to a review article, no allergic reactions to stevia have been reported [12].

Theoretically, patients with known allergy or hypersensitivity to the daisy family (Asteraceae/Compositae) should avoid stevia. Other members of the daisy family include ragweed, chrysanthemums, marigolds, and many other herbs.

ADVERSE EFFECTS/POST-MARKET SURVEILLANCE

***General*:** Stevia appears to be well tolerated [13]. Long-term stevioside therapy was not associated with significant adverse effects in some publications [13, 14].

***Cardiovascular*:** Stevioside (250mg) capsules given thrice daily decreased the systolic and diastolic blood pressure in patients with hypertension after three months of therapy in one study [13]. In rats, intraperitoneal injection of 25mg/kg stevioside exhibited antihypertensive effects [33]. However, four weeks' consumption of 1,000mg daily rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure [34].

***Endocrine*:** Stevioside (0.5mg/kg) lowered the blood glucose levels in STZ-induced diabetic rats in one study [16]. Glucose levels were decreased in a number of human clinical trials [4, 17].

***Gastrointestinal*:** Patients taking 250mg or 500mg stevioside orally three times daily for one year or more in clinical trials reported experiencing nausea and abdominal fullness [13]. These effects resolved after the first week of treatment [14, 33].

***Musculoskeletal*:** Patients taking 250mg or 500mg stevioside orally three times daily for one year or more in clinical trials reported experiencing myalgia and muscle weakness [13]. These effects resolved after the first week of treatment [14, 33].

***Neurologic/CNS*:** Patients taking 250mg or 500mg stevioside orally three times daily for one year or more in clinical trials reported experiencing dizziness and asthenia [13]. These effects resolved after the first week of treatment [14, 33].

Other: Das *et al.* concluded that neither 0.5% stevioside nor 0.5% rebaudioside A is cariogenic in rats [35].

Precautions/Warnings/Contraindications

Avoid in individuals with a known allergy or hypersensitivity to the daisy family (Asteraceae/Compositae). Other members of the daisy family include ragweed, chrysanthemums, marigolds, and many other herbs. According to a review article, however, no allergic reactions to stevia have been reported [12].

Use cautiously in patients with hypotension or taking hypotensive drugs, since various human and animal studies have shown that stevioside decreases systolic and diastolic blood pressure [13-15].

Use cautiously in patients with hypocalcemia since rebaudioside A-induced insulin stimulation at high glucose concentrations disappears in the absence of extracellular Ca2+ [1].

Use cautiously in patients with hypoglycemia or taking hypoglycemic drugs or insulin, since various human and animal studies have shown that stevioside decreases blood glucose levels [4, 13, 15-17].

Use cautiously in patients with impaired kidney function or other kidney diseases, as animal studies have shown that higher doses of stevia affect renal activity and perfusion, sodium excretion, and urinary flow [18-20].

Use cautiously in patients using vasodilators, specifically calcium channel blockers, due to possible additve effects [14, 18, 36].

Pregnancy & Lactation

There is limited available information regarding the use of stevia in humans. Based on animal study, stevia may possibly be safe, although a recommendation cannot be made at this time. Traditionally, stevia has been used in Paraguay for the control of fertility. Investigation into the mechanism behind this effect showed that Stevia rebaudiana infusion had no specific toxicological effects on the cell cycle of Allium cepa L. meristems, which suggests that its proposed contraceptive properties may not be connected with chromosome cycle [2]. In hamsters, stevioside at a dose as high as 2.5g/kg of body weight per day affected neither growth nor reproduction [25].

There is currently a lack of information for stevia on the National Library of Medicine's Drugs and Lactation database (LactMed).

INTERACTIONS

Stevia/Drug Interactions

***Antidiabetic Agents*:** Based on human [4, 17] and animal [4, 17] evidence, stevioside and aqueous extracts of Stevia rebaudiana leaves may decrease glucose levels. Rebaudioside A, a glycoside found in stevia, has been found to stimulate the production and activity of insulin [1].

***Antihypertensives*:** Based on clinical observations in humans, stevioside may decrease systolic and diastolic blood pressure [13, 14]. Several animal studies support this observation [15, 33, 37]. However, consumption of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure [34].

***Anti Inflammatory Agents*:** Based on animal study, stevia appears to have anti-inflammatory activity [38]. The effects of concurrent use of anti-inflammatory agents and stevia are not clear.

***Antineoplastic Agents*:** In an *in vitro* study, isosteviol, a constituent of stevia, prevented the growth of human cancer cells [32]. The effects of concurrent use of antineoplastic agents and stevia are not clear.

***Antiviral Agents*:** *In vitro*, hot water extracts from Stevia rebaudiana inhibited the replication of all four serotypes of human rotavirus [11]. The effects of concurrent use of antiviral agents and stevia are not clear.

***Calcium Channel Blockers*:** Based on animal evidence, stevioside may act as a calcium antagonist [18]. In an animal study, verapamil (a calcium antagonist) tended to increase the renal and systemic effects of stevioside [18].

***Diuretics*:** Stevioside is secreted by renal tubular epithelia and induces diuresis and natriuresis [39] in animals. Theoretically, concurrent use of stevia and diuretics may cause additive effects.

***Fertility Agents*:** Traditionally, stevia is used by women in Paraguay to control fertility [2]. Based on animal evidence, chronic administration (60 days) of a Stevia rebaudiana aqueous extract may decrease male fertility [20]. However, in another animal study, high doses of stevioside did not appear to have an effect on growth and fertility of both sexes [25]. The effects of concurrent use of fertility agents and stevia are not clear.

***Sodium Monoketocholate (MKC)*:** Combined pretreatment with stevia and sodium monoketocholate yielded lower values of glycemia in mice compared with that measured after treatment with stevia alone [40].

***Vasodilators*:** Several animal studies indicate that steviol may act as a vasodilator [14, 36].

Stevia/Herb/Supplement Interactions

***Anti Inflammatory Herbs*:** Based on animal study, stevia appears to have anti-inflammatory activity [38]. The effects of concurrent use of anti-inflammatory agents and stevia are not clear.

***Antineoplastics*:** In an *in vitro* study, isosteviol, a constituent of stevia, prevented the growth of human cancer cells [32]. The effects of concurrent use of antineoplastic agents and stevia are not clear.

***Antivirals*:** *In vitro*, hot water extracts from Stevia rebaudiana inhibited the replication of all four serotypes of human rotavirus (HRV) [11]. The effects of concurrent use of antiviral agents and stevia are not clear.

***Diuretics*:** Stevioside is secreted by renal tubular epithelia and induces diuresis and natriuresis [39].

***Fertility Agents*:** Traditionally, stevia is used by women in Paraguay to control fertility [2]. A study in rats indicated that chronic administration (60 days) of a Stevia rebaudiana aqueous extract may decrease male rat fertility [20]. At a dose as high as 2.5g/kg in hamsters, stevioside had no effect on growth and fertility of both sexes [25]. The effects of concurrent use of fertility agents and stevia are not clear.

***Hypoglycemics*:** Based on human [4, 17] and animal [4, 17] evidence , stevioside and aqueous extracts of Stevia rebaudiana leaves may decrease glucose levels. Rebaudioside A, a glycoside found in stevia, has been found to stimulate the production and activity of insulin [1].

***Hypotensives*:** Stevioside (250-500mg) given thrice daily decreased systolic and diastolic blood pressure [13-15]. Several animal studies support this observation [33].

Vasodilator Herbs and Supplements: Several animal studies indicate that steviol may act as a vasodilator [36, 41].

Stevia/Food Interactions

Insufficient available evidence.

Stevia/Lab Interactions

Blood Pressure: Based on animal and human study, stevia may cause a decrease in blood pressure. After stevioside therapy, the systolic and diastolic blood pressure of hypertensive patients decreased ($p<0.05$) [13, 14]. The hypotensive effect in spontaneously hypertensive rats lasted for more than 60 minutes with a dose of 200mg/kg [15]. Several other animal studies support this observation [33].

***Glucose Level*:** Based on human [4, 17] and animal [4, 17] evidence , stevioside and aqueous extracts of Stevia rebaudiana leaves may decrease glucose levels. Rebaudioside A, a glycoside found in stevia, has been found to stimulate the production and activity of insulin [1].

***Glucose Tolerance Test*:** Based on human study in healthy volunteers, the extract of Stevia rebaudiana may increase glucose tolerance [17].

