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Review

Nutritional and therapeutic values of *Stevia rebaudiana*: A review

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***Stevia rebaudiana* is a nutrient rich natural sweetest plant of Asteraceae family. The leaves naturally contain diterpene glycosides stevioside, rebaudiosides A-F, steviolbioside and dulcoside, which are responsible for its sweet taste and have commercial value all over the world as sugar substitute in foods, beverages or medicines. It is a plant which offers sweetness with fewer calories and do not show any side effects after consumption on human health. *Stevia* has many pharmacological and therapeutic applications as suggested by many preclinical and some clinical studies; these are nontoxic and possess antioxidant, antimicrobial, antifungal and anticarcinogenic activity. In future *Stevia* is likely to become a major source of high potency low calorie sweetener for growing natural food market. This review article presents beneficial role of *Stevia* and its metabolites on health promoting properties.**

Key words: *Stevia rebaudiana*, steviosides, sweetner.

INTRODUCTION

Stevia rebaudiana (Bertoni) is an herb of the 950 genera of Asteraceae family. Centuries ago, Paraguay natives used the leaves of this small, herbaceous, semi-bushy, perennial shrub to sweeten their bitter drinks. Gaurani Indians extensively used this plant for more than 1500 years. Dr. Moises Santiago Bertoni discovered this plant in 1888 at Paraguay. In 1905, the plant was scientifically named as *S. rebaudiana* after a Paraguayan chemist Dr. Rebaudi. It was reported that there are around 150 species within the *Stevia* family including *Stevia dianthoidea*, *Stevia Phlebophylla, Stevia anisostemma, Stevia bertholdii, Stevia crenata, Stevia enigmatica, Stevia eupatoria*, *Stevia lemmonii, Stevia micrantha*, *Stevia ovata*, *Stevia plummerae*, *S. rebaudiana*, *Stevia salicifolia*, *Stevia serrata* and *Stevia viscida* with all plants being sweet but *rebaudiana* having the highest sweetness levels. It is also known as sweet herb, sweet leaf, honey leaf, candy leaf and honey yerba (Carakostas et al., 2008). Originating in the South American wild, it could be found growing in semi-arid habitat ranging from grassland to scrub forest to mountain terrain. When cultivated or growing naturally in fertile soil, the mature *Stevia* plant grows up to 65 cm (26 inches) to as tall as 180 cm (72 inches). It is a short day plant and flowering from January to March in the southern hemisphere. The flowers are white in color with a pale purple throat. They are small in size and arranged in the form of small corymbs (Goettemoeller and Ching, 1999; Singh and Rao, 2005). It prefers a sandy soil, requiring a warm sunny position. The suitable natural climate is semi-humid subtropical with temperature extremes from 21 to 43°C and average 24°C (Huxley, 1992). It is widely used in many parts of the world as sweetener and grown commercially in Central America, Korea, Paraguay,

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Brazil, Thailand and China (Mizutani and Tanaka, 2002; Kim et al., 2002, Jaroslav et al., 2006). Two French chemists in 1931 isolated the glycosides which is secondary metabolites responsible for the sweet taste of *Stevia* (Bridel and Lavielle, 1931). The chemical structure was established in 1952 as a diterpene glycoside. The leaves of *Stevia* contain a natural complex mixture of eight sweet diterpene glycosides, including isosteviol, stevioside, rebaudiosides (A, B, C, D, E, F), steviolbioside and dulcoside A (Rajasekaran et al., 2008; Goyal et al., 2010). Out of various steviol glycosides (SGs), stevioside and rebaudioside A are the major metabolites and these compounds are 250 to 300 times as sweet as sucrose (Allam et al., 2001; Mantovaneli et al., 2004; Debnath, 2008), pH-stable, heat-stable, not fermentable (Abdullateef and Osman, 2012) and posses health promoting potential. Along with sweetness, *Stevia* has some bitter aftertaste due to the presence of some essential oils, tannins and flavonoids (Phillips, 1987).

The *Stevia* leaves have sensory and functional properties superior to those of many other high-potency sweeteners and is likely to become a major source of natural sweetener for the growing food market (Goyal et al., 2010). It is commercially well known to exert beneficial effects on human health and has become an interesting area of research these days. Leaves of *S. rebaudiana* has many medical applications like antimicrobial (Satishkumar et al., 2008), antiviral (Kedik et al., 2009), antifungal (Silva et al*.,* 2008), anti-hypertensive (Chan et al., 1998; Lee et al., 2001; Hsieh et al., 2003), anti-hyperglycaemic (Jeppesen et al., 2002; Benford et al., 2006), anti-tumour (Satishkumar et al., 2008), anti-inflammatory, anti-diarrhoeal, diuretic, anti-human rotavirus activities (Das et al., 1992; Takahashi et al., 2001), anti-HIV (Takahashi et al., 1998), hepatoprotective (Mohan and Robert, 2009) and immunomodulatory effects (Jaroslav et al., 2006; Chatsudthipong and Muanprasat, 2009).

Toxicological studies have shown that secondary metabolites present in *Stevia* does not have teratogenic, mutagenic or carcinogenic effects and no allergic reactions have been observed after consuming it as an sweetener (Pol et al., 2007). The objective of this article is to review systematic literature and to summarize the nutritional, pharmacological and therapeutic applications of *S. rebaudiana* and its related compounds.

METABOLISM

S. rebaudiana leaves contain a zero-calorie *ent*-kaurene diterpene glycosides (stevioside and the rebaudiosides) 300 times sweeter than sucrose with superior solubility in water and a positive taste profile that are safely metabolized by the body without any effect (Soejarto et al., 1982; Megeji et al., 2005; 5. Geuns et al., 2007).

The major compounds of *Stevia* as steviol glycosides (SGs) are metabolized and eliminated through similar pathways in both humans and animals, has been studied by Genus et al. (2003, 2007). Rebaudioside A in the digestive tract is first metabolized by microbes in the colon to stevioside which is further converted into glucose molecule and steviol. The released glucose molecule is used by the bacteria in the colon and is not absorbed into the blood stream. The metabolized components essentially leave the body and there is no accumulation. The metabolism of steviol glycosides to steviol means that the metabolic equivalency of the different steviol glycosides permits to apply the findings from studies with stevioside to the safety evaluation of rebaudioside A, and thus to the safety of *Stevia* (Koyama et al., 2003). There *Stevia* species possess some differences between, it has been demonstrated that the conversion rate of rebaudiside A and stevioside are similar between rats and humans, with the conversion from stevioside to steviol more rapid than that of rebaudioside A to stevioside in both species. Moreover, quantitative and qualitative similarities have been found between the organisms in the gut (microflora) of rat and the human body (Wingard et al., 1980).

A study on the human digestive tract demonstrates that steviol is not altered or changed at either high or low concentrations as observed through human faeces, indicating that steviol is in fact the final product of *Stevia* metabolism (Koyama et al., 2003). The study also showed that the majority of steviol glycosides are absorbed and glucuronidated (a bond intended to help them clear out of the blood) in the liver. The newly bonded glucuronide is released in the blood and filtered by the kidneys into the urine. Small amounts of glucuronidate that remain in the colon are excreted through fecal matter. Tests with stevioside compounds and the effect of gastric juices and digestive enzymes on them show their failure to degrade or rearrange the compounds (Wingard et al., 1980). *In vitro* digestibility of steviosides by various digestive enzymes was examined by Hutapea et al. (1997), it was found that none of the enzymes digested the stevioside and intestinal microflora hydrolyzed it to both steviol and steviol-16, 17 alpha-epoxide. Later, steviol 16, 17 alpha-epoxide was then completely converted back into steviol, which further excreted from the body in urine as steviol glucuronide (Chatsudthipong and Muanprasat, 2009).

