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Stevia Rebaudiana - A Magical Sweetener

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Abstract: Stevia (*Stevia rebaudiana* Bertoni) - a natural alternative to artificial sweetener belongs to Asteraceae family and contains over hundred phytochemicals including well characterized stevioside, rebaudioside A and steviol. It is well known for its application in treatment of many diseases like diabetes, candidacies, high blood pressure and weight loss in various Indian traditional system of medicine. The chemical extracts from its leaves is responsible for Stevia's sweetness and out of eight glycosides discovered so far, one called *stevioside* is considered the sweetest and has been tested to be approximately 300 times sweeter than sugar. At present in India there is no large scale mechanized production of Stevia due to difficulties in producing the crop through seeds. The conventional propagation through seeds is mainly confined in their wild states which show poor germination and genetic variability. However, the vegetative cutting propagation is not enough to meet the present requirement because of being too slow and having enough possibilities of accumulation of pathogen in the tissues. The seeds of *Stevia* show a very low germination percentage. The aim of this review article is to outline i) the limitation for its cultivation and how that has been overcome through micro propagation and ii) the chemistry of *Stevia* leaf extracts that are safe to use and having antimicrobial, antibacterial, antiviral and anti-yeast activity. This report covers all the relevant literature published up to 2009.

Key words: Rebaudioside A · *Stevia rebaudiana* Bertoni · Steviol · Stevioside

INTRODUCTION

Stevia is a genus of about 200 species of herbs and shrubs in the sun flower family (Asteraceae). It grows up to 1 m tall and has leaves 2-3 cm long. The plant is indigenous to the northern regions of South America and is still found growing wild in Brazil and Paraguay. It is grown commercially in many parts of Brazil, Paraguay, Uruguay, Central America, Israel, Thailand and China but its cultivation has now become popular world wide. For hundreds of years, indigenous 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 heartburn and to help lower uric acid levels. It is only in the sixteenth century, when the Europeans came to know its medicinal value and started using it in herbal tea. Since then stevia has been used widely throughout Europe and Asia.

The leave extracts of *Stevia,* 300 times the sweetness of sugar has documented properties of antibacterial, antifungal, anti-inflammatory, antimicrobial, antiviral, antiyeast, cardiotonic, diuretic, hypoglycemia and hence a boon to diabetic people, hypotensive tonic and vasodilator. The leaves contain diterpene glucosides with a sweet taste but which are not metabolized and contain no calories. Stevia has a negligible effect on blood glucose but enhancing glucose tolerance [1]. The fresh leaves have a nice liquorice taste and hence it is an attractive natural sweetener to diabetics and others like on carbohydrate-controlled diets. Humans with no side effect have extensively tested it on animals. 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 powder in their feed experienced a doubling of serum calcium [2, 3].

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Cultivation of Stevia: Jeevan herbs, an Indian Agrofarm is one of the pioneer cultivators of stevia in India and is dedicated to high quality, organic cultivation of stevia along with other medicinal plants. Its leaves are about 5 cm long and 2 cm wide and are planted crosswise. When cultivated, the stevia can become 1.0 m high but in the wild type, the height of the plant varies from 40-80 cm. Stevia can be grown in relatively poor soil. The plants can be used for commercial production for 8 years at a stretch of which four harvests of vegetative parts takes place six times a year. The roots remained in place can be used for regeneration. The dry weight can vary from 15-35 g plant^{-1}.

Planting Material and Land Preparation: There are about 90 varieties of *Stevia rebaudiana* developed all around world depending upon the different climatic requirements. The land sites are ploughed and/or cultivated twice to prepare a fairy smooth firm-planting surface. Transplants from cutting are superior but Stevia can also be propagated from seeds in plug trays placed in the green house for a period of 7-8 weeks, though the process is rather expensive.

Stevia plug plants are planted into the field on either 53 cm or 61 cm row spacing with a total plant density in the order of 100,000 plants per hectare. Different climatic conditions influence stevia cultivable through out the year except four times when it is extremely hot or cold. The plants appear to have low nutrient requirements; generally, the stevia plant requires frequent shallow irrigation. Normally, one applies irrigation if the stem tips are drooping at least one time per week.