Stevia/Nutrient Depletion

***Glucose*:** Based on human [4, 17] and animal [4, 17] evidence , stevioside and aqueous extracts of Stevia rebaudiana leaves may decrease glucose levels. Rebaudioside A, a glycoside found in stevia, has been found to stimulate the production and activity of insulin [1].

MECHANISM OF ACTION

Pharmacology

***Constituents*:** Stevia rebaudiana leaves accumulate a mixture of at least eight different steviol glycosides, with stevioside (a diterpenic carboxylic alcohol with three glucose molecules) and rebaudioside A being the most abundant [9, 42-46]. Steviol and isosteviol (ent-16-ketobeyeran-19-oic acid) are two products of enzymatic hydrolysis of stevioside [32, 47]. The sterol fraction of Stevia rebaudiana Bertoni contains the following sterols: stigmasterol (45.8%), beta-sitosterol (39.4%), and campesterol (13.1%) [48]. Four steviol (ent-kaurene-type diterpenoid) glycosides, stevioside, rebaudiosides A and C, and dulcoside A, have been isolated from Stevia rebaudiana Bertoni [38].

Stevioside, an inhibitor of long-chain fatty acid transport, was observed to inhibit both ketogenesis and [14C] CO2 production from [1-14C] palmitate (100-300mcM) in an isolated and hemoglobin-free perfused rat liver [49].

The components responsible for the sweet properties of the plant are glycosides of steviol, primary stevioside (ent-13-hydroxykaur-16-en-18-oic acid), which may be 250-300 times sweeter than sucrose and rebaudiosides A and C [28].

***General*:** In an *in vitro* assay, Stevia rebaudiana natural products inhibited oxidative phosphorylation, ATPase activity, NADH-oxidase activity, succinate-oxidase activity, succinate dehydrogenase, and L-glutamate dehydrogenase [50]. Based on animal study, intravenous doses of 50, 100 and 200mg/kg stevioside did not alter serum catecholamines in anesthetized animals [15]. UDP-glucose flavonoid glucosyltransferase (srUFGT) in Stevia rebaudiana is 1419bp in length, encoding 473 deduced amino acids with a predicted molecular mass of 53.2kDa [51]. The products of *in vitro* translation from an expression vector had anthocyanidins and steviol glucosyltransferase activity. The recombinant UDP-glucosyltransferase may participate in the synthesis of steviol glycosides. The results support the hypothesis that the flavonoid glucosyltransferases, which have a broad substrate specificity, may be not only involved in flavonoid glucosylation but may also play a role in producing the water-soluble steviol-glycosides in *S. rebaudiana*.

The stoichiometric relationship between the formation of steviol and the utilization of ent-KA and cofactors confirmed the equation ent-KA + NADPH + H (+) + O2-->steviol + NADPH(+) + H2O [52].

An *in vitro* study showed that two terpene cyclases (-)-copalyl diphosphate synthase (CPS) and (-)-kaurene synthase (KS) are part of the steviol glycoside biosynthetic pathway and that Stevia rebaudiana has recruited two genes to secondary metabolism from a highly regulated pathway involved in hormone biosynthesis [53].

Fragments of a 62 year-old leaf of S. rebaudiana exhibited a potent and prolonged sensation of sweetness, thereby indicating the stability of its sweet ent-kaurene glycoside constituents to drying, preservation, mounting, and storage [54].

***Anticancer Activity*:** In an *in vitro* study, isosteviol, a constituent of stevia, prevented the growth of human cancer cells, with LD50 values of 84-167mcM, and 500mcg of the compound caused a marked reduction in TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation (inhibitory effect, 53.0%) [32].

***Antifertility Activity*:** Traditionally, stevia has been used in Paraguay for the control of fertility. Investigation into the mechanism behind this effect showed that Stevia rebaudiana infusion had no specific toxicological effects on the cell cycle of Allium cepa L. meristems, which suggests that its proposed contraceptive properties may not be connected with chromosome cycle [2].

***Anti-inflammatory and Immunomodulatory Activity*:** Based on animal study, stevia appears to have anti-inflammatory activity. Four steviol (ent-kaurene-type diterpenoid) glycosides, stevioside, rebaudiosides A and C, and dulcoside A, have been isolated from Stevia rebaudiana Bertoni. These compounds showed strong inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice. The 50% inhibitory dose of these compounds for TPA-induced inflammation was 54.1-291.6mcg per ear. Furthermore, at 1.0 and 0.1mg per mouse of stevioside mixture, the mixture of these compounds markedly inhibited the promoting effect of TPA (1mcg/mouse) on skin tumor formation initiated with 7,12-dimethylbenz[a] anthracene (50mcg per mouse) [38].

Stevioside at 1mM suppressed ipopolysaccharide (LPS)-induced release of TNF-alpha and IL-1beta and slightly suppressed nitric oxide release in THP-1 cells without exerting any direct toxic effect, whereas steviol at 100mcM did not [7]. Activation of IKKbeta and transcription factor NF-kappaB were suppressed by stevioside, as demonstrated by Western blotting. Furthermore, only stevioside induced TNF-alpha, IL-1beta, and nitric oxide release in unstimulated THP-1 cells. Release of TNF-alpha could be partially neutralized by anti-TLR4 antibody. This study suggested that stevioside attenuates synthesis of inflammatory mediators in LPS-stimulated THP-1 cells by interfering with the IKKbeta and NF-kappaB signaling pathway, and stevioside-induced TNF-alpha secretion is partially mediated through TLR4.

In an *in vitro* study, isosteviol prevented the growth of human cancer cells, with LD50 values of 84-167mcM, and 500mcg of the compound caused a marked reduction in TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation (inhibitory effect, 53.0%) [32].

***Antimicrobial Activity*:** At high concentrations, both stevioside and steviol showed toxic effects on Salmonella typhimurium TA 98 and TA 100 [47]. From aerial parts of Stevia triflora DC, ombuoside and the synthetic derivatives octa-acetylombuoside, ombuine, and retusine showed antimicrobial activity against several strains of Gram-positive and Gram-negative bacteria and the yeast Candida albicans, using the agar diffusion method [9]. It has been proposed that the presence of free hydroxyl groups, either alcoholic or phenolic, is an important chemical feature for the expression of flavonol antimicrobial activity. A fermented aqueous extract from Stevia rebaudiana Bertoni showed strong bactericidal activity towards a wide range of food-borne pathogenic bacteria including enterohemorrhagic Escherichia coli O157:H7 [55]. The colony-forming ability of the food-borne pathogenic bacteria tested so far was reduced to <10-7 when exposed to ≥40% (v/v) solutions of the fermented extract at 37ºC for two hours. The active principle(s) of the fermented stevia extract were bactericidal under acidic conditions.

Anti-human rotavirus (HRV) activity of hot water extracts from Stevia rebaudiana was examined and found to inhibit the replication of all four serotypes of HRV *in vitro*. The study results suggest that Stevia rebaudiana extracts may bind to 37 kD VP7 and interfere with the binding of VP7 to the cellular receptors by steric hindrance, which results in the blockade of the virus attachment to cells [11].