ACCEPTABLE DAILY INTAKE (ADI)

Globally, scientists have concluded that *Stevia* sweeteners are safe for people of all ages. *Stevia* leaf or extracted forms like stevioside, rebaudioside A and steviol glycosides was approved by US FDA as a dietary supplement considered (Generally recognized as safe) rating in the US (GRAS Notification 287 for Steviol Glycosides with Rebaudioside A and Stevioside as Principal Components) as appears to have an adequate

daily intake (ADI) of 25 mg/kg (Genus et al., 2003) (following 100-fold safety factor, commonly seen in ADI values) in rats which is around 7.9 mg/kg in humans. Xili et al. (1992) also calculated the acceptable daily intake (ADI) of stevioside which is 7.9 mg/kg body weight. However, this ADI should be considered as a minimum value as the authors did not test concentrations of stevioside higher than 793 mg/kg body weight. The routine daily human consumption of 5 to 6 mg of *Stevia* leaf extract as a dietary sweetener per kg of body weight is safe (Carakostas et al., 2008). Rebaudioside-A has been used for years throughout the world as a natural non-nutritive sweetening alternative to sucrose and other nutritive variants (Goyal et al., 2010). The Dietary Supplement Health and Education Act (DSHEA) passed in the United States in 1994 were also approved steviol glycosides (SGs) to be used as a functional ingredient in dietary supplements (Williams and Burdock, 2009). Minutes of the Tenth Meeting of Food Authority held on 20th September, 2012 at 11:00 h at FDA Bhavan, New Delhi approved the use of steviol glycoside as an artificial sweetener in various foods.

Currently, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a thorough scientific review of all the available scientific data and concluded *Stevia* sweeteners are safe for use in foods and beverages, an acceptable daily intake of steviol glycoside of up to 4 mg/kg of body weight was recommended.

The European Food Safety Authority (EFSA) in 2010 assessed the safety of steviol glycosides from *Stevia* and established an Acceptable Daily Intake (ADI) for their safe use. Daily Intake (ADI) of 4 mg/kg body weight is expressed as steviol equivalents (Carakostas et al., 2008). The ADI is listed in units of mg per kg of body weight." The European Commission on 11th November 2011, allowed the usage of steviol glycosides as a food additive which will probably lead to wide-scale use in Europe (Stoyanova et al., 2011).

COMPOSITION

A number of natural products have been isolated from *S. rebaudiana* Bertoni, more than 100 compounds have been identified from this species, the best known are steviol (ent-13-hydroxykaur-16-en-19-oic acid and its glycosides) and its glycosides stevioside, rebaudioside A-F, steviolbioside, dihydroisosteviol, rubusoside and dulcoside A. Savita et al. (2004) analysed the leaves of *S. rebaudiana* on dry weight basis and calculated the energy value of 2.7 kcal g^{-1}. Structurally, stevioside (13-[2-O-ß Dglucopyranosyl-X-glucopyran-osyl) oxy] kaur-16-en-19- oic-acid ß-D- glucopyranosyl ester) is a glycoside with a glucosyl and a sophorosyl residue attached to the aglycone steviol, which has a cyclopentanon hydrophenanthrene skeleton. *Stevia* is a nutrient rich herb containing substantial amount of other nutrients, like 80 to 85% water, protein, fibre, aminoacids, free sugars, iminosugar steviamine and its (-)-steviamine enantiomer, lipids, essential oils, ascorbic acid, beta carotene, riboflavin, thiamine, austroinulin, sterebins A-H, nilacin, rebaudi oxides, gibberellic acid, indole-3-acetonitrile, apigenin, quercetin, isoquercitrin, luteolin, miocene, kaempferol, stigmasterol, xanthophyllus, umbeliferone, chlorogenic acid, caffeic acid, dicaffeoylquinic acid, chromium, cobalt, magnesium, iron, potassium, phosphorus and trace elements (Komissarenko et al., 1994; Choudhary and Bandyopadhyay, 1999; Konoshima and Takasaki, 2002; Sharma et al., 2006; Jayaraman et al., 2008; Esmat and Ferial, 2009; Hu et al., 2010). The nutritional profiles of the leaves of *S. rebaudiana* are shown in Tables 1 to 8.

THERAPEUTIC VALUES OF *S. REBAUDIANA*

The ancient Ayurvedic system of medicine has a long history regarding the use *of S. rebaudiana* (Megeji et al., 2005). Leaves of *S. rebaudiana* has been recommended as a treatment against various chronic and non-chronic diseases like diabetes, cardiovascular disease, cancer, renal disease, obesity, inflammatory bowel disease and dental caries.

Glucoregulation

Diabetes mellitus (DM) is a group of diseases characterized by hyperglycemia and varying degrees of an insufficient insulin effect. According to the World Health Organization (WHO, 2004), there are approximately 177 million people with diabetes worldwide. The global prevalence of diabetes will go up from 8.6% in 2012 to 9.8% in 2030 and the numbers of people affected with diabetes will go up from 285 to 435 million. India leads the world with the largest number of diabetic subjects, earning the dubious distinction of being termed the "diabetes capital of the world".

Stevia leaf extract has been used traditionally in the treatment of diabetes (Megeji et al., 2005; Soejarto et al., 1982). Their ingestion causes a slight suppression of plasma glucose levels and significantly increased glucose tolerance in normal adult humans (Curi et al., 1986). Steviol glycosides have an enhancing effect on insulin secretion by directly acting on β-cells without altering the K^+ - ATP channel activity and cAMP level in the islets, thus documenting stevioside and steviol as potent antihyperglycemic agents (Jeppesen et al., 2000). Stevioside regulate blood glucose levels by enhancing not only insulin secretion, but also insulin utilization in insulin-deficient rats; which was due to decreased phosphoenolpyruvate carboxykinase (PEPCK) gene expression in rat liver by stevioside's action of slowing down gluconeogenesis suggested by Chen et al. (2005). Study conducted in diabetic humans, where a single acute dose of stevioside (1,000 mg) was able to reduce

Table 1. Amount of sweet glycosides in *Stevia rebaudiana* leaves (% of the leaves dry weight).

Glycoside	Reference						
	Kinghorn and Soejarto (1985)	Crammer and Ikan (1987)	Kolb et al. (2001)	Gardana et al. (2010)	Goyal et al. (2010)	Atteh et al. (2011)	Jaworska et al. (2012)
Stevioside	5-10	3-10	3.78-9.75	5.8	9.1	6.5	2.0
Steviol	ND	ND	ND	ND	ND	ND	0.70
Steviolbioside	ND	ND	ND	ND	ND	ND	1.2
Rebaudioside A	2-4	1.0	1.62-7.27	1.8	3.8	2.3	5.0
RebaudiosideB	ND	ND	ND	ND	ND	ND	0.50
Rebaudioside C	1-2	ND	ND	1.3	0.6	ND	2.0
Rebaudioside D	ND	ND	ND	ND	ND	ND	3.3
Dulcoside A	0.4-0.7	0.2	ND	ND	0.3	ND	1.0

Table 2. Proximate analysis of dried *Stevia rebaudiana* leaves (g 100 g^{-1} dry weight basis).

