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Review article
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A REVIEW ON NATURAL SWEETENER PLANT – STEVIA HAVING MEDICINAL AND COMMERCIAL IMPORTANCE

IZVJEŠTAJ O BILJCI STEVIA, PRIRODNOM ZASLAĐIVAČU, LJEKOVITE I KOMERCIJALNE VAŽNOSTI

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ABSTRACT

Stevia is a perennial herb that belongs to the Asteraceae family. It is a natural sweetener plant and estimated to be 300 times sweeter than cane sugar. The leaves of stevia are the source of diterpene glycosides, viz. stevioside and rebaudioside. Stevioside is regenerated as a valuable natural sweetening agent because of its relatively good taste and chemical stability. Now it is being cultivated in Japan, Taiwan, Philippines, Hawaii, Malaysia and overall South America for food and pharmaceutical products. Products can be added to tea and coffee, cooked or baked goods, processed foods and beverages, fruit juices, tobacco products, pastries, chewing gum and sherbets. Health and safety issues have been extensive by considered and in the past 20 years media took significant attention in the US regions. The direct effect of stevioside on transport activity of glucose in skeletal muscle study divulged that insulin action on muscle glucose transport might be improved due to the low concentration of stevioside, signifying that stevioside has the imminent action in the glucose transport system in skeletal muscle. Also, it has a potential commercial value and that is why private and public biotechnology companies are producing stevia in huge quantity and marketing its products.

Key words: Stevioside rebaudioside, pharmacological consequence

SAŽETAK

Stevia je biljka trajnica iz porodice Asteraceae. Ona je prirodni zaslađivač i procjenjuje se da je 300 puta slađa od šećerne trske. Listovi Stevije su izvor diterpen glikozida, tj. steviozida i rebaudiozida. Steviozid se regenerira kao vrijedno prirodno sredstvo za zaslađivanje zbog relativno dobrog okusa i kemijske stabilnosti. Danas se uzgaja u Japanu Tajvanu, Filipinima, Havajima,

Malaziji i po čitavoj Južnoj Americi za prehrambene i farmaceutske proizvode. Proizvodi se mogu dodati u čaj i kavu, kuhane i pečene proizvode, prerađenu hranu i piće, voćne sokove, duhanske proizvode, kolače, gumu za žvakanje i napitke. O njima se uvelike raspravljalo u vezi zdravlja i sigurnosti, a u zadnjih 20 godina mediji su posvećivali značajnu pozornost na području SAD-a. Izravno djelovanje steviozida na transport glukoze u proučavanju mišića skeleta otkrivaju da se djelovanje inzulina na transport glukoze u mišiću može poboljšati zahvaljujući niskoj koncentraciji steviozida, ističući da steviozid ima trenutno važno djelovanje u sustavu transporta glukoze u mišiću skeleta. Isto tako, ona ima potencijalne komercijalne vrijednosti pa zato privatne i javne biotehnološke kompanije proizvode Steviju u ogromnim količinama i prodaju njezine proizvode.

Ključne riječi: Steviozid, rebaudiosid, farmakološke važnosti

INTRODUCTION

Stevia, a natural sweetener plant having medicinal and commercial importance is being used all over the world. *Stevia rebaudiana* Bertoni is the botanical name of stevia. It is a perennial shrub belongs to the (Asteraceae) Compositae family. Stevia is native to Paraguay and Brazil and it is often referred to as ‘‘the sweet herb of Paraguay’’. It is also known as “honey yerba” and “honeyleaf” and by some other variations of these names. The mature plant grows up to 65-centimetres (26 inches) to as tall as 180 cm (72 inches) when cultivated or growing naturally in fertile soil. It is a short day plant and flowering from January to March in the southern hemisphere. 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). Stevia grows in areas with up to 1375mm of rain a year. Although plants are not very frost resistant, it can be grown as half-hardy annuals in Britain, starting them off in a greenhouse and planting them out after the last expected frosts .The chronological records show that stevia leaves have been used for hundreds of years by the Guarani Indians and they named the plant Ka'a He'e The main use was as a sweetener, particularly in their green tea, branded as maté. It was also used in medicine or as a snack. Stevia’s leaf is estimated to be 150 to 300 times sweeter than refined sugar. Detail information about Stevia, its botanical aspects, sweet and non-sweet constituents, variations