***Cardiovascular Effects*:** Previous animal studies have shown that stevioside has an antihypertensive effect [13]. A clinical trial conducted has shown that after three months of stevioside therapy, the systolic and diastolic blood pressure of hypertensive patients decreased (systolic: 166.0 ± 9.4-152.6 ± 6.8mmHg; diastolic: 104.7 ± 5.2-90.3 ± 3.6mmHg, $p<0.05$) [13]. In another clinical trial, stevioside produced statistically significant decreases in mean [with ± standard deviation] SBP and DBP compared with baseline (SBP, from 150 [7.3]-140 [6.8]mmHg; DBP, from 95 [4, 2]-89 [3.2]mmHg; $p<0.05$) and compared with placebo ($p<0.05$) [14]. Stevioside given intravenously at doses of 50, 100 and 200mg/kg to conscious spontaneously hypertensive rats exhibited a dose-dependent hypotensive effect on both systolic and diastolic blood pressure [15]. In another animal study, stevioside at the concentrations of 50, 100 and 200mg/kg were administered intraperitoneally rats [37]. A hypotensive effect of stevioside administered intraperitoneally was noted in different strains of rats at the dose of 50mg/kg. Blood pressure returned to previous levels after the drug was discontinued for 2-3 days. Drinking of 0.1% stevioside solution in mature rats could have antihypertensive effect and also prevented hypertension in immature rats [37]. An animal study showed that intraperitoneal injection of stevioside 25mg/kg has antihypertensive activity in spontaneously hy-

pertensive rats through its vasorelexation effect mediated mainly through Ca2+ influx inhibition [33]. Another study in dogs confirms the belief that the hypotensive mechanism is likely due to inhibition of the Ca2+ influx [14]. A rat study indicates that vasodilatation induced by isosteviol is related to the opening of small conductance calcium-activated potassium (SK(Ca)) and ATP-sensitive potassium (K(ATP)) channels [36]. Another study by Wong *et al*. indicates that a decrease of [Ca2+]i in A7r5 cells by isosteviol is mainly mediated by the selective opening of K(ATP) channels or SK(Ca) channels. Alteration in the Kv channel also plays a critical role in the inhibitory action of isosteviol [56]. In a long-term study of type 2 diabetic rats fed 0.025g x kg(-1) x d(-1) of stevioside (purity >99.6%) for six weeks, stevioside exhibited an antihyperglycemic effect (incremental area under the glucose response curve [IAUC]): 985 ± 20 (stevioside) versus 1,575 ± 21 (control) mM/L x 180 minutes, ($p<0.05$) [57]. Stevioside caused a pronounced suppression of both the systolic and the diastolic blood pressure.

An infusion of CaCl2 in rats prepared with stevioside induced a marked attenuation of the vasodilating responses of stevioside, suggesting that stevioside may act as a calcium antagonist [18]. Rebaudioside A-induced insulin stimulation at high glucose disappears in the absence of extracellular Ca2+ [1].

***Endocrine Effects*:** Antihyperglycemic effects have been observed [58]. Recently, it was demonstrated that stevioside stimulates the insulin secretion both *in vitro* and *in vivo*. Abudula *et al*. evaluated the effect of rebaudioside A on the insulin release from mouse islets [1]. Rebaudioside A (10-16 to 10-6M/L) dose-dependently stimulated the insulin secretion in the presence of 16.7mM/L of glucose ($p<0.05$). The stimulation of insulin release occurs at a concentration of 10-14M/L rebaudioside A, and maximal insulin response was obtained at 10-10M/L ($p<0.01$). Rebaudioside A stimulates insulin secretion in a glucose-dependent manner (3.3-16.7mM/L) and only potentiates insulin secretion at glucose >6.6mM/L. The effect of rebaudioside A is critically dependent on the presence of extracellular Ca2+, i.e., rebaudioside A-induced insulin stimulation at high glucose disappears in the absence of extracellular Ca2+. In conclusion, rebaudioside A possesses insulinotropic effects and may serve a potential role as treatment in type 2 diabetes mellitus. In one animal study, stevioside 0.5mg/kg was observed to regulate blood glucose levels by enhancing insulin secretion, as well as insulin utilization in insulin-deficient rats by slowing down gluconeogenesis and thus decreasing phosphoenol pyruvate carboxykinase gene expression in rat liver [16]. Curi *et al*. showed that stevia extract increased glucose tolerance and decreased plasma glucose levels during the test and after overnight fasting in all healthy volunteers [17].

In another clinical trial, stevioside reduced postprandial blood glucose levels in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism [4]. After examining the influence of stevioside on the glycogen levels of fasted rats, Hubler *et al*. concluded that stevioside exerts a stimulatory action on hepatic glycogen synthesis under gluconeogenic conditions [59]. In a long-term study of type 2 diabetic rats, fed 0.025g x kg(-1) x d(-1) of stevioside (purity >99.6%) for six weeks, stevioside exhibited an antihyperglycemic effect (incremental area under the glucose response curve [IAUC]): 985 ± 20 (stevioside) versus 1,575 ± 21 (control) mM/L x 180 minutes, ($p<0.05$) [57]. It also enhanced the first-phase insulin response and concomitantly suppressed the glucagons. Bolus injections of stevioside (0.025g x kg(-1)) did not induce hypoglycemia. Stevioside augmented the insulin content in the beta-cell line, INS-1. Stevioside may increase 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 down-regulate phosphodiesterase 1 (PDE1) estimated by 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. An earlier animal study by Jeppesen *et al*. proposes that stevioside exerts antihyperglycemic, insulinotropic, and glucagonostatic actions in type 2 diabetic rats [60]. In an animal study, acute oral stevioside (SVS) increased whole-body insulin sensitivity, and low concentrations of SVS (0.01-0.1mM/L) modestly improved *in vitro* insulin action on skeletal muscle glucose transport in both lean and obese Zucker rats [58]. These results indicate that one potential site of action of SVS is the skeletal muscle, which is the major site of glucose disposal.

An animal study evaluated the effect of mice pretreatment with two commercial products of Stevia rebaudiana Bertoni (200mg/kg of Stevita (Stevita Co., Inc., Arlington, Texas) (stevia) and the other with 20mg/kg of Clear Steviosides liquid (Stevita Co., Inc., herbal supplement, Brazil) (stevioside)) on blood glucose concentration [61]. Blood glucose levels in mice pretreated with stevia and stevioside were lower compared with control. Also, a smaller increase in this parameter compared to control was registered with pretreated mice in the glucose-tolerance test, pretreatment with stevioside being again more effective. Another study was made of the combined effect of two commercial products of Stevia rebaudiana Bertoni and sodium monoketocholate (MKC) on blood glucose concentration in mice [40]. It was found that the combined pretreatment yielded lower values of glycemia compared with that measured after treatment with stevia alone.

Steviol, isosteviol and glucosilsteviol have also been found to decrease glucose production and inhibit oxygen uptake in rat renal cortical tubules [62]. The sweet principle stevioside, and steviolbioside, however, were without effect on gluconeogenesis and oxygen uptake.

Stevioside decreases the effects of atractyloside on glycolysis, glycogenolysis, gluconeogenesis, and oxygen uptake in rat livers [63]. Stevioside acts directly on pancreatic beta-cells *in vitro* to secrete insulin, actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity [64].

The effects of the active principles of S. rebaudiana (SR) on endocrine parameters of male rats were studied upon chronic administration (60 days) of a concentrated, crude extract of its leaves, starting at prepubertal age (25-30 days old) [65]. Results showed that the SR-treated group did not differ from the control group, with exception to the seminal vesicle weight.

***Kidney Effects*:** To evaluate the effect of crude extract of Stevia rebaudiana on renal water, Na+ and K+ excretion in male Wistar rats (250-350g each) under antidiuresis or water diuresis conditions were evaluated [20]. During intravenous infusion of the extract (0.05mg per min per 100g) no differences were detected in mean arterial pressure or renal hemodynamics parameters. In contrast, fractional water and sodium excretion and solute clearance increased in both groups of animals. In antidiuresis rats, the extract increased reabsorption of water by the collecting duct, and in water-diuresis animals, the extract increased free water clearance. The data suggest preferential action of the extract in the proximal tubular cells involved with the salt transport mechanism.

Oral administration of stevia extract, corresponding to 2.67g of dry leaves per day for 30 days, resulted in a decrease in mean arterial pressure in both the normotensive (N) and hypertensive rats (H). Glomerular filtration rate was constant in the N rats and increased in the H rats after stevia treatment. Normotensive and hypertensive rats presented an increase in renal plasma flow following oral stevia administration. Stevia administration provoked an increase in urinary flow in both N and H animals. Sodium excretion increased in N and H animals after stevia treatment. These results are consistent with impairment of a renal autoregulation mechanism in this hypertensive model after stevia administration. In conclusion, it was shown that stevia extract, at doses higher than used for sweetening purposes, may be a vasodilator agent in normotensive and hypertensive animals [19].

Several earlier studies by Melis *et al.* [18, 39, 66, 67] suggest that oral administration to rats of an aqueous extract of stevia dried leaves may induce systemic and renal vasodilation, causing hypotension, diuresis, and natriuresis in rats.