Component	Reference						
	Tadhani and Subhash (2006)	Goyal et al. (2010)	Kaushik et al. (2010)	Mishra et al. (2010)	Serio (2010)	Abou-Arab et al. (2010)	Atteh et al. (2011)
Moisture	ND	4.65	7.7	7	ND	5.37	ND
Protein	20.4	11.2	12	10	11.2	11.40	16.0
Fat	4.34	1.9	2.7	3	5.6	3.73	2.6
Ash	13.1	6.3	8.4	11	ND	7.41	15.5
Carbohydrate	35.2	ND	ND	52	53	61.9	ND
Crude fibre	ND	15.2	ND	18	15	15.5	6.8

Table 3. Fatty acid composition of *Stevia rebaudiana* leaf oil (g 100 g^{-1}).

Fatty acids	Reference	
	Tadhani and Subhash (2006)	Atteh et al. (2011)
Palmitic acid (C16)	27.51	29.5
Palmitoleic acid (C16-1)	1.27	3.0
Stearic acid (C18)	1.18	4.0
Oleic acid (C18-1)	4.36	9.9
Linoleic acid (C18-2)	12.40	16.8
Linolenic acid (C18-3)	21.59	36.2

the postprandial area under the curve (AUC) of glucose by 18% relative to control (1 g corn starch) and appeared to benefit the insulin:glucose ratio in serum by 40% (Gregersen et al., 2004). The medicative effects of medium-polar (benzene:acetone, 1:1 v/v) extract of leaves from *S. rebaudiana* (family Asteraceae) on alloxan-induced diabetic rats was studied. Medium-polar leaf extract of *S. rebaudiana* (200 and 400 mg/kg) produced a delayed but significant ($P < 0.01$) decrease in the blood glucose level, without producing condition of hypoglycemia after treatment, together with lesser loss in the body weight as compared with standard positive control drug glibenclamide (Misra et al., 2011). Stevioside also enhances glucose-stimulated insulin secretion, but does not affect fasting insulinemia (Xiao and Hermansen, 2005; Chen et al., 2006). In a 6-week study, stevioside-fed diabetic rats displayed significantly enhanced first-phase insulin responses with concomitant suppression of glucagon secretion and attenuation of blood glucose concentration excursions (Jeppesen et al., 2003). The effects of *Stevia* leaves and its extracted polyphenols and fiber on streptozotocin induced diabetic rats were studied

Table 4. Amino acid composition of *Stevia rebaudiana* leaves (g 100 g^{-1} dry matter).

Amino acids	Reference	
Essential amino acid	**Abou-Arab et al. (2010)**	**Li et al. (2011)**
Arginine	0.45	0.81
Lysine	0.70	0.15
Histidine	1.13	0.34
Phenyl alanine	0.77	0.88
Leucine	0.98	1.30
Methinine	1.45	ND
Valine	0.64	0.94
Threonine	1.13	0.75
Isoleucine	0.42	0.72
Non-essential amino acid		
Aspartate	0.37	1.72
Serine	0.46	1.02
Glutamic	0.43	1.90
Proline	0.17	1.72
Glycine	0.25	0.85
Alanine	0.56	0.95
Cysteine	0.40	ND
Tyrosine	1.08	0.49

Table 5. Minerals content of dried *Stevia rebaudiana* leaves (mg 100 g^{-1}).

Component/ Mineral	Reference						
	Tadhani and Subhash (2006)	**Goyal et al. (2010)**	**Kaushik et al. (2010)**	**Mishra et al. (2010)**	**Serio (2010)**	**Abou-Arab et al. (2010)**	**Atteh et al. (2011)**
Calcium	1550	544	722	464.4	600	17.7	8.2
Phosphorus	350	318	ND	11.4	318	ND	2.6
Sodium	160	89.2	32.7	190	ND	14.93	0.7
Potassium	2510	1780	839	1800	1800	21.15	17.3
Iron	36.3	3.9	31.1	55.3	3.9	5.89	366
Magnesium	ND	349	ND	349	500	3.26	2.4
Zinc	6.39	1.5	ND	1.5	ND	1.26	20

Table 6. Water-soluble vitamins of Stevia rebaudiana leaves (mg/100 g dry base of extract).

Vitamins	Reference
	Kim et al. (2011)
Vitamin C	14.97
Vitamin B2	0.43
Vitamin B6	0.00
Folic acid	52.18
Niacin	0.00
Thiamine	0.00

and found that *Stevia* leaves have a significant role in alleviating damage in the streptozotocin-diabetic rats besides its hypoglycemic effect and it also reduce the risk of oxidative stress (Shivanna et al., 2013). Overall, *Stevia* possess the ability to increase the insulin effect on cell membranes, increase insulin production, stabilize glucagon secretion and blood sugar levels, and improve glucose tolerance to ingested carbohydrates and lower post-prandial blood sugar levels in both animals and humans. In other words, *Stevia* is shown to provide a comprehensive set of mechanisms that counter the mechanics of type II diabetes and its eventual complications. Thus, sugars can be replaced with steviol glycosides or stevioside of *Stevia* leaf to support healthy glucoregulation. The addition of leaves of *Stevia*, dried or in powder form in supplementary food products of diabetic patients aid in increasing the natural sweetness and also help in rejuvenating the pancreatic gland.

Blood pressure regulation

"Essential hypertension" is defined as an increase in blood pressure above certain measured levels. The definition of high blood pressure begins at a systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg. High blood pressure results in pathological changes accrue in medium sized and small arteries that cause further increases in blood pressure. The pathology is a thickening of the walls of these blood vessels so that effectively the diameter of the vessels is diminished. This causes the heart to work harder to pump enough blood to meet the demands of all the tissues increasing the risk for to heart attack or stroke. *Stevia* can be used as a heart tonic to normalize blood pressure levels, to regulate heartbeat, and for other cardiopulmonary indications. In humans, a hot water extract of the leaf has been shown to lower both systolic and diastolic blood pressure. Studies on *Stevia* extracts, as well as its isolated glycosides, demonstrate its hypotensive and diuretic action.

Stevia acts at the cell membrane level much in the same way as a type of medication known as a calcium channel blocking agent. These medicines are routinely prescribed to help control high blood pressure by relaxing the muscular walls of the arteries causing the elevation in blood pressure. Studies suggest that *Stevia* acts to relax arteries and lower blood pressure. Leaves of *S. rebaudiana* contain non-caloric sweeteners (steviol glycosides) whose consumption could exert beneficial effects on human health (Gardana et al., 2010). Glycosides present in *Stevia* possess valuable biological properties. Regular consumption of these compounds decreases the content of cholesterol in the blood (Atteh et al., 2008), improves cell regeneration and blood co-agulation, suppresses neoplastic growth and strengthens blood vessels (Barriocanal et al., 2008; Jeppesen et al., 2003; Maki et al., 2008; Wingard et al., 1980).

The use of stevioside results in a clinically significant hypotensive effect in spontaneously hypertensive rats, without adversely affecting their heart rates or serum catecholamine levels (Chan et al., 1998). Phytosterols present in the wax of *Stevia* leaves were found to respond against cardiovascular defects (Markovie et al., 2008).

