[Download free from www.ijplsjournal.com, Dec., 2010]

ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Stevia rebaudiana: A medicinal and nutraceutical plant and sweet gold for diabetic patients

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Abstract

Natural sweeteners that can substitute for sucrose have caught great attention due to the growing incidence of obesity and diabetes. Stevia (*Stevia rebaudiana*) is an exotic plant in our country. It has both economical and medicinal importance. Now a days it has become a major source of commercial sweetener for the growing natural food market in the future. This plant has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. The sugar or sucrose is the most popular sweetener in the world. However, for adverse health effects of sucrose and known artificial sweeteners, interest in and search for no calorie natural sweeteners has been intensified in recent years. Very fortunately, stevioside was found which can satisfy the urge for nurge for sweet consumption of diabetic subjects. The leaves of *Stevia rebaudiana* contain different steviol glycosides, the major constituent being stevioside. Stevioside is a diterpenoid glycoside, comprising an aglycone (steviol) and three molecules of glucose. In addition to stevioside several other sweet compounds such as steviobioside, rebaudioside A, B, C, D, E and ducoside A, were isolated from *Stevia rebaudiana.* This review discusses the potential of medicinal and nutritional importance of this wild herb for health care management and also describes its as an alternative for diabetic patients.

Keywords: Streptozotocin (STZ), Stevioside (SV), DM (diabetic rats), Mean arterial blood pressure (MABP),

Introduction

Diabetes is one of the most commonly occurring problems around the globe. Technically it is known as Diabetes Mellitus. It is the single most important metabolic disorder. This can affect nearly every organ system in the body. This is actually a disease in which there is uncontrolled increase of glucose or sugar level in the blood there by loading to many troubles. These disturbances in the insulin levels lead to the uncontrolled increase in glucose in the blood that can even be detected in the urin[1.] Diabetes mellitus is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed in this century, medical science cannot claim that it knows all that needs to be known about this disease, including its management. This is the main reason for the persistent interest all over the world to explore alternative remedies from the so-called "alternative systems" of medicine[2.] The disease was well known to the ancient Indian medical experts. All the renowned classic texts of Ayurveda like Charaka Samhita (1000 B.C.), Sushruta Samhita (600 B.C.) and subsequent works refer to this disease under the term *Madhumeha* or *Ikshumeha* (literally meaning sugar in the urine). Apart from detailed description of its etiopathogenesis (according to Ayurvedic concepts), the two types of diabetic patients (obese and lean) and a definite familial prediction to the disease are referred to in Ayurveda, besides the importance given to dietary regulations, physical exercises and baths, in addition to the use of a number of plant drugs in the management of the disease[3].There is an intense search for low calorie sweeteners and high potency in order to provide an alternative to sugar for its use in food and drugs[4]. Stevia is a natural non-calorie sweetener [5].

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ISSN: 0976-7126

The leaves of stevia contain deterpene glycoside viz. stevioside and rebaudioside with a sweet taste [6]. Stevioside extracted from the plant is 300-500 times sweetener than sugar. It is recommended for diabetes and has been extensively tasted on animals and has been used by humans with no side effects[7].

Stevioside is complex of three glucose molecules and one molecule of steviol aglycone, a diterpenic carboxylic alcohol[8]. Due to its noncalorie value, it has become popular as a sugar substitute in a variety of foods and beverages in Japan, Brazil, South Korea and Paraguay [9]. In addition to its use as a sweetener, several researchers have shown stevioside to have therapeutic value as contraceptive[10] and to have cardiovascular[11a] and metabolic effects. The leave extracts of stevia has documented properties of antibacterial, antifungal, anti-inflammatory, antimicrobial, antiviral, antiyeast, cardiotonic, diuretic, hypoglycemia and hence a boon to diabetic people[12].

The colonel propagation and antimicrobial activity of *Stevia rebaudiana* has described by Debnath [13]. They found in their studies that all the bacterial species viz. *E coli* (MT CC41), *B. subtilis* (MT CC 41), *S. mutans* (MT CC479) and *S. aureus* (MT CC 737) were inhabited by the *Stevia rebaudiana* extracts in various solvent although a few fungi showed inhibition to the leaf extracts. *Stevia rebaudiana* is the single sweetener which has antidiabetic property.

