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RESEARCH ARTICLE

STEVIOSIDE: A NATURAL SWEETENER HAVING POTENTIAL OF CONTROLLING GLUCOSE LEVELS IN DIABETIC PATIENTS

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ABSTRACT

Stevia rebaudiana (Bertoni) containing stevioside is a natural sweetener, which has antihyperglycemic property. The plant can also be used for treatment of number of ailments like hypertension and obesity. This article serves about structural details, biosynthetic pathway, toxicology and pharmacological action of stevioside in glucose metabolism. The metabolism of stevioside is also discussed in relation to possible formation of steviol. Toxicological studies reveal negligible effect of stevioside on human health. It concludes that stevioside is very much suited as sweetening agent for diabetic patients as it tends to potentiate the insulin secretion as well as for obese persons intending to lose weight. So far no allergic reactions have been documented with the use of stevioside.

INTRODUCTION

In plants a number of compounds derived from primary pathways make up the bulk of the plant. These are polysaccharides, sugars, proteins and fats, which are the building blocks for plant growth. On the other side, the secondary products like alkaloids, terpenoids, phenolics, steroids, flavonoids etc. although present at a much lower concentration are found in very large number of plants throughout the plant kingdom (Verpoorte *et al.,* 1999). Originally, secondary products are synthesized as end point in metabolism, with little specific role. However many of the compounds were shown to have an active turnover and now it is accepted that they have much more defined function in plants and are of human value (Alfermann *et al.,* 1995). There is a relationship between primary compounds, intermediary metabolism and the groups of secondary compound (Fig. 1). Secondary compounds are of significant importance to us, since they form the basis of aroma, flavouring and coloring of food, spices, drinks and beverages in our diet. When isolated from their host plants and purified, some secondary products provide very high value compounds as flavour additives, perfumes and pharmaceuticals. The last category is the most valuable, but production is often limited by inaccessibility of the source plant (Collin, 2001). *Stevia rebaudiana* (Bertoni) is a perennial herbaceous plant of family *Asteraceae* (*Compositae*) which consist of approximately 230 species native to certain regions of South America (Paraguay and Brazil). It is often referred as ''the sweet herb of Paraguay'' and the other common names are Sweet leaf, Khaa Jee, Caa-he-he, and it is locally called as honey leaf of Paraguay (Hanson and De Oliveira, 1993). It is a perennial semi-shrub, 12 inches in height and produces small white flowers. It is usually harvested from third month of its cultivation. *Stevia rebaudiana* was first described in the early 1900's. The chemistry of the *Stevia* as a sweetener has been the interest of chemists and later biochemists. In 1930s steviol, the precursor of the sweetener was identified. Later in 1950s and 1960s the structures of major active compounds, diterpenoid glycosides (steviol glycosides) were established. Eight diterpene glycosides with sweetening properties have been identified in the leaf tissue of the plant. These metabolites use same pathway as gibberellic acid, an important phytohormones (Singh and Rao, 2005). The two main glycosides, stevioside (traditionally 5-10% of the dry weight of the leaves) and rebaudioside A (R-A). There are also other related compounds including rebaudioside C (1-2%) and dulcoside A & C, as well as minor glycosides, including flavonoids, glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils (Putieva and Saatov, 1997). New minor compounds are still being discovered Fig 2. (a) Tabular representation of related compounds found in *Stevia rebaudiana.* Stevioside, the main sweet component in the leaves of *S. rebaudiana* tastes about 300 times sweeter than sucrose (0.4% solution) (Genus *et al.,* 2003). According to

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some reports, Paraguay leaves contain the highest concentration (9-13%) of stevioside/rebaudioside molecule;

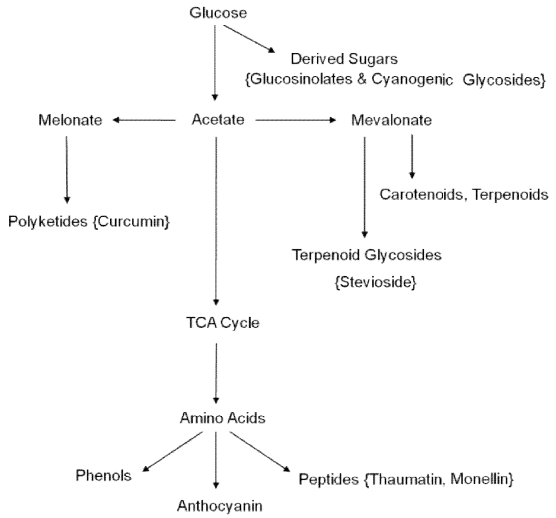

Fig. 1. Schematic diagram showing relationship between primary and secondary metabolites