Stevioside induces vasorelaxation (Lee et al., 2001; Wong et al., 2004; Liu et al., 2003). This effect was tested in a year-long randomized, double-blind, placebo-controlled study of 106 hypertensive subjects who consumed capsules containing either stevioside (750 mg daily) or placebo (Chan et al., 2000). Beginning after 3 months and persisting throughout the remaining 9 months of the study, the subjects consuming stevioside exhibited significantly greater decreases in systolic and diastolic blood pressures. No significant adverse effects occurred. In a longer 2-year study, compared to placebo, 1,500 mg of stevioside daily also produced significantly greater decreases in systolic and diastolic blood pressures in subjects with mild hypertension (Hsieh et al., 2003). Studies have shown that purified stevioside induces hypotension, diuresis, and natriuresis in rats and these effects are probably related to changes in prostaglandin activity (Melis et al., 1985). The effect of consumption of *Stevia* extract on 20 selected hypercholesterolemic women was studied and it was found that consumption of 20 ml extract in a glass of water (200 ml) helps in the reduction of bad cholesterol such as triglyceride and low-density lipoprotein (LDL) with significant increase in good cholesterol that is high-density lipoprotein (HDL), and it was concluded that *Stevia* extract had a hypolipidaemic effect and it maintains cardiovascular health (Sharma and Mogre, 2007). The previous studies prove the clinical efficacy of *Stevia* leaves in reducing chronic hypertension by relaxing arteries and help prevent the build up of calcium on artery walls.

Cancer

Cancer can be regarded as a disease of the body's cells. Its development involves damage to the DNA of the cells and this damage accumulates overtime. *Stevia* and its metabolites have been used for years throughout the world as a natural non-nutritive sweetening alternative to sucrose and other nutritive variants (Goyal et al., 2010). In addition, the toxicity of *Stevia* has been investigated extensively in both short- and long-term studies. Importantly, no serious toxic, genotoxic, or carcinogenic effects were detected in mammalian species and it is safe for human consumption (Aze et al., 1991; Toyoda et al., 1997).

Labdane sclareol, compound present in leaf extract of *Stevia* has anti-tumorous and cytotoxic properties (Kaushik et al., 2010). Studies have demonstrated the inhibitory effects of *Stevia* leaf extracts and their polyphenolic constituents on tumor promotion and initiation. Stevioside, the *Stevia* leaf aglycones, steviol and isosteviol, and their metabolites have been reported to inhibit tumor promotion by blocking Epstein-Barr virus early antigen (EBV-EA) induction (Akihisa et al., 2004) as well as by reducing tumor formation in the two-stage mouse skin carcinogenesis model following sequential exposure to 7,12-dimethylbenz [a]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Konoshima and Takasaki, 2002; Yasukawa et al., 2002; Takasaki et al., 2009). The hydrolysis product of stevioside, isosteviol, potently inhibits DNA replication and human cancer cell growth *in vitro* (with LD50 values of 84 to 167 µMol) (Mizushina et al*.,* 2005). The toxicity of rebaudioside-A was studied by bacterial reverse mutation test (Ames test) using standard *Salmonella typhimurium* as well as *Escherichia coli*, there was no statistically significant increase in the number of relevant colonies

exposed to rebaudioside-A at concentrations up to 5000 µg/plate. In the Ames test, rebaudioside-A was found to be non-mutagenic in these two bacterial strains. Rebaudioside-A was evaluated for mutagenic potential in cultured human lymphocytes, this test did not induce a statistically significant increase in the incidence of chromosomal aberrations or polyploidy in cultured Chinese Hamster V79 cells after 4 and 20 h treatments at any of the doses tested with or without S9 metabolic activation. Additionally, rebaudioside-A was shown not to cause any signs of toxicity in male Wistar rats after being administered a single dose of 2000 mg/kg of body weight and observed for 16 h post dosing (Williams and Burdock, 2009). Effect of stevioside against tumor was examined and stevioside slowed the tumor promoting agent (TPA) induced tumor promotion in a skin carcinogenesis in mice (Nakamura et al., 1995). Toxic and carcinogenic effects of stevioside was studied on initiation and promotion of urinary bladder, results showed that pre-neoplastic or neoplastic lesions development was not enhanced in urinary bladders by stevioside while studies performed with the dose effect of bladder carcinogenicity of *N*-nitrosobutyl-*N*- (4-hydroxybutyl) amine (Hagiwara et al., 1984). Subsequently, no neoplastic or pre-neoplastic lesions were observed in any tissue (Xili et al*.*, 1992). Steviol and stevioside were tested for mutagenicity in *S. typhimurium* strains TA98 and TA100 and for chromosomal effects on cultured human lymphocytes; results revealed that steviol did not exhibit mutagenicity in either TA98 or TA100, with or without metabolic activation. However, stevioside was not mutagenic at concentrations up to 25 mg/plate, but showed direct mutagenicity to only TA98 at 50 mg/plate.

No significant chromosomal effect of stevioside and steviol was observed in cultured blood lymphocytes from healthy donors (Suttajit et al., 1993). Stevioside, a bioactive compound present in *Stevia* was found to be nonmutagenic in 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) (Matsui et al*.*, 1996). The toxicological effects of low concentrations of stevioside on apoptosis induced by serum deprivation using the PC12 cell system was studied by using DNA electrophoresis and TUNEL signal assays and on the basis of data it was found that stevioside enhanced apoptosis induced by serum deprivation and this enhancement was caused by increased expression of Bax and of cytochrome c released into the cytosol which suggest that stevioside affects the regulation of the normal apoptotic condition (Takahashi et al*.,* 2012).

Renal function

Globally, there are nearly 70 million people with kidney disease of varying severity levels. Chronic kidney disease resulted in 400,000 deaths in 1990 and 735,000 deaths in 2010 (Lozano, 2012). The kidneys are essential organs for maintaining many aspects of the internal environment of the body. The main function of kidney is to maintain homeostatic balance with respect to fluids, electrolytes, and organic solutes. Various disease conditions may affect the kidney and disturb the normal functioning of the nephrons.

Melis (1992) studied the effect of stevioside from the leaves of *S. rebaudiana* on renal function of normal and hypertensive rats. Analysed stevioside acts as a typical systemic vasodilator which provoked hypotension, diuresis and natriuresis in both the normal and hypertensive rats. Constant administration of stevioside in both normal and hypertensive rats increase the glomerular filtration rate (GFR) and renal plasma flow (RPF) which was due to vasodilation of both the afferent and efferent arterioles. Study was designed to explore the direct effect of stevioside on transepithelial transport of p-aminohippurate (PAH) in isolated S2 segments of rabbit proximal renal tubules using *in vitro* micro-perfusion. Findings suggest that stevioside, at a pharmacological concentration of 0.70 mM, inhibits transepithelial transport of PAH by interfering with the basolateral entry step, the rate-limiting step for transepithelial transport. The lack of effect of stevioside on transepithelial transport of PAH on the luminal side and its reversible inhibitory effect on the basolateral side indicate that stevioside does not permanently change PAH transport and should not harm renal tubular function at normal human intake levels (Jutabha et al., 2000).

Yuajit et al. (2013) studied the inhibitory effect and detailed mechanisms of steviol and its derivatives on cyst growth using a cyst model in Madin-Darby canine kidney (MDCK) cells. Results revealed that steviol retards MDCK cyst progression, first by directly inhibiting cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel activity and second by reducing CFTR expression, in part, by promoting proteasomal degradation of CFTR. Steviol and its analogs represent promising natural plant-based drug candidates for treatment of polycystic kidney disease.