The plant is also used for treatment of a number of aliments like hypertension and hyperlipidemia. Extracts of the leaves of the plant, *Stevia rebaudiana* Bertoni have been used for many years in traditional South American treatment of diabetes. Oral intake of extracts slightly suppresses plasma glucose during healthy subjects. A 35% reduction in blood glucose is also observed in diabetic subjects after oral intake of *Stevia rebaudiana* Bertoni extracts[14.] Possible treatment of osteoporosis has been suggested by observation that eggshell breakage can be reduced by 75% by adding a small percentage of stevia leaf powder to chicken feed and those pigs given 2.0% stevia leaf power in their feed experienced a doubling of serum calcium[15].

Anti Hyperglycemic Effects

Many experiments have been conducted to study the antidiabetic effect of stevioside and clarify whether stevioside participates in glycemic action of extract of *Stevia rebaudiana* in different cell lines. Jeppensen *et al*., examined potential Antihyperglycemic effect in type-2 diabetic gotokakizaki (GK) rats. Rats were fed 0.025 $g.Kg^{-1}.d^{1}$ of Stevioside for 6 weeks. An intra-arterial catheter was inserted into the rats after 5 weeks and conscious rats were subjected to arterial tolerance test ($2.0g.Kg^{-1}$) during week 6. They used adult male GK and male Wistar rats from Japan. Stevioside had an Antihyperglycemic effect (incremental area under the glucose response curve (IAUC). Stevioside augmented the insulin content in the β cell lines, INS-1, Stevioside may increase the insulin secretion, in part by induction of genes involved in glycolysis[16.]

Kinghorn and Soejarto, has also described that there is a popular use of herbal and alternative medicine for the treatment of diabetes. They showed that extracts from *Stevia rebaudiana* has long been used for the treatment of diabetes in South America[17]. An early study showed that 0.5 g% of stevioside and 10% of powdered stevia leaves in both high-carbohydrate and high-fat deist given to rats caused a significant reduction in blood glucose level following 4 weeks of treatment[18].Subsequently, the effect of aqueous extract of stevia leaves on glucose tolerance test was investigated in humans following intake of aqueous extracts of stevia leaves, 5 g% at 6 h intervals for 3 days. There was a significant decrease in plasma glucose level during glucose tolerance test. The effects of stevioside and steviol on glucose absorption have been investigated [19]. Their studies suggests that stevioside at doses of 1mm and 5 mm does not inhibit intestinal glucose absorption in hamster jejunum, whereas 1mm steviol inhibits glucose absorption by about 30% but does not affect the activity of intestinal $Na^{-}K^{+}$ ATPase. There are reductions in intestinal mucosal ATP content and absorptive surface area.

The effect of stevioside on glucose synthesis has been studied in two types of diabetic rats, type-1 (insulin dependent) and type-2 (insulin independent). Stevioside lowers the high blood glucose levels in both type-1 and type-2 diabetic rats. The hyperglycemic effect of stevioside on streptozotocin (STZ) induced diabetic rats following oral intake of stevioside (1, 2 or 10 mg/kg/BW/Day) for 15 days[20].

ISSN: 0976-7126

Table1: Effect of Stevioside and extracts of *Stevia rebaudiana* on the serum insulin and plasma glucagon concentrations in both normal and diabetic rats

S/No.	Group	Serum insulin concentration (µlU/mL)	Plasma glucagon concentration (pg/mL)
1.	Control(n=7)	5.13±0.19	45.89±6.59
2.	Control-SVS (n=8)	6.19±0.15	48.30± 6.62
3.	Control-SR (n=8)	5.17±0.36	47.08±4.95
4.	Dm(n=8)	2.66±0.19	76.04±5.38
5.	Dm-SVS(n=8)	3.29±0.11	75.21±3.12
6.	Dm-SR(n=9)	3.87±0.45	49.43±3.45

All values are mean ±SEM[20].