Pests and its Control: Insect pest pressures other than cutworms are minimal. Septoria diseases can cause considerable damage to the stevia crop. A survey of the literature reports the occurrence of only a few fungal diseases on *S. rebaudiana*. These include *Erysiphe cichoracearum*, *Rhizoctonia solani*, *Sclerotium dephinii*, *Septoria steviae*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *A. steviae* [4].

Harvesting and Drying: Generally it can be scheduled when plants are 40-60 cm in height. Shorter days induced flowering optimum yield and stevioside quality and quantity is best just prior to flowering.

Drying of the woody stems, soft green leaf material is completed immediately after harvesting utilizing a drying wagon or a kiln. Depending upon weather condition and density of loading, it generally takes 24-48 hours to dry stevia at 40°C to 50°C. An estimated 21,500 kg ha^{-1} of green weight is dried down to 6000 kg ha^{-1} of dry weight. "India has suitable climate for stevia cultivation. In spite of this, stevia cultivation has not been taken up on a large scale. On the contrary, China is dominating the market and it grows 80 per cent of the world's stevia leaf, while Japanese commercial products such as diet soft drinks sop up 2,000 tonnes of stevia extract a year (41 per cent of the global stevia market).

Economics of Stevia and its Principle Advantages: It is the stevioside and rebaudiocide content in the Stevia leaves that determines the price and marketability

Table 1: Economics of Stevia Cultivation

Provisional cost to cultivate stevia in one acre of land	First Year	Second Year
Green manuring	US $ 40	----------
Compost-5 trolleys @ US$ 10 each	US $ 50	----------
Vermi compost1000 kg @ US$ 0.07 per kg	US $ 70	----------
Land preparation + Bed raising		
Drip irrigation	US $ 60	----------
Planting material 30,000 plants @ US$ 0.08	US $ 600	----------
Sowing	US $ 2400	----------
Weeding		
Irrigation and supervision	US$ 40	----------
Harvesting and drying	US $ 60	US $ 60
Packing and transportation & miscellaneous	US $ 50	US $ 50
	US $ 100	US $ 100
	US $ 230	US $ 230
Total Cost	US $ 3700	US $ 440
Total output- 2250 kg of dry leaves @ US$ 1.8	US $ 4050	US $ 4050
Net profit	US $ 350	US $ 3610

of Stevia. The economics of Stevia cultivation are given in Table 1. Some of the principle advantages of stevia cultivation are given below:

- Stevia is a completely natural non-synthetic product: Stevioside (the sweetener) contains absolutely no calories.
- The leaves can be used in their natural state.
- It has enormous sweetening power; only small quantities need to be used.
- The plant is non-toxic.
- The leaves as well as the pure stevioside extract can be cooked.
- Stable when heated upto 200°C.
- Non-fermentative.
- Flavour enhancing.
- Clinically tested and frequently used by humans without negative effect.

***In vitro* Propagation:** The seeds of stevia show a very low germination percentage. Propagation by seeds does not allow the production of homogeneous populations, resulting in great variability in important features like sweetening levels and composition [5]. Vegetative propagation too is limited by lower number of individuals that can be obtained simultaneously from a single plant [6]. Due to the above mentioned difficulties, tissue culture is the only alternative for rapid mass propagation of stevia plants. Plant tissue culture technology may help to conserve rare and endangered medicinal plants. Many important medicinal herbs have been successfully propagated *in vitro*, either by organogenesis [7] or by somatic embryogenesis.