of the naturally occurring sweeteners to improve the taste can be found in the recent excellent book by Kinghorn (2002). Not only the Stevia plant but also its extracts have been used for several years as a sweetener in South America, Asia, Japan, China and in different countries of the EU. In Brazil, Korea and Japan Stevia leaves, stevioside and highly refined extracts are officially used as a low calorie sweetener (Mizutani and Tanaka, 2002; Kim et al., 2002). Presently in the US, leaf or extracted forms of stevia is permitted as a dietary supplement. A number of well-known food safety and regulatory agencies from around the world have made their apprehension with stevia based ingredients accurately known for many years (JECFA, 1999; SCF, 1985; FDA, 2007). It has also been reported that *S. rebaudiana*, as a non-calorie first natural sweetener used in medicinal green teas for treating heart burn and other ailments (Vanek *et al.*., 2001), even though there are more than 200 species of the genus *Stevia*, only *S. rebaudiana* gives the sweetest essence (Savita *et al.*., 2004). Japanese have been using stevia and its products in cooked or baked goods, processed foods and beverages, fruit juices, tobacco products, pastries, chewing gum and sherbets (Brandle and Rosa, 1992).

HISTORY OF STEVIA

Stevia leaves were used by indigenous peoples in Paraguay and Brazil since before recorded history (Lee, 1979; Soejarto, 2002). In the 1887, M. S. Bertoni, a Botanist was the first European to document stevia and later on in 1931, French chemists extracted stevioside, the main sweet component in the form of an extremely sweet, white crystalline compound. Afterwards stevia was considered to utilize as a sweetener for food shortages experienced by Britain during World War II, conversely, interest faded when sugar again became available. Japan used stevia in replace of saccharin after it was banned in the 1970 s and stevia sweeteners have been consumed in Japan in large amount than in any other country. In North America and Europe, stevia began to use as herbal product and started available in the market In the 1970s and 1980s and its extracts have been allowed for using as a dietary enhancement in the US in 1994 (FDA, 1995). In Europe it was not permitted in market or for other use due to the lack of proper reports or documentation. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed steviol glycosides in the 58th, 63rd, and 68th meetings. JECFA established both temporary specifications and

a temporary ADI for steviol glycosides of 0-2 mg/kg bw/day. Along with this JECFA requested human studies conducted in normotensive and hypotensive subjects to answer questions about potential blood pressure lowering effects and also in relation to insulin-dependent and insulin-independent diabetes to know the effects on glucose homeostasis. After getting sufficient information, the European Commission's Scientific Committee on Food (SCF) reviewed and suggested that it would reduce the safety factor to 100 and make the ADI permanent (SCF, 1985, 1999a,b).