Name of compound	R1	R2	Relative sweetening power*
Steviol	H	H	Nd
Steviolbioside	H	β-Glc-β-Glc (2 → 1)	100–125
Stevioside	β-Glc	β-Glc-β-Glc (2 → 1)	250–300
Rebaudioside A	β-Glc	β-Glc-β-Glc (2 → 1) | β-Glc (3 → 1)	350–450
Rebaudioside B	H	β-Glc-β-Glc (2 → 1) | β-Glc (3 → 1)	300–350
Rebaudioside C (Dulcoside B)	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)	200–300
Rebaudioside E	β-Glc-β-Glc (2 → 1)	β-Glc-β-Glc (2 → 1)	250–300
Rebaudioside F	β-Glc	β-Glc-β-Xyl (2 → 1) | β-Glc (3 → 1)	Nd
Dulcoside A	β-Glc	β-Glc-α-Rha (2 → 1)	50–120

Glc: glucose; Rha: rhamnose; Xyl: xylose; nd: not determined
*Respect with sucrose = 1.

Fig 2. (a) Tabular representation of related compounds found in *Stevia rebaudiana*

China contains only 5-6% whereas Indian *Stevia* stand midway between the two. Under Indian climatic conditions, stevioside concentration was found about 9.08% of the dry weight of the leaves (Patil *et al.,* 1996; Chalapathi, 2001). Due to the sweetness and supposed therapeutic properties of *Stevia* leaves, it has attracted considerable economic and scientific interests. Japan was the first country in Asia to market stevioside as a sweetener in food and drug industry. Since then, cultivation of the elite plant has expanded to several countries in Asia to United State of America, Canada and Europe (Brandle and Rosa, 1992). This compound is synthesized as a secondary product in plant biosynthetic pathways and is of high economic value. Use of stevioside as artificial sweetener has increased dramatically due to health concern such as dental caries, obesity and diabetes (Genus, 2003) and hence, considered to be the molecule of interest.

Molecule of interest, Stevioside

Stevioside (13- [(2- O-β- D- glucopyranosyl –α -D-glucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester is a diterpenic carboxylic alcohol with three glucose molecules, having molecular weight of 804.9 and molecular formula $C_{38}H_{60}O_{18}$ (Fig. 2b). Extracts of *Stevia rebaudiana* leaves and its processed substances, including stevioside, have been used as a sugar substitute to sweeten a variety of foods, including beverages, confectionery, pickled vegetables and seafood in Japan and other parts of the world. Stevioside is the main sweet component. In addition to stevioside, several other sweet compounds such as steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside A were isolated from leaves of *Stevia rebaudiana* (Kennelly, 2002; Starrat *et al.,* 2002). All these isolated diterpenoid glycoside possess same chemical structure of steviol except difference in residues of carbohydrate at position C-13 and C-19 (Shibata *et al.,* 1995). Along with sweetness, stevioside possess some bitter and undesirable aftertaste (Jakinovich *et al.,* 1990). However, this problem can be solved by enzymatic modification or altering the ratio of stevioside to rebaudioside A (Labov *et al.,* 1991; Yamamoto *et al.,* 1994).

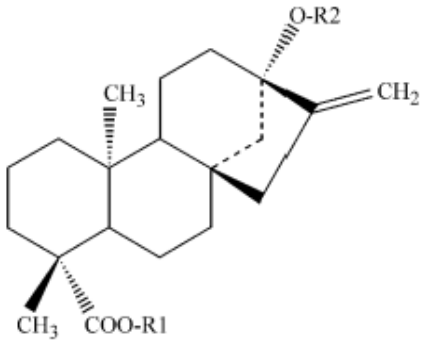

Fig 2. (b) Structure of stevioside

Recent clinical studies suggest that stevioside can reduce blood glucose levels in Type II diabetics and blood pressure in mildly hypertensive patients (Hsieh *et al.,* 2003; Gregersen *et al.,* 2004). It also has advantages for those suffering from obesity, heart disease, and dental caries (Kinghorn and Soejarto, 1985) and has been found to be antihypertensive (Chan *et al.,* 2000; Lee *et al.,* 2001), antihyperglycemic (Jeppesen *et al.,* 2002; Lailerd *et al;* 2004), antioxidant (Xi *et al.,* 1998), anti-human rotavirus (Takahashi *et al.,* 2001), and anti-inflammation and antitumor promoting (Yasukawa *et al.,* 2002). Stevioside has also been reported to influence glucose metabolism (Toskulkao *et al.,* 1995; Suanarunsawat *et al.,* 1997) and renal function (Jutabha *et al.,* 2000). Besides that it also possess anti-fungal and antibacterial properties. Stevioside is highly stable at high temperature (100°C) and at pH range of 3 to 9 (Kinghorn and Soejarto, 1985).