Mutagenicity: Another study indicates that stevioside and steviol are neither mutagenic nor clastogenic *in vitro* at limited doses [27]. Whereas stevioside demonstrates no mutagenic activity in a variety of test systems, the aglycone, steviol (13-hydroxy-ent-kaurenoic acid), is mutagenic toward the Salmonella typhimurium strain TM677 in the presence of a metabolic activating system [6, 30]. Pezzuto *et al.* demonstrated that steviol shows a dose-dependent positive response in forward mutation assay using Salmonella typhimurium TM677 in the presence of a metabolic activation system [6, 30]. Studies were carried out to identify the genuine mutagenic active substance from among the eight steviol derivatives. 15-Oxo-steviol was found to be mutagenic at 1/10 the level of steviol itself under the presence of S9 mixture. Interestingly, specific mutagenicity of the lactone derivative under the presence of S9 mixture was 10 times lower than that of the lactone derivative without the addition of S9 mixture [31].

PHARMACODYNAMICS/KINETICS

Absorption: In the rat intestine, stevia mixture components are first degraded and then absorbed as steviol [68].

Metabolism: Stevia mixture, stevioside, and rebaudioside A appeared to be hydrolyzed to steviol by human intestinal microflora [3, 42]. Interestingly, human intestinal microflorae were not able to degrade steviol. Furthermore, stevioside and rebaudioside A did not influence the composition of fecal cultures; among the selected intestinal groups, bacteroides were the most efficient in hydrolyzing stevia sweeteners to steviol [3].

According to a report by Ishii-Iwamoto *et al.* [69], stevioside is not metabolized in the isolated perfused rat liver.

HISTORY

Extracts of leaves of the plant Stevia rebaudiana Bertoni have been used for many years in traditional treatment of diabetes in South America [1], particularly by the Guaraní tribes of Paraguay and Brazil.

The Swiss botanist Moisés Santiago Bertoni described the stevia plant with its distinctive sweet taste in 1899. In 1931, two French chemists isolated the glycosides that imbue stevia with its sweet flavor.

Stevioside, a natural plant glycoside isolated from the plant Stevia rebaudiana, has been commercialized as a sweetener in Japan and Brazil for more than 20 years [13, 14]. In 1971, the Japanese firm Morita Kagaku Kogyo Co., Ltd., produced the first commercial stevia sweetener in Japan. As of 1996, Japan consumed more stevia than any other country, with stevia comprising 40% of its sweetener market. As of 1996, China was deemed the world's largest exporter of stevioside.

In 1991, the U.S. Food and Drug Administration (FDA), in what some claimed to be a controversial move, labeled stevia as an “unsafe food additive,” and restricted its import. Stevia and its extracts are not Generally Recognized as Safe (GRAS) nor approved as food additives by the FDA. Stevia may be imported only if “explicitly labeled as a dietary supplement or for use as a dietary ingredient in a dietary supplement.”

Stevia was restricted in the United States until 1995, when the Dietary Supplement Health and Education Act (DSHEA) required the FDA to modify its stance. Although stevia may be marketed as a dietary supplement under the DSHEA, products that are labeled as using S. rebaudiana plant parts or extracts as flavoring agents, sweeteners, or other food additive purposes are deemed as “unsafe” because “available toxicological information on stevia is inadequate to demonstrate its safety.”

Regulatory agencies in Europe (i.e., The Scientific Committee on Food for the European Commission) have not approved use of stevia as a food additive. As of September 2006, in Canada, the Natural Health Products Directorate accepted the addition of stevia to natural health products as a

sweetening agent provided that the stevia content does not surpass specific medicinal dosage limits. Since June 2008, stevia has been approved as a dietary supplement and sweetener for food and beverages in Australia and New Zealand, but remains banned in Singapore and Hong Kong.

In December 2008, the FDA granted the steviol glycoside rebaudioside A (reb-A) GRAS (generally recognized as safe) status as a general-purpose sweetener for food and drink. Coca-Cola and PepsiCo have both announced their intentions to use reb-A in beverages as a zero-calorie sweetener. Coca-Cola also announced its plans to market rebiana-sweetened products in 12 countries that allow stevia's use as a food additive.

EVIDENCE TABLE

Condition	Study Design	Author, Year	N	Statistically Significant?	Quality of Study 0-2=poor 3-4=good 5=excellent	Magnitude of Benefit	ARR	NNT	Comments
Hypertension	Randomized controlled trial	Hsieh, 2003	174	Yes	4	Large	NA	NA	Two-year study. Difficult to generalize because only Chinese patients were studied.
Hypertension	Randomized controlled trial	Chan, 2000	106	Yes	3	Large	NA	NA	Difficult to generalize because only Chinese patients were studied.
Hypertension	Randomized controlled trial	Barriocanal, 2008	76	No	3	NA	NA	NA	Patients received the steviol glycoside stevioside: 250mg three times daily.
Hypertension	Randomized controlled trial	Ferri, 2006	18	No	3	NA	NA	NA	Two -year study using up to 15mg/kg of crude stevioside obtained from the leaves of *Stevia rebaudiana* Bertoni.
Hyperglycemia	Clinical trial	Gregersen, 2004	12	Yes	0	Medium	NA	NA	Acute, paired crossover study, small sample size.
Hyperglycemia	Clinical trial	Curi, 1986	16	Yes	0	Small	NA	NA	Normal volunteers, not randomized, no control, poor design.

*See APPENDIX B for an explanation of columns in Natural Standard Evidence Table.

EVIDENCE DISCUSSION

Hypertension

Summary: Stevioside is a natural plant glycoside isolated from the plant Stevia rebaudiana, which has demonstrated antihypertensive effects in animal studies (dogs, rats). Two randomized controlled studies have investigated stevioside at doses of 250-500mg, three times daily for up to two years, with statistically significant, lasting decreases in blood pressure in hypertensive patients. Both studies were conducted in Chinese patients, and there is debate as to whether stevia would have similar results in different populations. However, two recent studies did not find any statistically significant changes in blood pressure when compared to placebo. Nonetheless, stevia appears to have no major side effects, and more research is warranted to compare its effectiveness with the current standard of care.

Evidence: Hsieh *et al*. [14] investigated the long-term (two-year) efficacy and tolerability of stevioside in Chinese hypertensive patients (aged 20-75 with systolic blood pressure [SBP] 140-159mmHg and diastolic blood pressure [DBP] 90-99mmHg) in a multicenter, randomized, placebo controlled trial. Secondary objectives were to determine the effects of stevioside on left ventricular mass index (LVMI) and quality of life (QOL). Patients took capsules containing 500mg of stevioside powder or placebo three times daily for two years. Blood pressure was measured, LVMI was determined by two-dimensional echocardiography, and QOL was assessed using the Medical Outcomes Study 36-Item Short-Form Health Survey. One hundred seventy four patients (87 men, 87 women) were enrolled in the study, and 168 completed it: 82 (42 men, 40 women; mean age [±SD]: 52 [7] years) in the stevioside group and 86 (44 women, 42 men; mean age: 53 [7] years) in the placebo group. After two years, the stevioside group had statistically significant decreases in mean (SD) SBP and DBP compared with baseline (SBP, from 150 [7.3] to 140 [6.8]mmHg; DBP, from 95 [4.2] to 89 [3.2]mmHg; $p<0.05$) and compared with placebo ($p<0.05$). Based on the patients' records of self-monitored

blood pressure, these effects were noted beginning approximately one week after the start of treatment and persisted throughout the study. There were no statistically significant changes in body mass index or blood biochemistry, and the results of laboratory tests were similar in the two groups throughout the study. No statistically significant difference in the incidence of adverse effects was noted between groups. QOL scores with stevioside were statistically significantly improved overall compared with placebo ($p<0.001$). Neither group had a statistically significant change in mean LVMI. However, after two years, 6/52 patients (11.5%) in the stevioside group had left ventricular hypertrophy (LVH), compared with 17/50 patients (34.0%) in the placebo group ($p<0.001$). Of those who did not have LVH at baseline, 3/46 patients (6.5%) in the stevioside group had developed LVH after two years, compared with 9/37 patients (24.3%) in the placebo group ($p<0.001$). The authors conclude that in Chinese patients with mild hypertension, oral stevioside significantly decreased SBP and DBP compared with placebo. QOL was improved, and no statistically significant differences in adverse effects between groups were noted. This study uses appropriate randomization and blinding techniques; however, the study population does not address the efficacy or safety of stevia in other populations.