CHEMICAL INGREDIENTS

As a replacement sugar, natural sweetner have trapped more consideration to the researchers and stevia is assumed as the main substitutinal natural sourse of sugar. Though total chemical composition of stevia is still unavailable, a range of stevia species has been studied by biochemists and biotechnologists for its chemical constituents and out of 110 species, only 18 were found having this features (Soejarto et al., 1982). Stevioside, a diterpenoid glycoside (encompassing steviol, an aglycone) is the main component in stevia. The leaf is the main source of stevioside (Geuns, 2003; Along with stevioside, some other sweet compounds such as steviobioside, rebaudioside A, B, C, D, E and dulcoside A were also found in the leaf of stevia. According to Shibata and coworkers (1995) report all of these diterpenoid glycosides encompasses the identical chemical backbone structure (steviol) but having a bit differ in the carbohydrate residues at C13 and C19 positions. The chemical backbone of stevioside and its derivatives are shown in the Fig. 1. The percentage of components were also studied and those were stevioside 5-10% of total dry weight, rebaudinoside A 2-4%, rebaudinoside C 1-2% and dulcoside A 0.4-0.7% (Wood et al., 1955). The structural formulation of stevioside derivatives and its sweetness fold compared to sucrose is studied well (Crammer and Ikan, 1986; Geuns, 2003) which are enlisted in the table 1. Along with sweetness, stevia has some bitter aftertaste due to the presence of some essential oils, tannins and flavonoids and it was noticed that stevioside and rebaudioside A is responsible to some extent for the aftertaste, albeit role of rebaudioside A is considerably less than of stevioside (Phillips, 1987). Stevioside has been extracted and its products were also prepared such as Ngowatana (1997)

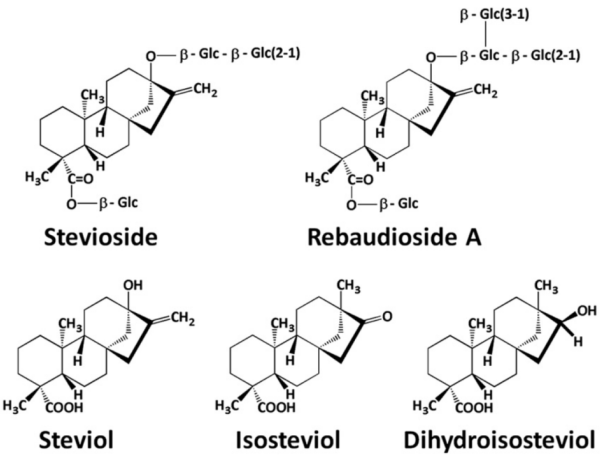

Figure 1: Chemical backbone of stevioside, the main compound found in the leaf of stevia and some other interrelated compounds.

Table 1: Structural derivatives of stevioside and its related compounds and sweetness fold than sugar. (Crammer and Ikan, 1986; Geuns, 2003)

Compound	R1 chain	R2 chain	Fold change of sweetness
Stevioside	β- Glc	β- Glc- β-Glc (2→1)	300
Steviolbioside	H	β- Glc- β-Glc (2→1)	100-125
Rebaudioside A	β- Glc	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	250-450
Rebaudioside B	H	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	300-350
Rebaudioside C	β- Glc	β- Glc- α-Rha (2→1) | β- Glc- (3→1)	50-120
Rebaudioside D	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	250-450
Rebaudioside E	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1)	150-300
Dulcoside A	β- Glc	β- Glc- α-Rha (2→1)	50-120

estracted stevioside as white fine powder form which is highly hygroscopic. More to the point of stevioside, some other compounds were also identified in stevia plant, like 80-85% water, ascorbic acid, beta carotene, riboflavin, thiamine, gibberellic acid, indole-3-acetonitrile, isoquercitrin, kaempferol, stigmasterol, xanthophyllus, umbeliferone, chlorogenic acid, caffeic acid, chromium, cobalt, magnesium, iron, potassium and phosphorus (Sharma et al., 2006).

BIOLOGICAL AND PHARMACOLOGICAL CONSEQUENCE OF STEVIA

Stevia leaves were used to use by Paraguayan Guarani Indians in tea for sweetening and as medicines by chewing the leaves and are used to drink stevia tea several times in a day but there was no side effects. Hence, health and safety issues have been extensive consideration and in the past 20 years media took significant attention in the US regions (Tsanava et al., 1991). Along with this researchers tried to know the biological and physiological mechanisms of stevia compounds' in human health.

BIOLOGICAL ACTIVITIES OF STEVIA

Among varied range of chemical compounds of stevia, a number of biological activities has also been studied which are listed in the table 2.