Obesity

Obesity is the most common nutritional disorder; it is a state of excess accumulation of fat in the body. In clinical terms, obesity is a condition of excess body weight more than 20% above the ideal body weight. In recent years, overweight and obesity have increased markedly and contributing factors include a social environment that supports physical inactivity, excessive food consumption, and unhealthy food choices. Overweight and obesity, is a major risk factor associated with a wide number of health problems including hypertension, hyperlipidemia, diabetes, surgical risks, pulmonary and renal problems, pregnancy complications and certain type of cancer.

Increased consumption of sugar leads to several nutritional and medicinal problems such as obesity. Regular consumption of sugar-sweetened snacks and beverages may cause metabolic disorders, such as obesity. Therefore, an efficacious weight management strategy is to substitute sugar with low calorie sweeteners (Stephen et al., 2010). *Stevia* leaves contain zero-calorie *ent*-kaurene diterpene glycosides (stevioside and rebaudiosides) that are not metabolized to produce energy and taste 300 times sweeter than sucrose (Soejarto et al., 1982; Megeji et al., 2005; Walter and Soliah, 2010). In human studies, the measured sweetness of 1 g of crude extract of *Stevia* leaf dissolved in water ranged from 100 to 150 times that of equivalent concentrations of sucrose (Cardello et al., 1999). *Stevia* sweeteners in foods and beverages offer low calorie alternative substitute of sugar, assist with weight control and weight loss by restricting or controlling calorie intake in the diet. Ingestion of steviol in high doses showed a reduction in body weight as experimented in rats (Curry and Roberts, 2008).

Leaves of *S. rebaudiana* can also be used as a functional food ingredient and prove beneficial to dietetic practice. In fact, “replacing intake of added sugars with non-nutritive sweeteners could result in a deficit of 380 cal/day or 1 pound of weight loss in 9 to 10 days, if intake was at 95 g (24 tsp) daily” (8 Position of JADA). *Stevia* can be used in place of sugar as they provide fewer calories per gram than sugar which is not completely absorbed by digestive system. Consumption of *Stevia* leaves and extract reduce the carving for sweet and fatty foods and are useful in weight loss programme (Jain et al., 2007).

Inflammatory bowel disease (IBD)

IBD is a group of inflammatory conditions of the colon and small intestine. The two major forms are Crohn’s disease and ulcerative colitis. The onset of IBD occurs most often in patients between the ages of 15 and 30 years, and both sexes are equally affected. In each case, the cause is unknown but genetic predisposition and immune and autoimmune phenomena are involved (Kornbluth et al., 1998).

Stevia and its polyphenolic compounds steviol and stevioside exert anti-inflammatory effects on colonic epithelial cells. Shiozaki et al. (2006) conducted the study on animals and observed that stevioside inhibit intestinal smooth muscle contraction, stimulation of which is linked to hypermotility-associated diarrhea. Pariwat et al. (2008) studied the stevioside and its similar compounds steviol, dihydroisosteviol, isosteviol and isosteviol 16-oxime, on cAMP-regulated chloride (Cl) secretion in human T84 colonic epithelial cells line and *in vivo* for their antidiarrheal efficacy, results showed that steviol and its analogs, inhibited cAMP activated Cl secretion in intact T84 cells in a dose-dependent manner. The stevioside ineffectiveness could be due to its molecular bulkiness, rendering it relatively impermeable to cell membranes, and thereby exhibiting a promising agent in antidiarrheal treatment. Similar compounds of dihydroisosteviol could be a new class of cystic fibrosis transmembrane conductance regulator inhibitors that may be useful for further development as antidiarrheal agents.

Dental caries

Dental caries, also known as tooth decay, is the most prevalent chronic diseases of people worldwide and individuals are susceptible to this disease throughout their lifetime. It is an oral infectious disease in which organic acid metabolites produced by the metabolism of oral microorganisms lead to gradual demineralization of tooth enamel, followed by rapid proteolytic destruction of the tooth structure. Bacteria are an essential part of the tooth decay process. Several microorganisms are capable of fermenting dietary carbohydrate. *Streptococcus mutans* is the most prevalent followed by *Lactobacillus casein* and *Streptococcus sanguis*.

Regular consumption of nutritive sweeteners also known as caloric sweeteners or sugars provide energy in the form of carbohydrate, causes cavities which encourage the growth of harmful bacteria in the mouth contributing to plaque formation and gingivitis. There is a requirement to substitute sucrose with natural sweetener which should be nutritionally appropriate and not being detrimental to the overall general health of the individual (Matsukubo and Takazoe, 2010). *Stevia*, as a non nutritive sweetener are zero- or low-calorie alternatives to nutritive sweeteners, such as table sugar possess bacteriostatic and bacteriocidal properties benefit oral health by eliminating the cause of dental decay and gingivitis. *Stevia* is a natural sucrose substitute with high nutritional value beneficial in the battle against dental caries. Extract of *Stevia* leaves and its major secondary metabolites, steviol, isosteviol, stevioside and rebaudioside A, B, C and E are noncariogenic and have been found to inhibit glucan induced aggregation of cariogenic organism, Thus *Stevia* have potential of providing oral health benefits (Wu et al., 1998). Studies suggested that development of dental caries in rat pups are triggered in presence of sucrose solution while it is not with stevioside (Das et al., 1992). The major cariogenic organism, *S. mutans,* experiences growth suppression and secretes less acid when grown on media containing stevioside than when grown on sucrose, glucose or fructose media (Grenby, 1991).

MARKETING AND USE

The excessive sugar intakes have become a major health concern all over the world. This means that majority of our society is at risk for a high number of precarious

health conditions that can lead to various chronic diseases (Anton et al., 2010). The major culprit along with solid fats is a surplus of sugar, which on average provides 35%, or nearly 800 calories/person/day. These disproportionate amounts create elevated levels of at risk members in our society. There is a fundamental need for an alternative sweetener in place of sugar, or other chemical sweeteners. It may have taken a long time for many countries to decide, but today, throughout the world including India, *Stevia* is growing successfully. It has certainly earned the right to be considered a safe, natural sugar substitute and alternative sweetener used as a functional food ingredient to sweeten a diverse variety of consumer products such as soft drinks, tea, coffee, ice-creams, confectionery and bakery, etc. As of 2011, natural sweeteners made from extract of the *Stevia* plant have taken about 10% of the US consumer market for table-top sugar substitutes, only nine months after being approved by the US Food and Drug Administration. GLG Life Tech, an international *Stevia* supplier has taken the initiative to introduce *Stevia* in Indian market and facilitated its production and extraction. *Stevia* costs 30% less than current sugar prices and its growing popularity has drawn Coca-Cola Company to introduce, develop and commercialize *Stevia* in its leading brands. Relative to sucrose, the potent sweetness intensities of these glycosides have projected them as cost effective sucrose substitute which demonstrates how a slight alteration in a person's diet can drastically change their health risk.

CONCLUSION

The sweet herb *S. rebaudiana* (Bertoni) has a valuable future and is extensively used in various areas of the world. *Stevia* and its metabolites have commercial value in number of countries as sugar substitutes in foods, beverages and medicines. Studies have reported the health promoting of this natural herb *Stevia* which is well known as therapeutic agent and an efficient medication for curing chronic diseases. Researchers need to work more on *Stevia* for clinical evidences and demonstration of metabolic pathways regarding benefits to explore its full potential.