Chan *et al*. conducted a multicenter, randomized, double-blind, placebo controlled study in Chinese hypertensive subjects with diastolic blood pressure between 95 and 110mmHg and ages ranging from 28-75 years. After a four-week, single-blind placebo washout phase, 106 patients were randomized to receive either stevioside 250mg (N=60; men 34, women 26; mean age ± SD, 54.1 ± 3.8 years) or placebo (N=46; men 19, women 27; mean age ± SD, 53.7 ± 4.1 years) three times daily for one year. After three months, the systolic and diastolic blood pressure of the stevioside group decreased (systolic: 166.0 ± 9.4-152.6 ± 6.8mmHg; diastolic: 104.7 ± 5.2-90.3 ± 3.6mmHg, $p<0.05$), and the effect persisted during the whole year. Blood biochemistry parameters, including lipid and glucose, showed no statistically significant changes. The authors reported that no significant adverse effects were observed, and quality of life assessment showed no deterioration. The authors conclude that oral stevioside is a well-tolerated and effective modality that may be considered as an alternative or supplementary therapy for patients with hypertension [13]. This study has appropriate blinding, although description of randomization was unclear, and the study population may preclude generalization.

Barriocanal conducted a randomized double-blind, placebo controlled trial to evaluate the effects of steviol glycosides on blood glucose and on blood pressure (BP) in subjects with type 1 diabetes and type 2 diabetes, and subjects without diabetes and with normal or low-normal BP levels [22]. Seventy-six subjects (30 with Type 2 diabetes, 16 with Type 1 diabetes, and 30 without diabetes and normal or low-normal BP levels (120/80mmHg in at least two measurements taken on different days) were randomly allocated to active treatment (the steviol glycoside stevioside: 250mg three times daily) or to placebo treatment and followed-up for three months. Post-treatment systolic BP, diastolic BP, glucose, and glycated hemoglobin (HbA1c) were not significantly different from baseline measurements, except for the placebo Type 1 diabetics group, where a statistically significant difference was observed for systolic BP and glucose. No side effects were observed in the two treatment groups. This study shows that oral steviol glycosides, taken as sweetener, are well tolerated and have no pharmacological effect.

Ferri *et al*. conducted a randomized controlled trial to assess the antihypertensive effects of crude stevioside obtained from the leaves of Stevia rebaudiana Bertoni [21]. Patients with previously untreated mild hypertension underwent a placebo phase for four weeks. The volunteers selected in this phase were randomly assigned to receive either capsules containing placebo over 24 weeks or crude stevioside 3.75mg/kg per day (for seven weeks), 7.5mg/kg per day (for 11 weeks) and 15.0mg/kg per day (for six weeks). All capsules were prescribed twice a daily, 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 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 blood pressure 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 15.0mg/kg per day did not show an antihypertensive effect.

Hyperglycemia

***Summary*:** Stevia has been widely used for diabetes in South America, and animal studies have had promising results. One small trial investigated stevia in normal volunteers and found decreases in plasma glucose levels during the test and after overnight fasting. Another small study evaluated stevia in type 2 diabetics and found stevioside reduces postprandial blood glucose and tends to potentiate insulin secretion, but further research is necessary to draw any firm conclusions. Better-designed trials would help determine whether stevia's effect on glucose tolerance would aid in the prevention of diabetes and other hyperglycemic conditions.

***Evidence*:** Gregersen *et al*. conducted an acute, paired crossover trial in 12 type 2 diabetic patients to evaluate the effects of stevioside on blood glucose response [4]. A standard test meal was supplemented with either 1g of stevioside or 1g of maize starch (control). Blood samples were drawn at 30 minutes before and for 240 minutes after ingestion of the test meal. Compared to control, stevioside reduced the incremental area under the glucose response curve by 18% ($p=0.013$). The insulinogenic index (AUC(i,insulin)/ AUC(i,glucose)) was increased by approximately 40% by stevioside compared to control ($p<0.001$). Stevioside tended to decrease glucagon levels, while it did not significantly alter the area under the insulin, glucagon-like peptide 1, and glucose-dependent insulinotropic polypeptide curves. In conclusion, stevioside reduces postprandial blood glucose levels in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism.

The effect of aqueous extracts of Stevia rebaudiana leaves on a glucose tolerance test was investigated in 16 normal volunteers [17]. Aqueous extracts of five grams of leaves were administered to volunteers at regular six-hour intervals for three days. Glucose tolerance tests were performed before and after extract administration. A second group of six normal volunteers who ingested an aqueous arabinose solution was also studied to eliminate possible stress effects. The extract of Stevia rebaudiana increased glucose tolerance. The extract decreased plasma glucose levels during the test and after overnight fasting in all volunteers.

APPENDIX A

Natural Standard Evidence-Based Validated Grading Rationale™:

- Grades reflect the level of available scientific evidence in support of the efficacy of a given therapy for a specific indication.
- Expert opinion and folkloric precedent are not included in this assessment, and are reflected in a separate section of each review ("Strength of Expert Opinion and Historic/Folkloric Precedent").
- Evidence of harm is considered separately; the below grades apply only to evidence of benefit.

Level of Evidence Grade	Criteria
A (Strong Scientific Evidence)	Statistically significant evidence of benefit from >2 properly randomized trials (RCTs), OR evidence from one properly conducted RCT AND one properly conducted meta-analysis, OR evidence from multiple RCTs with a clear majority of the properly conducted trials showing statistically significant evidence of benefit AND with supporting evidence in basic science, animal studies, or theory.
B (Good Scientific Evidence)	Statistically significant evidence of benefit from 1-2 properly randomized trials, OR evidence of benefit from >1 properly conducted meta-analysis OR evidence of benefit from >1 cohort/case-control/non-randomized trials AND with supporting evidence in basic science, animal studies, or theory.
C (Unclear or conflicting scientific evidence)	Evidence of benefit from >1 small RCT(s) without adequate size, power, statistical significance, or quality of design by objective criteria,* OR conflicting evidence from multiple RCTs without a clear majority of the properly conducted trials showing evidence of benefit or ineffectiveness, OR evidence of benefit from >1 cohort/case-control/non-randomized trials AND without supporting evidence in basic science, animal studies, or theory, OR evidence of efficacy only from basic science, animal studies, or theory.
D (Fair Negative Scientific Evidence)	Statistically significant negative evidence (i.e., lack of evidence of benefit) from cohort/case-control/non-randomized trials, AND evidence in basic science, animal studies, or theory suggesting a lack of benefit.
F (Strong Negative Scientific Evidence)	Statistically significant negative evidence (i.e. lack of evidence of benefit) from >1 properly randomized adequately powered trial(s) of high-quality design by objective criteria.*
Lack of Evidence†	Unable to evaluate efficacy due to lack of adequate available human data.

* Objective criteria are derived from validated instruments for evaluating study quality, including the 5-point scale developed by Jadad *et al*., in which a score below 4 is considered to indicate lesser quality methodologically [70].
† Listed separately in reviews in the "Historical or Theoretical Uses which Lack Sufficient Evidence" section.

APPENDIX B

Explanation of Columns in Natural Standard Evidence Table:

1	2	3	4	5	6	7	8	9	10
Condition	**Study Design**	**Author, Year**	**N**	**Statistically Significant?**	**Quality of study 0-2=poor 3-4=good 5=excellent**	**Magnitude of Benefit**	**Absolute Risk Reduction (ARR)**	**Number Needed to Treat (NNT)**	**Comments**

Condition

Refers to the medical condition or disease targeted by a therapy.

Study Design

Common types include:

Randomized Controlled Trial (RCT): An experimental trial in which participants are assigned randomly to receive either an intervention being tested or placebo. Note that Natural Standard defines RCTs as being placebo-controlled, while studies using active controls are classified as equivalence trials (see below). In RCTs, participants and researchers are often blinded (i.e., unaware of group assignments), although unblinded and quasi-blinded RCTs are also often performed. True random allocation to trial arms, proper blinding, and sufficient sample size are the basis for an adequate RCT.

Equivalence Trial: An RCT which compares two active agents. Equivalence trials often compare new treatments to usual (standard) care, and may not include a placebo arm.

Before and After Comparison: A study that reports only the change in outcome in each group of a study, and does not report between-group comparisons. This is a common error in studies that claim to be RCTs.

Case Series: A description of a group of patients with a condition, treatment, or outcome (e.g., 20 patients with migraine headache underwent acupuncture and 17 reported feeling better afterwards). Case series are considered weak evidence of efficacy.

Case-control Study: A study in which patients with a certain outcome are selected and compared to similar patients (without the outcome) to see if certain risk factors/predictors are more common in patients with that outcome. This study design is not common in the complementary & alternative medicine literature.