ANTI HYPERGLYCEMIC EFFECT

Some metabolic syndrome such as diabetes which is caused due to the insulin abnormalities, pancreatic alpha cell dysfunction and comparative glucosan excess (DeFronzo, 1988; Unger, 1997) are the foremost public health anxiety in the present world. Stevia products (stevioside) could be admired as herbal or alternative medicine. Undoubtedly, stevia extracts have been extensively used for diabetes treatment in South America (Kinghorn and Soejarto, 2002). Study documented by Susuki *et al.* (1977) confirmed the hyperglycemic effect of stevioside i.e. 0.5 g % of stevioside and 10 g % of fine products of stevia leaves in equally high carbohydrate and high-fat diets showed a significant reduction in blood glucose level at the subsequent 4 weeks of

Table 2. Biological activities of stevia (*Stevia rebaudiana*) compounds

Compound	Activity tested	Model of test	Dosage	Result	Organism tested and remarks
Stevoside Rebaudiosides A-C Steviolbioside Dulcoside A	Toxic Effect (general)	Oral Mice	2 g/kg 2 g/kg 2 g/kg 2 g/kg	Inactive	
Steviol	Genotoxic Effect	Oral Mice	250 mg/kg 500 mg/kg 1000 mg/kg 2000 mg/kg	Inactive	DNA of the stomach, colon, liver, kidney and testis were not damaged.
Stevioside	Mutagenic Activity	Cell Culture	50 mg	Inactive	*Salmonella typhimurium* TA98 & TA100 by the in vitro Ames test.
Steviol	Mutagenic Activity	Cell Culture	2 mg	Inactive	*Salmonella typhimurium* TA98 & TA100 by the in vitro Ames test.
Stevioside	Antireproductive Activity	Oral Hamster Female	0.5 g/kg 1 g/kg 2.5 g/kg	Inactive	No abnormalities in growth and fertility. No effect on pregnancy and no fetal abnormalities seen.
Stevioside	Antihyperglycemic Activity	IV Rat	0.2 g/kg	Active	Stevioside, with glucose, suppressed glucose response (648 stevioside vs 958 control), increased insulin response and suppressed the glucagon level in a type 2 diabetic rat model.
Stevioside	Antihyperglycemic Activity	Oral Rat	0.025 g/kg	Active	In rat model for a type 2 diabetic, stevioside had an antihyperglycemic effect, enhanced first-phase insulin response and suppressed glucagon levels.
Stevioside	Insulin Enhancement	Rat	0.025 g/kg	Active	Augmented insulin content in the beta-cell line INS-1.
Stevioside	Insulinotropic Activity	In vitro - mouse islet cells	1nmol/L	Active	Enhanced insulin secretion in the presence of 16.7 mmol/L glucose. Only potentiated insulin secretion at or above 8.3 mmol/L glucose.
Stevioside	Pancreatic beta-cell stimulati	Cell culture	1-100 micromol/L	Active	Potentiated insulin secretion from INS-1 cells. Insulin secretion effect deemed to occur via direct action on beta cells.

treatment to rats. Consequently, aqueous extract of stevia leaves on glucose tolerance was also investigated and observed that ingestion of aqueous extract (5 g % at 6 h interval for 3 days) showed considerable decrease in plasma glucose level throughout glucose tolerance test (Curi et al., 1986). Thus, stevia is a good natural source as an alternative of artificial sugar.

CONSEQUENCE ON INSULIN SENSITIVITY AND GLUCOSE TRANSPORT

Stevioside and steviol has the direct effect on beta cells to secrete insulin. Type 2 diabetes is the main occurrence worldwide (Kolterman et al., 1980). Effects of stevioside are well studied for intensifying insulin sensitivity with fructose (Chang *et al.* 2005; Elliott et al 2002; Lailerd et al 2004). Predilection of insulin for the stimulation of glucose clearance is significantly prejudiced on those rats which indicate the turn down in insulin sensitivity in peripheral tissues allied to insulin resistance (Elliott *et al.*., 2002). The direct effect of stevioside on transport activity of glucose in skeletal muscle study divulged that insulin action on muscle glucose transport might be improved due to the low concentration of stevioside, signifying that stevioside has the imminent action in the glucose transport system in skeletal muscle (Lailerd *et al.*., 2004). Experiments were carried out for insulin sensitivity by Gregersen et al., 2004 with 1g of stevioside given to type 2 diabetic subjects and signified that is postprandial blood glucose levels is decreased with reference to 18%. Similar studies have also been studied with another important component, rebaudioside by Maki et al.(2008) and Barriocancl et al (2008).