Pathway for stevioside biosynthesis

In *Stevia*, kaurene is converted to steviol and then glycosylated to form the stevioside and other related compounds. Kaurene is the main precursor compound which

is synthesized in chloroplast and then transported to endoplasmic reticulum whereby multiple step of enzymatic action it goes to Golgi body and then finally deposited to vacuoles (Fig. 3). Steviol glycosides have close relation with gibberellins as they are produced from a branch of gibberellic acid (GA) biosynthetic pathway. The final step of gibberellic acid (GA) biosynthesis before the branch point of steviol production is the formation of (-)-kaurenoic acid from kaurene catalyzed by (-)-kaurene oxidase (KO). In *Stevia*, (-)-kaurenoic acid is converted into the tetracyclic diterpene steviol, which then proceeds through a multistep glucosylation pathway to form the various steviol glycosides (Humphery *et al.,* 2006).

Steviol is synthesized from the precursor geranygeranyl diphosphate, which is formed by deoxyxylulose 5-phosphate pathway, by the involvement of enzymes 1-deoxy-d-xylulose-5-phosphate synthase (DXS) and 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR) (Totte *et al.,* 2000). The activity of two different terpene cyclases (-)-copalyl diphosphate synthase (CPS) and (-)-kaurene synthase (KS) result in the formation of (-)-kaurene, in a three step reaction by (-)-kaurene oxidase (KO) to form (-)-kaurenoic acid (Humphery *et al.,* 2006). Elaborating the spatial organization of enzyme playing role in formation of steviol, kaurene oxidase, enzyme of the cytochrome P-450 family is main interest that catalyses the three step oxidation of (-)-kaurene to form (-)-kaurenoic acid.

It has dual role in both synthesis of gibberellins and steviol and found to be located on the endoplasmic reticulum (ER). Kaurene synthase was found to be located in the chloroplast stroma (Sun and Kamiya, 1994; Helliwell *et al.,* 2000). Kaurene synthase produces the reaction intermediate (-)-kaurene that has a low polarity and is therefore likely to partition into membranes. (-)-kaurene must then move from its site of synthesis in the chloroplast stroma, out through the membranes and into the ER membrane where it would be accessible to kaurene oxidase. Then the role comes for kaurene oxidase that catalyzes the formation of kaurenoic acid by oxidizing kaurene at C-19 position and hydroxylated product of kaurenoic acid at C-13 position by kaurene acid hydroxilase (KAH) gives steviol which facilitate the attachment point for sugar side chain (Kim *et al.,* 1996). Steviol is then glucosylated by a series of UDP-glucosyltransferases (UGTs), three of which have recently been identified and characterized are UGT85C2, UGT74G1 and UGT76G1 (Richman *et al.,* 2005). UDP-glucosyltransferases catalyze the transfer of a glucose molecule to an acceptor molecule thus altering its solubility, activity, toxicity or transport.

Stevia UGTs suggests that they are highly region specific and recognize a particular substructure of the acceptor molecule (Hansen *et al.,* 2003; Lim *et al.,* 2003; Achnine *et al.,* 2005; Richman *et al.,* 2005). The glucosyltransferases play major role in the latter part of the pathway producing a variety of steviol glycosides and were found to be located in the cytosol (Humphery *et al.,* 2006). The C-13 alcohol is successively glucosylated, yielding steviol-monoside and then steviol-bioside, further C-19 carboxylate is glucosylated, which finally result into stevioside (Shibata *et al.,* 1991).