Cohort Study: A study which assembles a group of patients with certain baseline characteristics (for example, use of a drug), and follows them forward in time for outcomes. This study design is not common in the complementary & alternative medicine literature.

Meta-analysis: A pooling of multiple trials to increase statistical power (often used to pool data from a number of RCTs with small sample sizes, none which demonstrates significance alone but in aggregate can achieve significance). Multiple difficulties are encountered when designing/reviewing these analyses; in particular, outcomes measures or therapies may differ from study to study, hindering direct comparison.

Review: An author's description of his or her opinion based on personal, non-systematic review of the evidence.

Systematic Review: A review conducted according to pre-specified criteria in an attempt to limit bias from the investigators. Systematic reviews often include a meta-analysis of data from the included studies.

P: Pending verification.

Author, Year

Identifies the study being described in a row of the table.

N

The total number of subjects included in a study (treatment group plus placebo group). Some studies recruit a larger number of subjects initially, but do not use them all because they do not meet the study's entry criteria. In this case, it is the second, smaller number that qualifies as N. N includes all subjects that are part of a study at the start date, even if they drop out, are lost to follow-up, or are deemed unsuitable for analysis by the authors. Trials with a large number of drop-outs that are not included in the analysis are considered to be weaker evidence for efficacy. (For systematic reviews the number of studies included is reported. For meta-analyses, the number of total subjects included in the analysis or the number of studies may be reported.) P = pending verification.

Statistically Significant?

Results are noted as being statistically significant if a study's authors report statistical significance, or if quantitative evidence of significance is present (such as p values). P = pending verification.

Quality of Study

A numerical score between 0-5 is assigned as a rough measure of study design/reporting quality (0 being weakest and 5 being strongest). This number is based on a well-established, validated scale developed by Jadad *et al.* (Jadad AR, Moore RA, Carroll D, *et al*. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Controlled Clinical Trials 1996;17[1]:1-12). This calculation does not account for all study elements that may be used to assess quality (other aspects of study design/reporting are addressed in the "Evidence Discussion" sections of reviews).

A Jadad score is calculated using the seven items in the table below. The first five items are indications of good quality, and each counts as one point towards an overall quality score. The final two items indicate poor quality, and a point is subtracted for each if its criteria are met. The range of possible scores is 0 to 5.

Jadad Score Calculation	
Item	Score
Was the study described as randomized (this includes words such as randomly, random, and randomization)?	0/1
Was the method used to generate the sequence of randomization described and appropriate (table of random numbers, computer-generated, etc)?	0/1
Was the study described as double blind?	0/1
Was the method of double blinding described and appropriate (identical placebo, active placebo, dummy, etc)?	0/1
Was there a description of withdrawals and dropouts?	0/1
Deduct one point if the method used to generate the sequence of randomization was described and it was inappropriate (patients were allocated alternately, or according to date of birth, hospital number, etc).	0/-1
Deduct one point if the study was described as double blind but the method of blinding was inappropriate (e.g., comparison of tablet vs. injection with no double dummy).	0/-1

Magnitude of Benefit

This summarizes how strong a benefit is: small, medium, large, none, or P (pending verification). If results are not statistically significant "NA" for "not applicable" is entered. In order to be consistent in defining small, medium, and large benefits across different studies and reviews, Natural Standard defines the magnitude of benefit in terms of the standard deviation (SD) of the outcome measure. Specifically, the benefit is considered:

- Large: if >1 SD
- Medium: if 0.5 to 0.9 SD
- Small: if 0.2 to 0.4 SD

In many cases, studies do not report the standard deviation of change of the outcome measure. However, the change in the standard deviation of the outcome measure (also known as effect size) can be calculated, and is derived by subtracting the mean (or mean difference) in the placebo/control group from the mean (or mean difference) in the treatment group, and dividing that quantity by the pooled standard deviation (Effect size=[Mean Treatment – Mean Placebo]/SDp).

Absolute Risk Reduction (ARR)

This describes the difference between the percent of people in the control/placebo group experiencing a specific outcome (control event rate), and the percent of people in the experimental/therapy group experiencing that same outcome (experimental event rate). Mathematically, Absolute risk reduction (ARR) equals experimental event rate minus control event rate. ARR is better able to discriminate between large and small treatment effects than relative risk reduction (RRR), a calculation that is often cited in studies ([control event rate – experimental event rate]/control event rate). Many studies do not include adequate data to calculate the ARR, in which cases "NA" is entered into this column. P = pending verification.

Number Needed to Treat (NNT)

This is the number of patients who would need to use the therapy under investigation, for the period of time described in the study, in order for one person to experience the specified benefit. It is calculated by dividing the Absolute Risk Reduction into 1 (1/ARR). P = pending verification.

Comments

When appropriate, this brief section may comment on design flaws (inadequately described subjects, lack of blinding, brief follow-up, not intention-to treat, etc.), notable study design elements (crossover, etc.), dosing, and/or specifics of study group/sub-groups (age, gender, etc). More detailed description of studies is found in the "Evidence Discussion" section that follows the "Evidence Table" in Natural Standard reviews.

REFERENCES

[1] Abudula, R.; Jeppesen, P.B.; Rolfsen, S.E.; Xiao, J.; Hermansen, K. Rebaudioside A potently stimulates insulin secretion from isolated mouse islets, studies on the dose-, glucose-, and calcium-dependency. *Metabolism*, **2004**, *53*(10), 1378-1381.

[2] Schvartzman, J.B.; Krimer, D.B.; Moreno, A.R. Cytological effects of some medicinal plants used in the control of fertility. *Experientia*, **1977**, *33*(5), 663-665.

[3] Gardana, C.; Simonetti, P.; Canzi, E.; Zanchi, R.; Pietta, P. Metabolism of stevioside and rebaudioside A from Stevia rebaudiana

extracts by human microflora. *J. Agric. Food Chem.*, **2003**, *51*(22), 6618-6622.

[4] Gregersen, S.; Jeppesen, P.B.; Holst, J.J.; Hermansen, K. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism*, **2004**, *53*(1), 73-76.

[5] Keyler, D.E.; Baker, J.I.; Lee, D.Y.; Overstreet, D.H.; Boucher, T.A.; Lenz, S.K. Toxicity study of an antidipsotropic Chinese herbal mixture in rats, NPI-028. *J. Altern. Complement. Med.*, **2002**, *8*(2), 175-183.

[6] Pezzuto, J.M.; Nanayakkara, N.P.; Compadre, C.M.; Swanson, S.M.; Kinghorn, A.D.; Guenthner, T.M.; Sparnins, V.L.; Lam, L.K. Characterization of bacterial mutagenicity mediated by 13-hydroxy-ent-kaurenoic acid (steviol) and several structurally-related derivatives and evaluation of potential to induce glutathione S-transferase in mice. *Mutat. Res.*, **1986**, *169*(3), 93-103.

[7] Boonkaewwan, C.; Toskulkao, C.; and Vongsakul, M. Anti-Inflammatory and Immunomodulatory Activities of Stevioside and Its Metabolite Steviol on THP-1 Cells. *J. Agric. Food Chem.*, **2006**, *54*(3), 785-789.

[8] Chatsudthipong, V.; Muanprasat, C. Stevioside and related compounds, therapeutic benefits beyond sweetness. *Pharmacol. Ther.*, **2009**, *121*(1), 41-54.

[9] Amaro-Luis, J. M.; Adrian, M.; Diaz, C. Isolation, identification and antimicrobial activity of ombuoside from Stevia triflora. *Ann. Pharm. Fr.*, **1997**, *55*(6), 262-268.

[10] Carino-Cortes, R.; Hernandez-Ceruelos, A.; Torres-Valencia, J.M.; Gonzalez-Avila, M.; Arriaga-Alba, M.; Madrigal-Bujaidar, E. Antimutagenicity of Stevia pilosa and Stevia eupatoria evaluated with the Ames test. *Toxicol. In Vitro*, **2007**, *21*(4), 691-697.

[11] Takahashi, K.; Matsuda, M.; Ohashi, K.; Taniguchi, K.; Nakagomi, O.; Abe, Y.; Mori, S.; Sato, N.; Okutani, K.; Shigeta, S. Analysis of anti-rotavirus activity of extract from Stevia rebaudiana. *Antiviral Res.*, **2001**, *49*(1), 15-24.