OTHER POTENTIAL EFFECTS

Along with insulin sensitivity, stevioside and related compounds has its potential effects as anti inflammatory, anticancer effects and research findings suggested that it can also be used as anti diarrheal therapeutics. Inflammation is correlated with some common diseases, for example inflammatory bowel disease (Bamias and Cominelli, 2007; Cho, 2008), autoimmune diseases (Atassi and Casali, 2008), atherosclerosis (Niessner et al., 2007), cancer (Niessner et al., 2007) and diarrhea and malabsorption (Kelly, 1999). Data showed the potential effects of stevioside such as skin inflammation was provoked by 12-0-

tetradecanoylphorbol-13-acetate (TPA) was inhibited by steviol glycosides collectively with stevioside (Yasukawa et al., 2002). Correspondingly, an anti-tumor study of stevioside was examined and stevioside slowed the TPA-induced tumor promotion in a skin carcinogenesis in mice (Nakamura et al., 1995).

TOXICITY, CARCINOGENICITY STUDIES OF STEVIA

Historical uses of stevia and structural behaviours of its two main component, Stevioside and rebaudioside in stevia is approved as non toxic category while studied the toxicity under acute oral condition due to its historical uses and structural behaviours (Medon et al., 1982; Toskulkao et al., 1997). High doses of orally ingested steviol showed a reduction in body weight as experimented in rats (Curry and Roberts, 2008) but no strong evidence of systematic toxicity was acknowledged (Carakostas et al., 2008). Also, dose related body weight diminution is toxicologically momentous studied by Toyoda et al. (1997) and findings showed that in the case of high doses i.e. 2000 mg/kg bw/day in males and 2,400 mg/kg bw/day in females. Hagiwara et al. (1984) studied the carcinogenic effects of stevioside of urinary bladder initiation and promotion however; pre-neoplastic or neoplastic lessions 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. Subsequently, no neoplastic or pre-neoplastic lesions were observed in any tissue (Xili et al. 1992.)

BIOTECHNOLOGICAL RESEARCHES OF STEVIA

Biotechnological approaches such as *in vitro* plant tissue culture methods have been applied for the multiplication of stevia all over the world via organogenesis or embryogenesis from different explants for instance axillary shoots, leaves (Ferreira and Handro, 1987 & 1988), stem tips (Tamura et al., 1984), nodal segments (Ahmed et al., 2007), suspension cultures (Ferreira and Handro, 1988) and anthers (Flachsland et al., 1966) and stems (Miyagawa et al., 1984). Tissue culture studies of stevia have also been done in Bangladesh. Some plant tissue culture laboratories in universities and institutes like University of Rajshahi, University of Dhaka, Jahangirnagar University,

Chittagong University, and some private laboratories like BRAC tissue culture lab, Akafuji Agrotechnologies and specially Bangladesh sugarcane research institute kept a pioneer role in vitro plantlets production from different explants source and established in the field as well. Ahmed et al (2007) regenerated multiple shoots from nodal segments and found highest regeneration rate in MS medium supplemented with 1.5 mg/L BA + 0.5 mg/L Kn. For rooting 97.66% rooting was recorded on MS medium with 0.1 mg/L IAA. Uddin et al (2006) studied callus culture from leaf, nodal and inter-nodal segments on MS medium containing 2,4-D at 2, 3, 4 and 5 mg/L. Traditional uses of stevia has been comprehend by the modern scientific approaches, therefore it is important to study extensively for its anti microbial activities as much report is not available for stevia microbiological investigations. Few Molecular level investigations have done for example, Brandle et al. (2002) has been sequenced 5548 expressed sequence tags (ESTs) from Stevia leaf cDNA library and studied the association of the MEP pathway for steviol biosynthesis but not involved in the mevalonic acid (MVA) pathway. Some advanced molecular techniques is also being applied for stevia such as NMR studies was carried out for the conformation of the natural sweetener rebaudioside A by Steinmetz and Lin (2009).