The mechanism of glycemic action of SV and *Stevia rebaudiana* in both serum insulin and plasma glucagon levels is shown in table below. The serum insulin level in normal rats treated with stevioside or *Stevia rebaudiana* Bertoni was not significantly different from normal rats fed with water. The serum insulin level was raised from 2.66 ±0.19 ml U/Ml in normal diabetic rats to 3.29 ±0.11mlU/ml ($p<0.05$) in DM-stevioside and to 3.87±0.45 µlu/ml ($p<005$) in DM *Stevia rebaudiana* Bertoni.

Hypertension Effect

Early studies bath in animals and humans demonstrated that stevioside and stevia extract decreases mean arterial blood pressure (MABP) by including vasolilation and diuresis as well as naturiuresis, which leads to decreased plasma volume [21, 22]. The antihypertensive effect of crude stevia extract (2.67g of dry leaf/day) taken orally is time-dependent and requires prolonged administration. There is no significant change in blood pressure for first 20 days. Indeed the hypertensive effect of the extract was observed 40 and 60 days following administration[23]. Reduced blood pressure occurs in rats following repeated oral dose of stevioside at 25 mg/kg BW/ day for 6 weeks. A double blind, placebo controlled studies in Taiwan to hypertensive subjects in ranging from 28-15 years. Each subject was given capsule containing 250 mg stevioside or placebo three times daily and followed up at monthly intervals for one year. After three months the systolic and diastolic blood pressure of the stevioside group decreased by about six points and the effect persisted during the whole year.

Antirotavirus Activity of Stevia

Tokahashi *et al*., found that the *Stevia rebaudiana* had inhibitory activity against the replication of Anti-human rotavirus (HRV). Anti-human rotavirus activity of hot water extracts from inhibited the replication of all four serotypes of HRV in-vitro. They showed that the *Stevia rebaudiana* inhibited the binding of anti VP7 monoclonal antibody to HRV-infected MA-104 cells. The inhibitory components of *Stevia rebaudiana* were found to be heterogenous anionic polysaccharide with different in charges. The component analyses suggested that the purified fraction named as Stevian with the highest inhibitory activity consists of the anionic polysaccharide with molecular weight of 9800, and contains Ser and Ala as amino acids. Analyses of sugar residues suggest uronic acid(s) as sugar components. It did not contain amino and neutral sugars and sulfate residues[24].

Antioxidant Activity

Contents of flavonoid and other phenolic substance have been suggested to play a preventive role in the development of cancer and heart disease[25]. In the present study the Folin-Ciocalteu method was used to determine the total phenolic compound and flavonoid content of stevia leaves and callus. The phenolic compound in Stevia leaves and callus were extracted by using HCL -methanol. Total phenolic compounds was wound to be 25.18 and 35.86 mg/gram of stevia leaves and callus on dry weight basis, respectively flavonoid content was 31.99 mg/gram for stevia callus on dry weight basis. They also selected the FRAP and DPPH assay to evaluate the antioxidant activities of leaves and callus of stevia. Gallic acid was the strongest antioxidant in both water and methanol whereas trolox was proved to be a weak antioxidant in water. The ICSO for Gallic acid, trolox and BHA observed was 11.04, 41.04 and 57.14ug /ml respectively[26].

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Table 1: Inhibitory effect of *Stevia rebaudiana* against rotaviruses on plaque formation

Serotype	Strain	EC50 (dilution)a	CC50 (dilution)b	Selectivity index
1.	Wa 121 (pH 2)	118 35	32 35	4 4
2.	DS-1 S2	137 153	32 32	4 5
3.	MO	138	32	4
4.	Hochi	114	32	4
Rhesus rotavirus	SA11	32	32	nd

a. EC50 was expressed as the mean value from triplicate experiments of plaque assays.
b. CC50, cytotoxicity of SE was determined by an 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay in the MA104 cell culture which was exposed to SE for 1 h (adsorption period) and incubated for 3 days. CC50 was expressed as the reciprocal of dilution of SE that reduced the absorbance of control cells (without SE) by 50%c. nd, not determined[24].