Stevia rebaudiana plants were a kind gift from a certified nursery. It was identified and characterized and *in vitro* work was initiated. Shoot apex, nodal and of leaf explants ranging in size from 1.0 to 1.2 cm were collected from a young growing plant. The mother plants were maintained in the green house under a temperature of 28±5°C, at a relative humidity of 50% and partial shade conditions. They were watered twice a day. After excision they were rinsed in a running tap water for 20 min and immersed in Tween 20 solution for 10 minutes, after three washes with doubled distilled water, further sterilization was carried out in the laminar air flow chamber using 0.1% (w/v) $HgCl_2$ for 5 minutes the explants were then rinsed three times with sterile water. Inoculation of stevia explants was done on MS medium [8], supplemented with cytokinins and auxins used singly and in combinations. The pH of the nutrient medium was adjusted to 5.8 and 0.8% agar was added prior to autoclaving. The cultures were maintained a temperature of 25°C and 16 h photoperiod. Various concentrations of growth regulators, viz. 6-benzyladenine purine (BAP), kinetin (Kn), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) were tried for shoot proliferation and callus induction.

Stevioside: Biochemical Aspects: The stevioside is the main sweetening compound found in the leaf of plant *Stevia rebaudiana* (Bert) Bertoni (from 5- 15% dry weight), followed by ribaudioside (3-6%). The stevioside and the other stevia glycosides have high chemical stability because of their tridimensional chemical form which produces resistance to acid and enzymatic hydrolysis ensuring their inalterability even under biochemical and physiological aspects.

Several other experiments have been performed to verify the metabolic action of the stevia diterpenic glycosides and its derivatives showed effects relative to the hydrolysis products of stevia glycosides, the aglycons stevial and isostevial, over isolated rat liver mitochondria. Wingard *et al*., [9] observed intestinal degradation of stevia sweeteners and the absorption of the aglycons. They also showed that the stevial is completely excreted by the biliary way, in the feces. These observations were confirmed by Nakayama *et al*., [10] who fed rats with 3H- stevioside and studied the radioactivity distribution in the body. They also showed that the stevioside is not absorbed by the intestinal tracts and after bacterial decomposition it produces the metabolites, stevial and glucose, which are absorbed in the cecal region. The stevial is excreted in the feces by the biliary way, completely bounded, probably by biliary mucopolysaccharides. They also demonstrated that there is no increase of stevial in the blood level and that this metabolite is recirculated by entero-hepatic way and is excreted in the feces. Extensive studies on chronic toxicity of stevioside and other stevia products were performed recently by Yamada *et al*., [11]. Male and female rats were fed daily with a ration containing 0.3 to 0.1% of stevioside and rebaudiaside for a period of 24 months. Biochemical anatoma- pathological and cariogenic tests were performed in 41 organs. The symptoms and alteration observed in the group tested did not differentiate from the control group. No dose effect relationship was observed in any animal, either even under high dose (1.0%).

Hypoglycemic Action: Chen *et al*., [12] studied the effect of stevioside on the glucose and insulin metabolism in two models of diabetes in rats, STZ- induced diabetic rats and NIDDM diabetic rats induced by feeding with fructose. Stevioside (0.5 mg kg^{-1}) lowered the blood glucose level in STZ induced diabetic rats, peaking at 90 min. Stevioside administered twice daily also demonstrated dose. Dependent effects in lowering the glucose levels in both diabetic rat models. It was demonstrated that stevioside was able to regulate blood glucose level by enhancing not only insulin secretion, but also insulin utilization in insulin deficient rats, the later was due to decreased PEPCK gene expression in rat liver by stevioside.

Antihyperglycemic Action: Extracts of stevia have been used for the treatment of diabetes in Brazil, although a positive effect on glucose metabolism has not been unequivocally demonstrated [13]. Lailerd *et al*., [14] hypothesize that supplementation with stevia stevioside to a test meal causes a reduction in post prandial blood glucose. Twelve type 2 diabetic patients were included in an acute, paired cross over study. A standard test meal was supplemented with either 1.0 g of stevioside or 1.0 g of maize starch (Control). Blood sample were drawn at 30 min. before and for 240 min. after ingestion of the test meal. Compared to control; stevioside reduced the increamental area under the glucose response curve. By 18% the insulinogenic index [AUC (i, insulin)/AUC (i, glucose)] was increased by approximately 40% by stevioside compared to control. Stevioside tended to decrease glucagon levels while it did not significantly alter the area under the insulin, glucagon like peptide and glucose dependent insulinotropic polypeptide curves. It has been concluded that stevioside reduces post prandial glucose levels in type 2 diabetic patients, indicating beneficial effects on the glucose metabolism.