[12] Geuns, J.M. Stevioside. *Phytochemistry*, **2003**, *64*(5), 913-921.

[13] Chan, P.; Tomlinson, B.; Chen, Y.J.; Liu, J.C.; Hsieh, M.H.; Cheng, J.T. A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. *Br. J. Clin. Pharmacol.*, **2000**, *50*(3), 215-220.

[14] Hsieh, M.H.; Chan, P.; Sue, Y.M.; Liu, J.C.; Liang, T.H.; Huang, T.Y.; Tomlinson, B.; Chow, M.S.; Kao, P.F.; Chen, Y.J. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension, a two-year, randomized, placebo-controlled study. *Clin. Ther.*, **2003**, *25*(11), 2797-2808.

[15] Chan, P.; Xu, D.Y.; Liu, J.C.; Chen, Y.J.; Tomlinson, B.; Huang, W.P.; Cheng, J.T. The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. *Life Sci.*, **1998**, *63*(19), 1679-1684.

[16] Chen, T.H.; Chen, S.C.; Chan, P.; Chu, Y.L.; Yang, H.Y.; Cheng, J.T. Mechanism of the hypoglycemic effect of stevioside, a glycoside of Stevia rebaudiana. *Planta Med.*, **2005**, *71*(2), 108-113.

[17] Curi, R.; Alvarez, M.; Bazotte, R.B.; Botion, L.M.; Godoy, J.L.; Bracht, A. Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. *Braz. J. Med. Biol. Res.* **1986**, *19*(6), 771-774.

[18] Melis, M.S.; Sainati, A.R. Effect of calcium and verapamil on renal function of rats during treatment with stevioside. *J. Ethnopharmacol.*, **1991**, *33*(3), 257-262.

[19] Melis, M.S. A crude extract of Stevia rebaudiana increases the renal plasma flow of normal and hypertensive rats. *Braz. J. Med. Biol. Res.*, **1996**, *29*(5), 669-675.

[20] Melis, M.S. Effects of chronic administration of Stevia rebaudiana on fertility in rats. *J. Ethnopharmacol.*, **1999**, *67*(2), 157-161.

[21] Ferri, L.A.; Alves-Do-Prado, W.; Yamada, S.S.; Gazola, S.; Batista, M.R.; Bazotte, R.B. Investigation of the antihypertensive effect of oral crude stevioside in patients with mild essential hypertension. *Phytother. Res.*, **2006**, *20*(9), 732-736.

[22] Barriocanal, L.A.; Palacios, M.; Benitez, G.; Benitez, S.; Jimenez, J.T.; Jimenez, N.; Rojas, V. 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. *Regul. Toxicol. Pharmacol.*, **2008**, *51*(1), 37-41.

[23] Carakostas, M.C.; Curry, L.L.; Boileau, A.C.; Brusick, D.J. Overview, the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. *Food Chem. Toxicol.* **2008**, *46*(Suppl 7), S1-S10.

[24] Aritajat, S.; Kaweewat, K.; Manosroi, J.; Manosroi, A. Dominant lethal test in rats treated with some plant extracts. *Southeast Asian J. Trop. Med. Public Health* **2000**, *31*(Suppl 1), 171-173.

[25] Yodyingyuad, V.; Bunyawong, S. Effect of stevioside on growth and reproduction. *Hum. Reprod.* **1991**, *6*(1), 158-165.

[26] Matsui, M.; Matsui, K.; Kawasaki, Y.; Oda, Y.; Noguchi, T.; Kitagawa, Y.; Sawada, M.; Hayashi, M.; Nohmi, T.; Yoshihira, K.; Ishidate, M.; Jr.; Sofuni, T. Evaluation of the genotoxicity of stevioside and steviol using six *in vitro* and one *in vivo* mutagenicity assays. *Mutagenesis*, **1996**, *11*(6), 573-579.

[27] Suttajit, M.; Vinitketkaumnuen, U.; Meevatee, U.; Buddhasukh, D. Mutagenicity and human chromosomal effect of stevioside, a sweetener from Stevia rebaudiana Bertoni. *Environ. Health Perspect.* **1993**, *101*(Suppl 3), 53-56.

[28] Brusick, D.J. A critical review of the genetic toxicity of steviol and steviol glycosides. *Food Chem.Toxicol.*, **2008**, *46*(Suppl 7), S83-S91.

[29] Sekihashi, K.; Saitoh, H.; Sasaki, Y. [Genotoxicity studies of stevia extract and steviol by the comet assay]. *J. Toxicol. Sci.*, **2002**, *27*(Suppl 1),1-8.

[30] Pezzuto, J.M.; Compadre, C.M.; Swanson, S.M.; Nanayakkara, D.; Kinghorn, A.D. Metabolically activated steviol, the aglycone of stevioside, is mutagenic. *Proc. Natl. Acad. Sci. USA*, **1985**, *82*(8), 2478-2482.

[31] Terai, T.; Ren, H.; Mori, G.; Yamaguchi, Y.; Hayashi, T. Mutagenicity of steviol and its oxidative derivatives in Salmonella typhimurium TM677. *Chem. Pharm. Bull. (Tokyo)*, **2002**, *50*(7), 1007-1010.

[32] Mizushina, Y.; Akihisa, T.; Ukiya, M.; Hamasaki, Y.; Murakami-Nakai, C.; Kuriyama, I.; Takeuchi, T.; Sugawara, F.; Yoshida, H. Structural analysis of isosteviol and related compounds as DNA polymerase and DNA topoisomerase inhibitors. *Life Sci.*, **2005**, *77*(17), 2127-2140.

[33] Lee, C.N.; Wong, K.L.; Liu, J.C.; Chen, Y.J.; Cheng, J.T.; Chan, P. Inhibitory effect of stevioside on calcium influx to produce antihypertension. *Planta. Med.*, **2001**, *67*(9), 796-799.

[34] Maki, K.C.; Curry, L.L.; Carakostas, M.C.; Tarka, S.M.; Reeves, M.S.; Farmer, M.V.; McKenney, J.M.; Toth, P.D.; Schwartz, S.L.; Lubin, B.C.; Dicklin, M.R.; Boileau, A.C.; Bisognano, J.D. The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. *Food Chem. Toxicol.* ***2008***, *46*(Suppl 7), *S40-S46*.

[35] Das, S.; Das, A.K.; Murphy, R.A.; Punwani, I.C.; Nasution, M.P.; Kinghorn, A.D. Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. *Caries Res.*, **1992**, *26*(5), 363-366.

[36] Wong, K.L.; Chan, P.; Yang, H.Y.; Hsu, F.L.; Liu, I.M.; Cheng, Y.W.; Cheng, J.T. Isosteviol acts on potassium channels to relax isolated aortic strips of Wistar rat. *Life Sci.*, **2004**, *74*(19), 2379-2387.

[37] Hsu, Y.H.; Liu, J.C.; Kao, P.F.; Lee, C.N.; Chen, Y.J.; Hsieh, M.H.; Chan, P. Antihypertensive effect of stevioside in different strains of hypertensive rats. *Zhonghua Yi. Xue. Za Zhi. (Taipei)*, **2002**, *65*(1), 1-6.

[38] Yasukawa, K.; Kitanaka, S.; Seo, S. Inhibitory effect of stevioside on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Biol. Pharm. Bull.*, **2002**, *25*(11), 1488-1490.

[39] Melis, M.S. Renal excretion of stevioside in rats. *J. Nat. Prod.*, **1992**, *55*(5), 688-690.

[40] Raskovic, A.; Jakovljevic, V.; Mikov, M.; Gavrilovic, M. Joint effect of commercial preparations of Stevia rebaudiana Bertoni and sodium monoketocholate on glycemia in mice. *Eur. J. Drug Metab. Pharmacokinet.*, **2004**, *29*(2), 83-86.

[41] Liu, J.C.; Kao, P.K.; Chan, P.; Hsu, Y.H.; Hou, C.C.; Lien, G.S.; Hsieh, M.H.; Chen, Y.J.; Cheng, J.T. Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. *Pharmacology*, **2003**, *67*(1), 14-20.

[42] Koyama, E.; Kitazawa, K.; Ohori, Y.; Izawa, O.; Kakegawa, K.; Fujino, A.; Ui, M. *In vitro* metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. *Food Chem. Toxicol.* **2003**, *41*(3), 359-374.