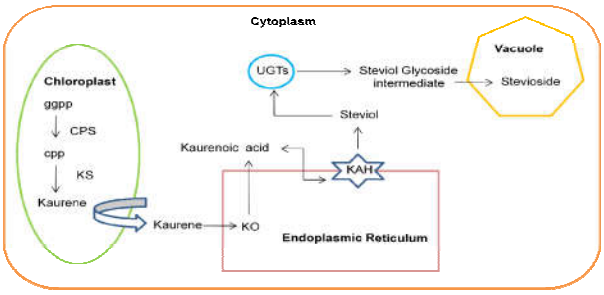

Fig. 3. Schematic representation to show the biosynthetic pathway of steviol glycoside

Pharmacokinetics of stevioside

Stevioside is relatively high molecular weight compound, oral stevioside uptake by the human body is extremely low (Yamamoto *et al.,* 1985; Bracht *et al.,* 1985; Koyama *et al.,* 2003b) and none of the digestive enzymes from the gastro intestinal tract of different animals and man are able to degrade stevioside into steviol, the aglycone of stevioside which appears to have a faster uptake (Wingard *et al.,* 1980; Hutapea *et al.,* 1997; Koyama *et al.,* 2003a, 2003b). In feeding experiments with rats and hamsters stevioside was metabolized to steviol by the bacterial flora of the caecum. Steviol was found in the blood of the animals with the maximum concentration occurring after 8 h (Nakayama *et al.,* 1986; Koyama *et al.,* 2003a). Moreover, bacteria isolated from the human colon are able to transform stevioside into steviol *in vitro* (Hutapea *et al.,* 1997; Koyama *et al.,* 2003a, 2003b). Taken together, these data indicate that in an oral ingestion of stevioside, it is steviol that is taken up by the intestine into the blood. Steviol appears to be the major metabolite of stevioside appearing into the blood circulation following oral ingestion. Liver seems to be the primary site of stevioside metabolism. Metabolism in liver occurs in two phases. Phase I reaction involves hepatic microsomal enzymes mainly cytochrome P450. Phase II reaction involves glucuronidation resulting in formation of water soluble steviol glucuronide which is soluble in water and eliminated via urine. However traces of only free steviol in stool and no stevioside or steviol glucuronide, suggest an additional route of steviol elimination. Steviol glucuronide is the common major metabolite found in circulation of both humans and rats. Biliary and urinary tracts appear to be the major routes for steviol glucuronide excretion. Studies done in humans have shown that urinary excretion seems to be major route in steviol glucuronide excretion (Cardoso *et al.,* 1996; Geuns *et al.,* 2006; Wheeler *et al.,* 2008). A recent metabolic study in humans showed that at 72 h after oral stevioside ingestion, steviol glucuronide excreted in urine and free steviol in feces accounts for 62% and 5.2% of the total dose of stevioside administered respectively (Wheeler *et al.,* 2008).

Pharmacology

WHO estimates that more than 346 million people worldwide have diabetes. This number is likely to more than double by 2030 without intervention. Almost 80% of diabetes deaths occur in low- and middle-income countries. Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030 (Ramachnadaran and Snehalatha, 2009). This metabolic syndrome has become major public health problem in industrialized and developing

countries. Type 2 diabetes mellitus is a chronic metabolic disorder resulting from defects in both insulin secretion from β-cells of islets and insulin action (DeFronzo, 1988). In addition to insulin abnormalities, pancreatic α-cell dysfunction and relative glucagon excess are involved (Unger, 1997). Postprandial hyperglycemia observed in type 2 diabetes is usually due to an increase in basal hepatic glucose production and a decrease in peripheral glucose disposal. Therefore, correction of this imbalance at either the entry or exit step of plasma glucose should help to correct this pathological condition.

Currently, there is a popular use of herbal and alternative medicine for the treatment of diabetes. Extracts of *S. rebaudiana* have been used for the treatment of diabetes; Stevioside suppresses the postprandial blood glucose level in type 2 diabetic subjects by an average of 18% (Gregersen, 2004). The circulating insulin levels tended to be increased by stevioside. In addition, stevioside, the major component of the extract, has a high sweetness with no calorie and only a small amount is needed for sweetening purposes. Thus, it should be a good alternative to sugar for diabetic patients. An early study showed that 0.5 g % of stevioside and 10 g % of powdered S*tevia* leaves in both high-carbohydrate and high-fat diets given to rats caused a significant reduction in blood glucose level following 4 weeks of treatment (Susuki *et al.,* 1977). The effects of stevioside and steviol on glucose absorption have been investigated using *in vitro* jejunal ring tissue and everted sac (Toskulkao *et al.,* 1995). However, stevioside does not interfere with glucose absorption. The effects of stevioside on glucose synthesis have 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. Hypoglycemic effect of stevioside on streptozotocin (STZ)-induced diabetic rats following oral intake of stevioside is mediated via its effect on phosphoenol pyruvate carboxy kinase (PEPCK), a rate-limiting enzyme for gluconeogenesis controlling glucose production in the liver. Stevioside decreases PEPCK mRNA and protein concentrations in a dose-dependent manner (Chen *et al.,* 2005). Thus, it seems likely that stevioside slows down gluconeogenesis in the liver via suppression of PEPCK gene expression leading to a decrease in plasma glucose level in diabetic rat.