Hypertension: The popularity of stevia continues to grow as more and more people found out about this amazing no calorie herbal sweetener. A double blind, placebo controlled studied in Taiwan to hypersentive subjects in ranging from 28-75 years. Each subject was given capsules containing 250 mg stevioside or placebo three times daily and followed up at monthly interval 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. Blood biochemistry including lipid and glucose showed no major changes [15,16].

Excretion and Biotransformation: The renal excretion of stevioside and its effect on the renal excretion of several other substances was studied in group of 10 male Wistar rats, which received intravenous in fusions of stevioside. No significant change in inulin clearance was observed, but there was a significant increase in *para*-aminohippuric acid clearance fractional sodium excretion, urinary flow as percent of glomerular filtration rate and glucose clearance. The author concluded that stevioside is secreted by the renal tubular epithelium and induces diuresis and natriuresis and a fall in renal tubular reabsorption of glucose [17].

Guens *et al*., [18] studied the metabolism of stevioside in chickens. Most of the stevioside was recovered untransformed in excreta with only 7.6% converted to steviol by broiler chickens or laying hens. No stevioside or steviol was detected in blood or in eggs. Guens *et al*., [19] also studied the excretion of stevioside in pigs. In pigs steviol but not stevioside was detected in the feces, indicating bacterial metabolism of stevioside to steviol. No steviol or stevioside was detected in blood.

Cardiovascular Action: A good deal of experimental work has been done on the effects of stevia and stevioside on cardiovascular functioning in man and animals. Some of this work was simply looking for possible toxicity, while some was investigating possible therapeutic action. In neither case have significant properties been found. 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 vary high doses [20]. The most curious finding is a dose dependent action on heart beat, with a slight increase appearing at lower doses, changing to a mild decrease at higher doses. In neither instance is the result remarkable nor is it extremely doubtful that humans would experience any effect at normal 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.

Anti Inflammatory and Immune Modulation: Boonkaewwan *et al*., [21] observed the effect of stevioside and steviol as anti-inflammatory and immune modulator. Stevioside at 1.0 mM significantly surprised lipopolysaccharide (LPS) induced released of TNF-α and IL-1β and slightly suppressed nitric oxide released in THP-1. Cells without exerting any direct toxic effect, whereas steviol at hundred micromolars did not. Activation of IKK-β and transcription factor NF-Kappa

B were suppressed by stevioside, as demonstrated by Western blotting. They suggested that stevioside attenuats synthesis of inflammatory mediators in LPS-stimulated THP-1 cells by interfering with the IKK-β and NF-Kappa B signaling pathway and stevioside induced TNF- α secretion is partially mediated through TLR-4. Ghanta *et al*., [22] has been reported that *Stevia rebaudiana* may be useful as a potential source of natural antioxidants.

Antihistamine Action: Histamine is a chemical substance existing widely in the tissues of animals, but excessive existence in a human body causes allergy, activates secretion of gastric acid, causes platelets aggregation and blood vessels contraction. Stevia extract liquid was found to detoxify histamine. Tatsuhiko *et al*., [23] found that extract of stevia was clinically useful for IgE related disease, atopic dermatitis or allergic dermatitis and has antihistaminic effects (H1 receptor). Kazuhiro *et al*., [24] showed stem extract of stevia contributed to the gastroprotective activity of the extract in animals fed dietary histamine by studying the contractile response of the smooth muscle of the guinea pig ileum.

Stevioside: Toxicological Studies

Acute Toxicology: In studies of the toxicity of stevioside given as single doses to rodents [25,26] no lethality was seen within 14 days after administration and no clinical signs of toxicity or morphological or histopathological changes were found. Krejci and Koechel [27] after intravenous administration of stevioside to pentabarbitol-anaesthetized dogs at a dose of 32.5 ìmol L^{-1} kg^{-1} bw (equivalent to 26 mg kg^{-1} bw), found no significant changes in any parameters of whole blood, plasma, or renal function and no significant alteration of the renal ultrastructure. The authors concluded that stevioside is totally devoid of acute extra renal effects (such as hypoxaemia, which could contribute to nephrotoxicity) and direct renal effects.