Table 2: Antioxidant activity of water and methanolic extracts of *Stevia rebaudiana* leaves and callus equivalent to gallic acid or ascorbic acid BHA or Trolox

Mg equivalent per gram on dry weight basis	Leaf extract		Callus extract	
	Water	Methanolic	Water	Methanolic
Gallic Acid	$09.66^{a} \pm 0.09$	$11.03^{b} \pm 0.47$	$09.44^{a} \pm 0.13$	$10.14^{a} \pm 0.18$
Ascorbic Acid	$25.70^{a} \pm 0.24$	$35.16^{c} \pm 0.49$	$25.11^{a} \pm 0.35$	$32.32^{b} \pm 0.56$
BHA	20.19 ± 0.19	$35.16^{b} \pm 1.49$	$19.72^{a} \pm 0.27$	$18.27^{a} \pm 0.32$
Trolox	38.24 ± 0.36	$37.40^{ab} \pm 1.58$	$37.36^{ab} \pm 0.51$	34.37 ± 0.60

(Values are a mean of three trials ± SEM (n=3))[26].

Anti-Inflammatory and Anticancerous Effects

There are evidences that show the anti-inflammatory effect of stevioside both in vitro and in vivo. Boonkaewwan *et al*., observed the effect of stevioside and steviol as anti-inflammatory agent. Stevioside at 1.0 mm significantly surprised lipopolysaccharidc (LPS) induced released of TNF-∝ and IL-1β and slightly suppressed nitric oxide released in THP-1. They suggested that stevioside attenuates synthesis of inflammatory mediatory in LPS-stimulated THP-1 cells by interfering with IKH-β and NF- kappa β signaling pathway and stevioside induced TNF -∝ secretion[27].

In addition, the anti-tumor effect of stevioside was investigated as TPA (12-0-tetradecanoylphorbol-13-acetate) is known to induce cancer formation in mammalian cells [28]. Stevioside inhibits TPA-induced tumor promotion in a skin cancer model of two stage carcinogenesis in mice. Mizushina *et al*., (2005) showed that isosteviol inhibits DNA polymerases and human DNA topoisamerase -II, Cellular targets for pharmacotherapy of cancer as well as inflammatory diseases.

Antimicrobial Activity

Jayaraman *et al*., has evaluated the antimicrobial and antitumor activity of *Stevia rebaudiana* leaf extracts[29]. They showed the antibacterial and antifungal activity by preparing nutrient broth (Hi Media) and by transferring a loopful of culture to 10 ml of nutrient broth and incubated at 37^{0}C for 24 hours for bacterial proliferation. The plant extract was introduced into the Agar-well and plates were incubated at 37^{0}C for 24 hours, the antibacterial activity of the

ISSN: 0976-7126

plant extract was determined by measuring the diameter of the inhibition zone. For determining the antifungal activity potato dextrose agar (Hi Media) was prepared and 1ml (50 mg/ml) of plant extract was added to the medium. Then cultures were placed, and all plates were incubated at 25^0 for 4 days[30].

Table 3: Antibacterial activity of the extracts of *Stevia rebaudiana* leaves[29]

Test	Zone of inhibition (mm)			
	Ethyl acetate	Acetone	Water	Chloroform
Staphylococcus aureus	10	19	-	-
Salmonella typhii	11	13	-	7
Escherichia coli	10	10	-	6
Bacillus subtilus	11	18	-	8
Aeromonas hydrophila	11	14	-	-
Vibrio cholerae	18	10	-	6