[43] Kinghorn, A.D.; Soejarto, D.D.; Nanayakkara, N.P.; Compadre, C.M.; Makapugay, H.C.; Hovanec-Brown, J.M.; Medon, P.J.; Kamath, S.K. A phytochemical screening procedure for sweet ent-kaurene glycosides in the genus Stevia. *J. Nat. Prod.*, **1984**, *47*(3), 439-444.

[44] Shibata, H.; Sawa, Y.; Oka, T.; Sonoke, S.; Kim, K.K.; Yoshioka, M. Steviol and steviol-glycoside, glucosyltransferase activities in Stevia rebaudiana Bertoni--purification and partial characterization. *Arch. Biochem. Biophys.*, **1995**, *321*(2), 390-396.

[45] Brandle, J.E.; Richman, A.; Swanson, A.K.; Chapman, B.P. Leaf Ests from Stevia rebaudiana, a resource for gene discovery in diterpene synthesis. *Plant Mol. Biol.*, **2002**, *50*(4-5), 613-622.

[46] Richman, A.; Swanson, A.; Humphrey, T.; Chapman, R.; McGarvey, B.; Pocs, R.; Brandle, J. Functional genomics uncovers three glucosyltransferases involved in the synthesis of the major sweet glucosides of Stevia rebaudiana. *Plant J.*, **2005**, *41*(1), 56-67.

[47] Klongpanichpak, S.; Temcharoen, P.; Toskulkao, C.; Apibal, S.; Glinsukon, T. Lack of mutagenicity of stevioside and steviol in Salmonella typhimurium TA 98 and TA 100. *J. Med. Assoc. Thai.* **1997**, *80*(Suppl 1), S121-S128.

[48] D'Agostino, M.; De Simone, F.; Pizza, C.; Aquino, R. [Sterols in Stevia rebaudiana Bertoni]. *Boll. Soc. Ital Biol. Sper.*, **1984**, *60*(12), 2237-2240.

[49] Constantin, J.; Ishii-Iwamoto, E.L.; Ferraresi-Filho, O.; Kelmer-Bracht, A.M.; Bracht, A. Sensitivity of ketogenesis and citric acid cycle to stevioside inhibition of palmitate transport across the cell membrane. *Braz. J. Med. Biol. Res.*, **1991**, *24*(8), 767-771.

[50] Kelmer, Bracht A.; Alvarez, M.; Bracht, A. Effects of Stevia rebaudiana natural products on rat liver mitochondria. *Biochem. Pharmacol.*, **1985**, *34*(6), 873-882.

[51] Ma, L.B.; Zhang, da B.; Chen, L.; Chen, M.C. Molecular cloning and characterization of Stevia rebaudiana UDP-glucosyltransferase. *Shi Yan. Sheng Wu Xue. Bao.*, **2003**, *36*(2), 123-129.

[52] Kim, K.K.; Sawa, Y.; Shibata, H. Hydroxylation of ent-kaurenoic acid to steviol in Stevia rebaudiana Bertoni--purification and partial characterization of the enzyme. *Arch. Biochem. Biophys.*, **1996**, *332*(2), 223-230.

[53] Richman, A.S.; Gijzen, M.; Starratt, A.N.; Yang, Z.; Brandle, J.E. Diterpene synthesis in Stevia rebaudiana, recruitment and up-regulation of key enzymes from the gibberellin biosynthetic pathway. *Plant J.* **1999**, *19*(4), 411-421.

[54] Soejarto, D.D.; Kinghorn, A.D.; Farnsworth, N.R. Potential sweetening agents of plant origin. III. Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. *J. Nat. Prod.* **1982**, *45*(5), 590-599.

[55] Tomita, T.; Sato, N.; Arai, T.; Shiraishi, H.; Sato, M.; Takeuchi, M.; Kamio, Y. Bactericidal activity of a fermented hot-water extract from Stevia rebaudiana Bertoni towards enterohemorrhagic Escherichia coli O157,H7 and other food-borne pathogenic bacteria. *Microbiol. Immunol.* **1997**, *41*(12), 1005-1009.

[56] Wong, K.L.; Yang, H.Y.; Chan, P.; Cheng, T.H.; Liu, J.C.; Hsu, F.L.; Liu, I.M.; Cheng, Y.W.; Cheng, J.T. Isosteviol as a potassium channel opener to lower intracellular calcium concentrations in cultured aortic smooth muscle cells. *Planta Med.*, **2004**, *70*(2), 108-112.

[57] Jeppesen, P.B.; Gregersen, S.; Rolfsen, S.E.; Jepsen, M.; Colombo, M.; Agger, A.; Xiao, J.; Kruhoffer, M.; Orntoft, T.; and Hermansen, K. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. *Metabolism*, **2003**, *52*(3), 372-378.

[58] Lailerd, N.; Saengsirisuwan, V.; Sloniger, J.A.; Toskulkao, C.; Henriksen, E.J. Effects of stevioside on glucose transport activity in insulin-sensitive and insulin-resistant rat skeletal muscle. *Metabolism*, **2004**, *53*(1), 101-107.

[59] Hubler, M.O.; Bracht, A.; Kelmer-Bracht, A.M. Influence of stevioside on hepatic glycogen levels in fasted rats. *Res. Commun. Chem. Pathol. Pharmacol.*, **1994**, *84*(1), 111-118.

[60] Jeppesen, P.B.; Gregersen, S.; Alstrup, K.K.; Hermansen, K. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects *in vivo*, studies in the diabetic Goto-Kakizaki (GK) rats. *Phytomedicine*, **2002**, *9*(1), 9-14.

[61] Raskovic, A.; Gavrilovic, M.; Jakovljevic, V.; Sabo, J. Glucose concentration in the blood of intact and alloxan-treated mice after pretreatment with commercial preparations of Stevia rebaudiana (Bertoni). *Eur. J. Drug Metab. Pharmacokinet.*, **2004**, *29*(2), 87-90.

[62] Yamamoto, N.S.; Kelmer Bracht, A.M.; Ishii, E.L.; Kemmelmeier, F.S.; Alvarez, M.; Bracht, A. Effect of steviol and its structural analogues on glucose production and oxygen uptake in rat renal tubules. *Experientia*, **1985**, *41*(1), 55-57.

[63] Ishii, E.L.; Bracht, A. Stevioside, the sweet glycoside of Stevia rebaudiana, inhibits the action of atractyloside in the isolated perfused rat liver. *Res. Commun. Chem, Pathol, Pharmacol.*, **1986**, *53*(1), 79-91.

[64] Jeppesen, P.B.; Gregersen, S.; Poulsen, C.R.; Hermansen, K. Stevioside acts directly on pancreatic beta cells to secrete insulin, actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity. *Metabolism*, **2000**, *49*(2), 208-214.

[65] Oliveira-Filho, R.M.; Uehara, O.A.; Minetti, C.A.; Valle, L.B. Chronic administration of aqueous extract of Stevia rebaudiana (Bert.) Bertoni in rats, endocrine effects. *Gen. Pharmacol.*, **1989**, *20*(2) 187-191.

[66] Melis, M.S. Chronic administration of aqueous extract of Stevia rebaudiana in rats, renal effects. *J. Ethnopharmacol.* **1995**, *47*(3), 129-134.

[67] Melis, M.S. Stevioside effect on renal function of normal and hypertensive rats. *J. Ethnopharmacol.*, **1992**, *36*(3), 213-217.

[68] Koyama, E.; Sakai, N.; Ohori, Y.; Kitazawa, K.; Izawa, O.; Kakegawa, K.; Fujino, A.; Ui, M. Absorption and metabolism of glycosidic sweeteners of stevia mixture and their aglycone, steviol, in rats and humans. *Food Chem. Toxicol.*, **2003**, *41*(6), 875-883.

[69] Ishii-Iwamoto, E.L.; Bracht, A. Stevioside is not metabolized in the isolated perfused rat liver. *Res. Commun. Mol. Pathol. Pharmacol.*, **1995**, *87*(2), 167-175.

[70] Jadad, A.R.; Moore, R.A.; Carroll, D.; Jenkinson, C.; Reynolds, D.J.; Gavaghan, D.J.; McQuay, H.J. Assessing the quality of reports of randomized clinical trials, is blinding necessary? *Control Clin. Trials*, **1996**, *17*(1), 1-12.

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