Short Term Studies of Toxicity: A 13-week study of toxicity was carried out in rats. They were given doses of 160, 310, 630, 1300 and 2500 mg kg^{-1} day^{-1}. None of the animals diet during the administration period, there was no difference in body weight gain between the control and treated groups during administration or in food consumption in the later part of the study. The activity of lactate dehydrogenase and the incidence of single-cell necrosis in the liver were increased in all groups of treated males. The authors considered these effects to be nonspecific because of the lack of a clear dose-response relationship, the relatively low severity and their limitation to males, other statistically significant differences in hematological and biochemical parameters were also considered to be of minor toxicological significance. The author concluded that a concentration of 5% in diet was a suitable maximum tolerable dose of stevioside for a two year in rats [28].

Sixteen broiler chicken and four laying hens were given diets containing stevioside (purity, >96%) at a concentration of 667 mg kg^{-1} of feed for 14 and 10 days, respectively. No significant differences were found in feed in take, body-weight gain and feed conversion [29].

Long Term Studies of Toxicity: Groups of 45 male and 45 female inbred Wistar rats were given diets containing stevioside (purity, 85%) at 0, 0.2, 0.6 or 1.2% (equivalent to 100, 300 and 600 mg kg^{-1} day^{-1}) for two years. The rats were evaluated for hematological, biochemical, pathological and histopathological parameters. All surviving animal were killed at two years. No treatment- related changes were observed in hematological, urinary, or clinical, biochemical values at any stages of study. The incidence and severity of non-neoplatic and neoplatic changes were unrelated to the concentration of stevioside in the diet. The study concluded that the acceptable daily intake of stevioside for humans was 7.9 mg kg^{-1} bw day^{-1}, on the basis of stevioside consumption of the rats during the first three months (the average for males and females being 7900 mg kg^{-1} day^{-1}).

In a study by Toyoda *et al*., [30,31] groups of 50 males and 50 females rats were given access ad libitum to diets containing stevioside (purity, 95.6%; stevioside was added to the powdered diet, which was than pelleted) at a concentration of 0, 2.5 or 5% (equal to doses of 0, 970 and 2000 mg kg^{-1} bw day^{-1} for males and 0, 1100 and 2400 mg kg^{-1} bw day^{-1} for females) for 104 weeks. The body weight gain of the treated animals was slightly depressed and a dose-response relationship was seen in males (2.3% and 4.4%) and females (2.4% and 9.2%) at the lowest and highest doses, respectively. Food consumption did not differ between the groups. The final survival rate of males receiving diets containing 5.0% stevioside was significantly decreased (60%) compared with that of the controls (78%). Absolute weights of the kidney were decreased in males and females at the highest dose; however, there was no significant histopathological evidence of neoplastic or non-neoplastic lesions attributable to treatment in any organ or tissue, except for a decreased incidence of mammary adenomas in females and a reduce severity of chronic nephropathy in males.

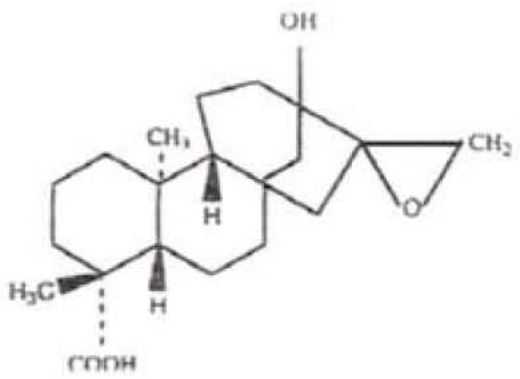

Fig. 1: Steviol- 16, 17 α-epoxide

Reproductive and Developmental Effect: Kuc and Planas [32] performed a study on arts to see if stevia had any contraceptive effect. The study was prompted by a rumor that Indian women in South America used the herb for contraceptive purposes. He observed long term reductions in the numbers offspring born to female rats administered of stevia solution. This study needs to be analyzed carefully as it involved a very high concentration, 10 ml of a dosage administered in about 20 minutes of a concoction derived by drying to a powder and boiling not just the leaves, but material from the stevia plant that would not ordinarily be consumed and utilized.