Das *et al*., has also reported the Comparative antimicrobial potential of different extracts of leaves of *Stevia rebaudiana* Bertoni leaf extracts, procured from Indian acidic and basic soil zones. Separately Stevia leaves were extracted with aqueous, methanol and ethanol solvents and their micro-biocides were diffusion technique compared against few selected gram positive (*Bacillus substilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhi*) by disc diffusion technique. The in vitro antimicrobial activity of aqueous, methanol and ethanol extracts of dried *Stevia* leaves (collected from acidic and basic soil field), stevia extracts showed high significant activities ($p< 0.001$) against *B. subtilis* and *S. aureus* whereas no activities found against *E. coil* and *S. typhi*. Among the extracts, only aqueous extract shows higher activities against *B. subtilis* and *S. aureus* (10.5 mm and 11.5 mm respectively) than methanolic and ethanolic extracts. However methanolic extract showed little higher activity against S. aureus (10.5 mm) than ethanolic extract (10.2 mm) at 300 mcg/ml concentration, whereas reverse activity shown against *B. subtilis* (9.9 mm for ethanolic extract and 9.8 mm for methanolic extract) at 300 mcg/ml concentration, but there were no significant variation in methanolic and ethanolic extracts against *B. subtilis* and *S. aureus*[31]. In other way, the extracts obtained from the basic soil zone the same trend followed as earlier where aqueous extract gave significant high activity than other two extracts. But interestingly, aqueous and ethanolic extracts showed activities against *S. typhi*, *B. subtilis* and *S. aureus*, whereas no activity shown against *S. typhi* with none of the former extracts collected from the acidic soil zone . Aqueous extract was significantly active against *S. typhi* (10.03 mm) at 300 mcg/ml concentration where as ethanolic extract was active significantly at 200 mcg/ml concentration (9.43 mm) but as per zone of inhibition measured, aqueous extract showed 9.7 mm where as ethanolic extract showed 9.43 mm at 200 mcg/ml concentration. These observations clearly highlighted that among the extracts, aqueous extract shown higher activities ($p<0.05$) against *S. typhi* (10.03 mm), *S. aureus* (11.23 mm) and *B. subtilis* (10.3 mm) followed by methanolic and ethanolic extracts. Methanolic extract showed higher activities against *S. aureus* and *B. substilis* (10.06 mm and 9.96 mm respectively) than ethanolic extracts for the same (9.49 mm and 9.68 mm respectively). Totally negative activity showed against *E. coli* with all the extracts collected from both the zones[31].

Cardiovascular Action

Cardiovascular action of stevia and stevioside on man and animals have been done when any action at all is observed, it is almost always a slight lowering of arterial blood pressure at low and normal doses, changing to a slight rise in arterial pressure at very high doses. The long term use of stevia would probably have a cardiotonic action, i.e. would produce a mild strengthening of the heart and vascular system[27].

Antihistamine Action

Histamine is a chemical substance existing widely in the tissues of animals, but excessive existence in a human body cases allergy, activates secretion of gastric acid, causes platelets aggregation and blood vessels contraction. Stevia extract liquid was found to detoxify histamine. It was found that extract of stevia was clinically useful for Age related disease, atopic dermatitis or allergic. Dermatitis and has antihistaminic effect (H1 receptor). Kazuhiro *et*

ISSN: 0976-7126

al., showed stem extract of Stevia contributed to the gastro protective activity of the extract in animal fed dietary histamine by studying the contractile response of the smooth muscles of the guinea pig ileum[32].

Conclusion

Stevia rebaudiana Bertoni is a small, perennial herb with green leaves that belongs to family Asteraceac. It grows primarily in the mountain range of Paraguay but over 150 various species of Stevia have been identified around the world[33]. Now days it has been used as natural sweetener substituting sugar which has no side effects and available as concentrated liquid, crushed leaf or concentrated white powder. It is recommended for diabetes and has been extensively tasted on animals and has been used by humans with no side effects. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future. For hundreds of years, indegenous peoples in Brazil and Paraguay have used the leaves of Stevia in their tea and food as a sweetener and also took it medicinally as a cardiotonic, for obesity, hypertension and heart burn and to help lower uric acid levels[34].The leave extract of Stevia has 300 times the sweetness of sugar has documented properties of antibacterial, antifungal, antiyeast, cardiotonic, diuretic, hypoglycemia and hence boon to diabetic people[12]. The fresh leaves have a nice liquorice taste and hence it is an attractive natural sweetener to diabetic and others like on carbohydrate controlled diets[15].

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