Effect of the *Stevia* on diabetes is broadly studied as acute/absolute and chronic antihyperglycemic agents. Insulin secretion in the body can be divided into two phases. First phase consists of basal insulin level which regulates the basal glucose level in the body. Second phase also known as reactive phase is characterized by insulin secretion in response to glucose intake. Pancreatic beta cell dysfunction is a major factor in the development of type 2 diabetes as beta cell function progressively declines a state of relative or absolute insulin deficiency develops. Type 2 diabetes mellitus does not develop until there is significant loss of beta cell function (Buchanan *et al.,* 2003; Del Prato and Marchetti, 2004). Pancreatic alpha cell dysfunction also plays an important role in the development of type 2 diabetes and progression of hyperglycemia. In patients with type 2 diabetes, impaired detection of glucose by alpha cells leads to reduced suppression of glucagon release that, in turn results in increased hepatic glucose output (Dunning *et al.,* 2005). Stevioside and steviol stimulate insulin secretion from mouse islets and INS-I cells. Classically insulinotropic agents are known to act on K^+ ATP channels like sulfonylurea which have hypoglycemia as major side effect. Stevioside and steviol seem to have an edge over the classic sulfonylureas, since the action of the diterpenes is not mediated via K^+ ATP channels Furthermore, the lack of insulin stimulatory effects at subnormal glucose levels may reduce or eliminate the risk of hypoglycemia. (Jeppesen *et al.,* 2000). Administration of stevioside in the fasting state had not caused hypoglycemia in the normal Wistar or the diabetic GK rat. Long-term oral stevioside treatment improves first-phase insulin response, suppresses glucagon levels, and has antihyperglycemic effects in the diabetic GK rat (Jeppesen *et al.,* 2002).

Some recent studies have shown that the acetyl-CoA carboxylase (ACC) plays a vital role for reactive phase for insulin secretion. Chronic hyperglycemia leads to glucotoxicity which is detrimental to pancreatic cells, causing impaired insulin secretion and cell turnover. During glucotoxity, ACC (acetyl-CoA carboxylase) gene expression, ACC protein, and phosphorylated ACC protein were increased in islet cells. Use of stevioside increases ACC gene expression and thus counter acts glucotoxicity via increased ACC activity (Chen *et al.,* 2007). Stevioside also counteracts lipid toxicity of beta islet cells but this action is independent of ACC (Chen *et al.,* 2006). Thus, it can be summarized that stevioside does not close ATP-sensitive potassium channels or affect the cAMP system in the cells at normal glucose levels, hence lowering the chances of arrhythmia and hypoglycemia. Stevioside increases ACC gene expression as well as ACC activity which protect islet cells from glucotoxicity. Stevioside increases insulin secretion via activation of phospholipases and protein kinase C (Chen *et al.,* 2007). The possible anti hyperglycemic action of *Stevia* and stevioside is shown in Fig. 4. The effect of stevioside depends largely upon plasma glucose level, especially when plasma glucose level is elevated. Hence, it found to be safe for normal individuals those not having diabetes.

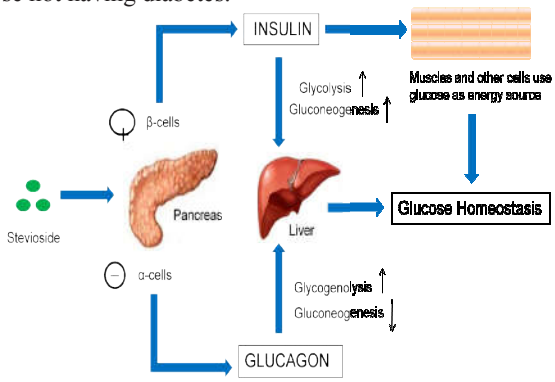

Fig.4. The possible anti-hyperglycemic actions of S*tevia* and stevioside

It inhibits glucagon secretion from α-cell of pancreas which affects glucose release. On the other hand, it stimulate glucose uptake by increasing insulin secretion from β-cell of pancreas. Besides the role of stevioside in anti-hyperglycemia, there are ample evidences showing its anti-hypertensive as well as anti-inflammatory action. Human studies have shown the effect of stevioside on cardiovascular system. Stevioside causes bradycardia (decrease in heart rate) and hypotension. The