USES OF STEVIA AND ITS PRODUCTS

Stevia has its legendary due to its various mode of actions such as, sweetener, hypoglycemic, hypotensive (lowers blood pressure), cardiotonic (tones, balances and strengthens the heart), antimicrobial activities (Taylor, 2005). Different studies and documents proved that stevia has its own and natural constituents which are very much helpful for human health. Among various uses, sweetener is the main use of stevia. Some ethnological uses has been recorded (Taylor, 2005) which are enlisted in the table 3.

COMMERCIALIZATION OF STEVIA

According to the document in the Dietary Supplement Health and Education Act (DSHEA) in 1994, in USA stevia did not have GRAS (Generally Recognized As Safe) status for eating, therefore, banned for human food. Then

Table 3. Ethnomedical uses of stevia. (Taylor, 2005)

Country	Ethnomedical uses
Brazil	Usually used for cavities, depression, diabetes, fatigue, heart support, hypertension, hyperglycemia, infections, obesity, sweet cravings, tonic, urinary insufficiency, wounds
Paraguay	Diabetes
South America	diabetes, hypertension, infections, obesity
United States	candida, diabetes, hypertension, hyperglycemia, infections, and as a vasodilator

Table 4. Commercially obtainable stevia products in USA market.

Product	Type	Manufacturer
Stevia	Crystals	At Stevia LLC (Valley Forge, PA, USA)
Stevia extracts	Powder	Life Extension Foundation (Fort Lauderdale, FL, USA)
JAJ Stevioside	Powder	JAJ Group, Inc. (Jacksonville, FL, USA)
Stevia liquid extract	Liquid	Baar Products, Inc. (Downingtown, PA, USA)
Stevia Dark Liquid Concentrate	Liquid concentrate	Stevia Now (Shrub Oak, Ny, USA)
Stevia Pure Powder Extracts	Powder extract	Stevia Now
Stevia Tablets	Tablets (100-400 mg)	Stevia Now

Doug Kinghorn of the Herb Research Foundation formed a review for American Herbal Products Association (AHPA) and on the basis of scientific evidence and historical use, stated that stevia was safe. So many researches and studies finally proved that stevia has no terrible efects in human and can be taken for having diverse medicinal uses of stevioside. Though stevia is the natural sweetest plant in the world since leaves contain diterpene glycoside which enriches sweet taste, it is not metabolized and calories free. GD Searle and Company which was later on bought by Monsanto in 1985 stated that more than 200 objective studies have found stevia as NutraSweet to be safe. The

papers were re-evaluated by relevant regulatory authorities like the FDA and as named Neotame is the new sweetener to be marketed by the company. Some available stevia products in USA market are enlisted in the table 4.

CONCLUSIONS

Stevia is now being used worldwide for having its various magnitudes. It has been proved that historically it has medicinal values and some stimulating actions. Among different chemical constituents, stevioside has a potential mode of actions in controlling type 2 diabetes. Therefore, peoples are used to ingest it without any confusion. Due to the demand, biotechnology companies are commercially producing stevia through tissue culture and marketing stevia in different form such as leaf powder, liquid and fresh leaves. Stevia has a natural sweetening activity and pharmaceutical properties, therefore, it can be concluded that some extensive high throughput biotechnological techniques should be implemented for the better known of stevia properties in animal health.

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