REFERENCES

Abdullateef RA, Osman M (2012). Studies on effects of pruning on vegetative traits in *Stevia rebaudiana* Bertoni (Compositae). Int. J. Biol. 4:146-153.

Abou-Arab A, Abou-Arab A, Abu-Salem MF (2010). Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia* rebaudiana Bertoni plant. Afr. J. Food Sci. 4:269–281.

Akihisa T, Hamasaki Y, Tokuda H, Ukiya M, Kimura Y, Nishino H (2004). Microbial transformation of isosteviol and inhibitory effects on Epstein-Barr virus activation of the transformation products. J. Nat. Prod. 67:407-410.

Allam AI, Nassar AM, Besheite SY (2001). Nitrogen fertilizer requirement of *Stevia rabaudiana* Bertoni under Egyptian condition. Egyptian J. Agric. Res. 79:1005-1018.

Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P, Williamson DA (2010). Effects of *Stevia*, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite 55:37-43.

Atteh J, Onagbesan O, Tona K, Buyse J, Decuypere E, Geuns J (2011). Potential use of *Stevia rebaudiana* in animal feeds. Arch. Zootec. 60:133-136.

Atteh J, Onagbesan O, Tona K, Decuypere E, Geuns J, Buyse J (2008). Evaluation of supplementary *Stevia* (*Stevia rebaudiana* Bertoni) leaves and stevioside in broiler diets: Effects on feed intake, nutrient metabolism, blood parameters and growth performance. J. Anim. Physiol. Anim. Nutr. 92:640–649.

Aze Y, Toyoda K, Imaida K, Hayashi S, Imazawa T, Hayashi Y, Takahashi M (1991). Subchronic oral toxicity study of stevioside in F344 rats. Eisei Shikenjo Hokoku 109:48-54.

Barriocanal L, Palacios M, Benitez G, Benitez S, Jimenez JT, Jimenez N (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans, a pilot study of repeated exposures in some normatensive and hypotensive individuals and in type 1 and type 2 diabetics. Regul. Toxicol. Pharmacol. 51:37–41.

Benford DJ, DiNovi M, Schlatter J (2006). "Safety Evaluation of Certain Food Additives: Steviol Glycosides"(PDF). WHO Food Additives Series (World Health Organization Joint FAO/WHO Expert Committee on Food Additives (JECFA) 54:140.

Bridel M, Lavielle R (1931). "Sur le principe sucre des feuilles de kaa-he-e (*Stevia* rebaundiana B)". Acad. Sci. 192:1123–1125.

Carakostas MC, Curry LL, Boileau AC, Brusick DJ (2008). Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem. Toxicol. 46:S1-S10.

Cardello HM, Silva MA, Damasio MH (1999). Measurement of the relative sweetness of *Stevia* extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Food Hum. Nutr. 54:119-30.

Chan P, Tomlinson B, Chen Y, Liu J, Hsieh M, Cheng J (2000). A doubleblind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br. J. Clin. Pharmacol. 50:215-220.

Chan P, Xu DY, Liu JC, Chen YJ, Tomlinson B, Huang WP, Cheng JT (1998). The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. Life Sci. 63:1679-1684.

Chatsudthipong V, Muanprasat C (2009). Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacol. Ther. 121:41-54.

Chen J, Jeppesen PB, Nordentoft I, Hermansen K (2006). Steviodose counteracts the glyburide-induces desensitization of the pancreatic beta-cell function in mice: Studies *in vitro*. Metabolism 55:1674-1680.

Chen TH, Chen SC, Chan P, Chu YL, Yang HY, Cheng JT (2005). Mechanism of the hypoglycemic effect of stevioside, a glycoside of *Stevia rebaudiana*. Planta Med. 71:108–113.

Choudhary K, Bandyopadhyay N (1999). Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. J. Ethnopharmacol. 64:179–184.

Crammer B, Ikan R (1987). Progress in the chemistry and properties of the rebaudiosides. In *Developments in Sweeteners*; Grenby, T. H., Ed.; Elsevier Applied Science: London, U.K. pp. 45-64.

Curi R, Alvarez M, Bazotte RB, Botion LM, Godoy JI, Bracht A (1986). Effect *of Stevia rebaudiana* on glucose tolerance in normal adult human. Braz. J. Med. Biol. Res. 19:771-774.

Curry LL, Roberts A (2008). Subchronic toxicity of rebaudioside A. Food Chem. Toxicol. 46:S11–S20.

Das S, Das AK, Murphy RA, Punwani IC, Nasution MP, Kinghorn AD (1992). Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. Caries Res. 26:363–366.

Debnath M (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. J. Med. Plant Res. 2:045-051.

Esmat AAA, Ferial MAS (2009). Evaluation of bioactive compounds of *Stevia rebaudiana* leaves and callus. Afr. J. Food Sci. 4:627–634.

Gardana C, Scaglianti M, Simonetti P (2010). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweetener

by ultra high performance liquid chromatography–mass spectrometry. J. Chromatogr. A. 1217:1463–1470.
Geuns JM, Augustijns P, Mols R, Buyse JG, Driessen B (2003). Metabolism of stevioside in pigs and intestinal absorption characteristics of stevioside, rebaudioside A and steviol. Food Chem. Toxicol. 41:1599-607.
Geuns JM, Buyse J, Vankeirsbilck A, Temme EH (2007). Metabolism of stevioside by healthy subjects. Exp. Biol. Med. 232:164-173.
Goettemoeller J, Ching A (1999). Seed germination in *Stevia* rebaudiana. Perspectives on new crops and new users. J Janick (Ed.), ASHS Press. Alexandria, VA.
Goyal S, Samsher, Goyal R (2010). *Stevia* (*Stevia rebaudiana*) a bio-sweetener: a review. Int. J. Food Sci. Nutr. 61:1-10.
Gregersen S, Jeppesen PB, Holst JJ, Hermansen K (2004). Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism 53:73-76.
Grenby TH (1991). Update on low-calorie sweeteners to benefit dental health. Int. J. Dent. 41:217-24.
Hagiwara A, Fukushima S, Kitaori M (1984). Effects of the three sweetener on rats urinary bladder carcinogenesis initiated by Nbutyl-N-(4-hydroxybutyl)-nitrosamine. Gann. 75:763–768.
Hsieh MH, Chan P, Sue YM, Liu JC, Liang TH, Huang TY, Tomlinson B, Chow MS, Kao PF, Chen YJ (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: A two-year, randomized, placebo-controlled study. Clin Ther. 25:2797-2808.
Hu XG, Bartholomew B, Nash RJ, Wilson FX, Fleet GW, Nakagawa S, Kato A, Jia YM, van Well R, Yu CY (2010). Synthesis and glycosidase inhibition of the enantiomer of (-)-*Stevia*mine, the first example of a new class of indolizidine alkaloid. Org. Lett. 2:2562-2565.
Hutapea AM, Toskulkao CH, Buddhasukh D, Wilairat P, Glinsukon TH (1997). Digestion of stevioside, a natural sweetener, by various digestive enzymes. J. Clin. Biochem. Nutr. 23:177-186.
Huxley A (1992). The New RHS Dictionary of Gardening. MacMillan Press. ISBN 0-333-47494-5.
Jain JL, Jain S, Jain N (2007). Fundamentals of biochemistry New Delhi: S. Chand & Co. Pub. Ltd. pp. 104–107.
Jaroslav P, Barbora H, Tuulia H (2006). Characterization of *Stevia rebaudiana* by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. J. Chromatogr. A. 1150:85-92.
Jaworska K, Krynitsky AJ, Rader JI (2012). Simultaneous analysis of steviol and steviol glycosides by liquid chromatography with ultraviolet detection on a mixed-mode column: application to *Stevia* plant material and *Stevia*-containing dietary supplements. J. AOAC Int. 95:1588-1596.
Jayaraman S, Manoharan M, Illanchezian S (2008). *In-vitro* anti-microbial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Trop. J. Pharm. Res. 7:1143–1149.
Jeppesen PB, Gregersen S, Alstrupp KK, Hermansen K (2002). Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects *in vivo*: studies in the diabetic goto-Kakizaki (GK) rats. Phytomedicine 9:9-14.
Jeppesen PB, Gregersen S, Poulsen CR, Hermansen K (2000). Stevioside acts directly on pancreatic β-cells to secrete insulin; Actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K^+ channel activity. Metabolism 49:208-214.
Jeppesen PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A, Xiao J, Kruhøffer M, Orntoft T, Hermansen K (2003). Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism 52:372-378.
Jutabha P, Toskulkao C, Chatsudthipong V (2000). Effect of stevioside on PAH transport by isolated perfused rabbit renal proximal tubule. Can. J. Physiol. Pharm. 78:737–744.
Kaushik R, Narayanan P, Vasudevan V, Muthukumaran G, Antony U (2010). Nutrient composition of cultivated *Stevia* leaves and the influence of polyphenols and plant pigements on sensory and antioxidant properties of leaf extracts. J. Food Sci. Tech. 47:27-33.
Kedik SA, Yartsev EI, Stanishevskaya IE (2009). Antiviral activity of dried extract of *Stevia.* Pharmaceut. Chem. J. 43:198–199.
Kim I, Yang M, Lee O, Kang S (2011). The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT – Food Sci. Technol. 44:1328–1332.
Kim J, Choi YH, Choi YH (2002). Use of stevioside and cultivation of *Stevia* rebaudiana in Korea. In: Kinghorn, A.D. (Ed.), *Stevia*, the Genus *Stevia*. Medicinal and Aromatic Plants-Industrial Profiles, Taylor and Francis, London and NY. 19:196–202.
Kinghorn A, Soejarto D (1985). Current status of stevioside as a sweetening agent for human use, *In*: Economic and medicinal plant research by Wagner H., Hikino H., Farnsworth N., (Eds.), Academic Press, London 1:1-52.
Kolb N, Herrera JL, Ferreyra DJ, Uliana RF (2001). Analysis of sweet diterpene glycosides from *Stevia rebaudiana:* improved HPLC method. J. Agric. Food Chem. 49:4538-4541.
Komissarenko NF, Derkach AI, Kovalyov IP, Bublik NP (1994). Diterpene glycosides and phenylpropanoids of *Stevia rebaudiana* Bertoni. Rast. Res. 1:53–64.
Konoshima T, Takasaki M (2002). Cancer-chemopreventive effects of natural sweetners and related compounds. Pure Appl. Chem. 74:1309-1316.
Kornbluth A, Sachar DB, Salomon P (1998). Crohn's disease. In: Feldman M, Sleisenger MH, Scharschmidt BF (eds.). Gastrointestinal and Liver disease, 6th ed. Philadelphia: WB Saunders.
Koyama E, Kitazawa K, Ohori Y, Izawa O, Kakegawa K, Fujino A, Ui M (2003). *In vitro* metabolism of the glycosidic sweeteners, *Stevia* mixture and enzymatically modified *Stevia* in human intestinal microflora. Food Chem. Toxicol. 41:359-374.
Lee CN, Wong K, Liu J, Chen Y, Chen J, Chan P (2001). Inhibitory effect of stevioside on calcium influx to produce anti-hypertension. Planta Med. 67:796-799.
Li G, Wang R, Quampah AJ, Rong Z, Shi C, Wu J (2011). Calibration and Prediction of Amino Acids in *Stevia* Leaf Powder Using Near Infrared Reflectance Spectroscopy. J. Agric. Food Chem. 59:13065–13071.
Liu JC, Kao PK, Chan P, Hsu YH, Hou CC, Lien GS, Hsieh MH, Chen YJ, Cheng JT (2003). Mechanism of the antihypertensive effect of stevioside in anesthetized dogs. Pharmacology 67:14-20.
Lozano R (2012). "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010". Lancet 380:2095–128.
Maki K, Curry L, Reeves M, Toth P, Mckenney J, Farmer MV (2008). Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food Chem. Toxicol. 46:47–53.
Mantovaneli ICC, Ferretti EC, Simões MR, Da Silva FC (2004). The effect of temperature and flow rate on the clarification of the aqueous *Stevia*-extract in a fixed-bed column with zeolites. Braz. J. Chem. Eng. 21:449-458.
Markovie IS, Darmati ZA, Abramovic BF (2008). Chemical composition of leaf extracts of *Stevia rebaudiana* Bertoni grown experimentally in Vojvodina. J. Serb. Chem. Soc. 73:283-297.
Matsui M, Matsui K, Kawasaki Y, Oda Y, Noguchi T, Kitagawa Y, Sawada M, Hayashi M, Nohmi T, Yoshihira K, Ishidate MJ, Sofuni T (1996). Evaluation of the genotoxicity of stevioside and steviol using six *in vitro* and one *in vivo* mutagenicity assays. Mutagenesis 11:573-9.
Matsukubo T, Takazoe I (2010). Sucrose substitutes and their role in caries prevention, Int. Dent. J. 56:119-130.
Megeji NW, Kumar JK, Singh V, Kaul VK, Ahuja PS (2005). Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. Curr. Sci. 88:801-804.
Melis MS (1992). Stevioside effect on renal function of normal and hypertensive rats J Ethnopharmacol. 36:213–217.
Melis MS, Macial RE, Sainati AR (1985). Effects of indomethacin on the action of stevioside on mean arterial pressure and renal function in rats. IRCS Med Sci. 13:1230-1231.
Mishra P, Singh R, Kumar U, Prakash V (2010). *Stevia* rebaudiana – A magical sweetener. Global. J. Biotech. Biochem. 5:62–74.
Misra H, Soni M, Silawat N, Mehta D, Mehta BK, Jain DC (2011). Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats. J. Pharm. Bioall. Sci. 3:242–248.
Mizushina Y, Akihisa T, Ukiya M, Hamasaki Y, Murakami NC, Kuriyama