hypotensive effect of the extract was observed 40 and 60 days following stevioside administration (Melis, 1996). Similarly, a slight hypotensive effect was observed in human subjects who received a tea prepared from *S. rebaudiana* (S*tevia* extract) daily for 30 days (Boeckh and Humboldt, 1981). The exact mechanism of stevioside anti-hypertensive action is not clear, however, the possible mechanism involves prostaglandin activity (Melis and Sainati, 1991b). In addition, anti-hypertensive action of stevioside occurs without changes in serum dopamine, norepinephrine and epinephrine levels, thus ruling out changes of sympathetic tone (Chan *et al.,* 1998). The anti-hypertensive effects of stevioside and S*tevia* extract could be partly due to their effects on plasma volume. Stevioside and extract of St*evia* are known to reduce the mean arterial blood pressure by inducing vasodilation (decreased TPR) and diuresis as well as natriuresis, which leads to decreased plasma volume (Melis, 1995; Melis and Sainati, 1991a, 1991b).

Stevioside blood pressure lowering-capacity is mainly observed in hypertensive subjects. Thus, there is a relatively low risk of development of hypotension in normal healthy human subjects at the amounts commonly used in the diet (Chatsudthipong *et al.,* 2009). Inflammation is an early host immune reaction mediated via immune cells and their cytokines. Stevioside induces pro-inflammatory cytokines (TNF-α, IL-1β) and nitric oxide (NO) production in un-stimulated human monocytic THP-1 cells. The induction of TNF-α, IL-1β, and NO may augment macrophage function and thus contribute to the enhancement of innate immunity. On the other hand, inhibition of TNF-α, IL-1β, and NO release in LPS- stimulated THP-1 cells, could be beneficial in pathological conditions resulting from the excess of TNF-α, IL-1β, and NO production, which may indicate an anti-inflammatory effect of stevioside (Boonkaewwan *et al.,* 2006).

Toxicology

The toxicology and safety of stevioside is must for its commercial use as a sweetener. Stevioside has been subjected to various assessments for safety, and to date, no serious toxic effects have been reported. An acceptable daily intake (ADI) of 7.9 mg stevioside/kg BW has been calculated as suggested by Joint FAO/WHO Expert Committee on Food Additive (JECFA, 2006). Stevioside and the crude extract of *S. rebaudiana* have been determined as non-mutagenic in many bacterial test systems, such as some test strains of *Salmonella typhimurium, Escherichia coli,* and *Bacillus subtillis*, either in the presence or absence of metabolic activation, which is mostly derived from the rat liver S9 fraction (Suttajit *et al.,* 1993). Stevioside has a very low acute oral toxicity in mice, rats and hamsters with an oral LD_{50} between 8.2 and 17 g/kg BW (Xili *et al*., 1992; Medon *et al.,* 1982; Toskulkao *et al.,* 1997). In hamsters, the LD_{50} of steviol (90% purity), an aglycone of stevioside, was 5.2 and 6.1 g/kg BW for male and female animals respectively. In rats and mice, the LD_{50} was above 15 g/kg BW demonstrating that the tested lethal dose in these animals is quite high and hence can be used for therapeutic purpose. (Toskulkao *et al*., 1997). Ingestion of stevioside by healthy individuals and those having diabetes mellitus or hypertension reported to produce no abnormality in liver and renal function (Hsieh *et al.,* 2003). Most studies demonstrate that oral stevioside, at an acceptable daily intake of 5mg/kg body weight, is safe and not carcinogenic (Carakostas *et al.,* 2008).

Future prospects

Till date most of the studies have been carried either *in vitro* or in some experimental animals. There are very few studies carried out in humans to date. More clinical studies in human are needed to establish the pharmaceutical value related to stevioside. The purity of the test compound should also be known. Stevioside appears to be completely metabolized to steviol prior to its absorption. Therefore, future work would be focused on steviol and its metabolism to identify the active compounds responsible for the observed effects of stevioside intake. The mechanism of action of stevioside and related compounds in human is still not very clear in terms of its pharmacological action. Most stevioside studies have conducted following oral administration. Intravenous injection of stevioside needs to be examined further. Most importantly safety of stevioside should be of main focus as per FDA instructions for its future use as drug and as non caloric sweetener for human diet.

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