I, Takeuchi T, Sugawara F, Yoshida H (2005). Structural analysis of isosteviol and related compounds as DNA polymerase and DNA topoisomerase inhibitors. Life Sci. 77:2127-2140.
Mizutani K, Tanaka O (2002). Use of *Stevia* rebaudiana sweeteners in Japan. In: Kinghorn, A.D. (Ed.), *Stevia*, the Genus *Stevia*. Medicinal and Aromatic Plants—Industrial Profiles, Taylor and Francis, London and NY 19:178-195.
Mohan K, Robert J (2009). Hepatoprotective effects of *Stevia rebaudiana* Bertoni leaf extract in CCl_4-induced liver injury in albino rats. Med. Arom. Plant Sci. Biotechnol. 3:59–61.
Nakamura Y, Sakiyama S, Takenaga K (1995). Suppression of syntheses of high molecular weight nonmuscle tropomyosins in macrophages. Cell Motil. Cytoskel. 31:273−282.
NeOfficial Journal of the European Union (2011). NeOfficial Journal of the European Union (11 November 2011) "COMMISSION REGULATION (EU) No 1131/2011"(PDF). p. 7. Retrieved 15 November 2011. "The CE regulation establishes steviol glycosides as food additive, and establishes maximum content levels in foodstuff and beverages."
Pariwat P, Homvisasevongsa S, Muanprasat C, Chatsudthipong V (2008). A natural plant-derived dihydroisosteviol prevents cholera toxin induced intestinal fluid secretion. J. Pharmacol. Exp. Ther. 324:798-805.
Phillips KC (1987). *Stevia*: Steps in developing a new sweetner, In: Grenby TH, editor Developments in sweetners New York pp. 1-5.
Pol J, Hohnová B, Hyötyläinen T (2007). Characterization of *Stevia* rebaudiana by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. J. Chromatogr. A. 1150:85–92.
Rajasekaran T, Ramakrishna A, Udaya Sankar K, Giridhar P, Ravishankar G (2008). Analysis of predominant steviosides in *Stevia rebaudiana* bertoni by liquid chromatography/electrospray ionization-mass spectrometry. Food Biotechnol. 22:179-188.
Satishkumar J, Sarvanan MM, Seethalakshmi I (2008). *In-vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Trop. J. Pharm. Res. 7:1143–9.
Savita S, Sheela K, Sunanda S, Shankar A, Ramakrishna P (2004). *Stevia rebaudiana* – A functional component for food industry. J. Hum Ecol. 15:261–264.
Serio L (2010). La *Stevia rebaudiana*, une alternative au sucre. Phytothérapie. 8:26–32.
Sharma N, Kaushal N, Chawla A, Mohan M, Sethi A, Sharma Y (2006). *Stevia rebaudiana*—A review. Agrobios Newsletter 5:46–48.
Sharma N, Mogre R (2007). Effect of *Stevia* intervention on lipid profile. In: On serving farmers and saving farming—India imperative and global perspective, GBPUA & T, Pantnagar, 10–12 January p. 85.
Shiozaki K, Fujii A, Nakano T, Yamaguchi T, Sato M (2006). Inhibitory effects of hot water extract of the *Stevia* stem on the contractile response of the smooth muscle of the guinea pig ileum. Biosci. Biotechnol. Biochem. 70:489-94.
Shivanna N, Naika M, Khanum F, Kaul VK (2013). Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana.* J. Diabetes Complicat. 27:103-113.
Silva PA, Oliveira DF, Prado NR, Carvalho DA, Carvalho GA (2008). Evaluation of the antifungal activity by plant extracts against *Colletotrichum gloeosporioides* PENZ. Ciência e Agrotecnologia 32:420–8.
Singh S, Garg V, Yadav D, Beg MN, Sharma N (2012). *In vitro* antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (Bertoni). Int. J. Pharm. Pharm. Sci. 4:468-473.
Singh SD, Rao GP (2005). *Stevia*: The herbal sugar of 21st century. Sugar Tech. 7:17-24.
Soejarto DD, Kinghorn AD, Farnsworth NR (1982). Potential sweetening agents of plant origin. III. Organoleptic evaluation of *Stevia* leaf herbarium samples for sweetness. J. Nat. Prod. 45:590-599.
Stephen DA, Corby KM, Hongmei H, Sandra C, William TC, Paula G, Donald AW (2010). Effects of *Stevia*, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite 55:37–43.
Stoyanova S, Geuns J, Hideg E, Van den Ende W (2011). The food additives inulin and stevioside counteract oxidative stress. Int. J. Food Sci. Nutr. 62:207–214.
Suttajit M, Vinitketkaumnuen U, Meevatee U, Buddhasukh D (1993). Mutagenicity and human chromosomal effect of stevioside, a sweetener from *Stevia rebaudiana* Bertoni. Environ. Health Perspect. 3:53-56.
Tadhani M, Subhash R (2006). Preliminary studies on *Stevia rebaudiana* leaves: Proximal composition, mineral analysis and phytochemical screening. J. Med. Sci. 6:321-326.
Takahashi K, Sun Y, Yanagiuchi I, Hosokawa T, Saito T, Komori M, Okino T, Kurasaki M (2012). Stevioside enhances apoptosis induced by serum deprivation in PC12 cells. Toxicol. Mech. Methods 22:243-249.
Takahashi K, Iwata Y, Mori S, Shigeta S (1998). *In-vitro* anti-HIV activity of extract from *Stevia rebaudiana*. Antiviral Res. 37:A59.
Takahashi K, Matsuda M, Ohashi K, Taniguchi K, Nakagomi O, Abe Y, Mori S, Sato N, Okutani K, Shigeta S (2001). Analysis of anti-rotavirus activity of extract from *Stevia rebaudiana*. Antiviral Res. 49:15–24.
Takasaki M, Konoshima T, Kozuka M, Tokunda H, Takayasu J (2009). Cancer preventive agents. Part 8: Chemopreventive effects of stevioside and related compounds. Bioorg. Med. Chem. 17:600-605.
Toyoda K, Matsui H, Shoda T, Uneyama C, Takada K, Takahashi M (1997). Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem. Toxicol. 35:597-603.
Walter JM, Soliah L (2010). Objective Measures of Baked Products Made with *Stevia*. J. Am. Diet. Assoc. 110:A54-A54.
Williams LD, Burdock GA (2009). Genotoxicity studies on a high-purity rebaudioside A preparation. Food Chem. Toxicol. 47:1831-1836.
Wingard R, Brown J, Enderlin F, Dale J, Hale R, Seitz C (1980). Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Cell Mol. Life Sci. 36:519–520.
Wong KL, Chan P, Yang HY, Hsu FL, Liu IM, Cheng YW, Cheng JT (2004). Isosteviol acts on potassium channels to relax isolated aortic strips of Wistar rat. Life Sci. 74:2379-2387.
World Health Organization (2004). Diabetes: The Cost of Diabetes. http://www.who.int/mediacentre/fact-sheets/fs236/en/, Accessed on December 5. pp. 627–634.
Wu CD, Johnson SA, Sriakantha R, Kinghorn AD (1998). Intense natural sweetener and their effect on cariogenic bacteria. J. Dental. Res. 77:283.
Xiao J, Hermansen K (2005). The mechanism underlying the insulintropic effect of stevioside- activation of acetyl-CoA carboxylase (abstract). Diabetes 54:A131.
Xili L, Chengjiany B, Eryi X, Reiming S, Yuengming W, Haodong S, Zhiyian H, (1992). Chronic oral toxicity and carcinogenicity study of stevioside in rats. Food Chem. Toxicol. 30:957–965.
Yasukawa K, Kitanaka S, Seo S (2002). Inhibitory effect of stevioside on tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two stage carcinogenesis in mouse skin. Biol. Pharm. Bull. 25:1488-90.
Yuajit C, Homvisasevongsa S, Chatsudthipong L, Soodvilai S, Muanprasat C, Chatsudthipong V (2013). Steviol Reduces MDCK Cyst Formation and Growth by Inhibiting CFTR Channel Activity and Promoting Proteasome-Mediated CFTR Degradation. doi:10.1371/journal.pone.0058871. PLoS ONE. 8:e58871.