Rebaudioside: Previously it has been demonstrated that stevioside exerts a direct insulinotropic action in isolated mouse islets and in clonal β-cell lines (INS-1 and INS-1E) and possesses antihyperglycemic effect. The question arises if rebaudioside A, which shares the main structure with stevioside, also possesses insulinotropic effects [33].

Stevioside completely hydrolyzed to steviol after 10 h of incubation with steviolbioside as an intermediate. Steviolbioside formation peaked at 2-4 h and steviol was first detected at 3-4 h of incubation. Rebaudioside A was completely hydrolyzed to steviol after 24 h of incubation, with steviolbioside as an intermediate. Steviolbioside was detected at 6-7 h and packed at 12-15 h of incubation. Steviol was unchanged by incubation with intestinal microflora after 72 h of incubation. Because stevioside and rebaudioside A are metabolites at different rates, toxicity assessment of stevioside can not definitively be extrapolated to assess the risk of rebaudioside A [34].

Hutapea *et al*., [35] reported that stevioside has a steviol-16, 17 α-epoxide metabolite (Figure 1) when incubated for 48 h with rat intestinal microflora. Epoxides are of concern because they are highly reactive with nucleophiles, such as DNA. Renwick and Tarka [36] speculated that the HPLC-UV (high performance liquid chromatography-ultra violet) increament used to detect the epoxide metabolite in the Hutapea study was not highly specific. However, given the possibility of epoxide formation from steviol and its glycosides based on their structure, the creation of an epoxide metabolite in the human system needs to be further investigated.

Wheeler *et al*., [37] conducted human metabolism studies that reported similar metabolic and elimination pathways (Figure 2) but not identical pharmacokinetics or rebaudioside A and stevioside. Healthy, adult, male subject received a single oral dose of 5 mg kg^{-1} of 98.7% pure rebaudioside A and 4.2 mg kg^{-1} of 96.6% pure stevioside. Plasma, urine and fecal sample were collected during a pre dose period and up to 72 h post dose. Both glycoside were hydrolyzed in the gastrointestinal tract in to steviol, which was absorb and conjugated to a glucuronide. Steviol glucuronide was predominantly excreted in the urine and accounted for 59% and 62% of the rebaudioside A and stevioside respectively. Steviol excreted in the urine only accounted for 0.04% and 0.02% of rebaudioside A and stevioside, respectively. Steviol glucuronide was not detectable in the feces, but steviol in the feces accounted for 4.8% and 5.2% of rebaudioside A and stevioside respectively.

In the human metabolism studies, rebaudioside A and stevioside had different pharmacokinetics results for certain parameters when steviol and steviol glucuronide were measured. For instance, there was a longer T_{max} (time of maximum observed plasma concentration) and lower C_{max} (maximum observed plasma concentration) of steviol glucuronide and steviol when the patients were administered rebaudioside A compared to stevioside. Stevioside toxicity studies may be a good way to predict the toxicity of rebaudioside A itself. The toxicity of rebaudioside A and stevioside should be studied individually since each will potentially be used as ingredients in human foods.

Steviol

Inhibitory Effect: An inhibitory effect of steviol, metabolite of the natural sweetener stevioside, on transepithelial transport of p-aminohippurate (J_{PAH}) was observed in isolated S_2 segments of rabbit renal proximal tubules using *in vitro* micro perfusion. Steviol, a glycons part of stevioside and many other natural glycosides, is one of the major metabolites of stevioside during its enzymatic hydrolysis [38]. In addition to its use as a sweetener, several researchers have shown stevioside to

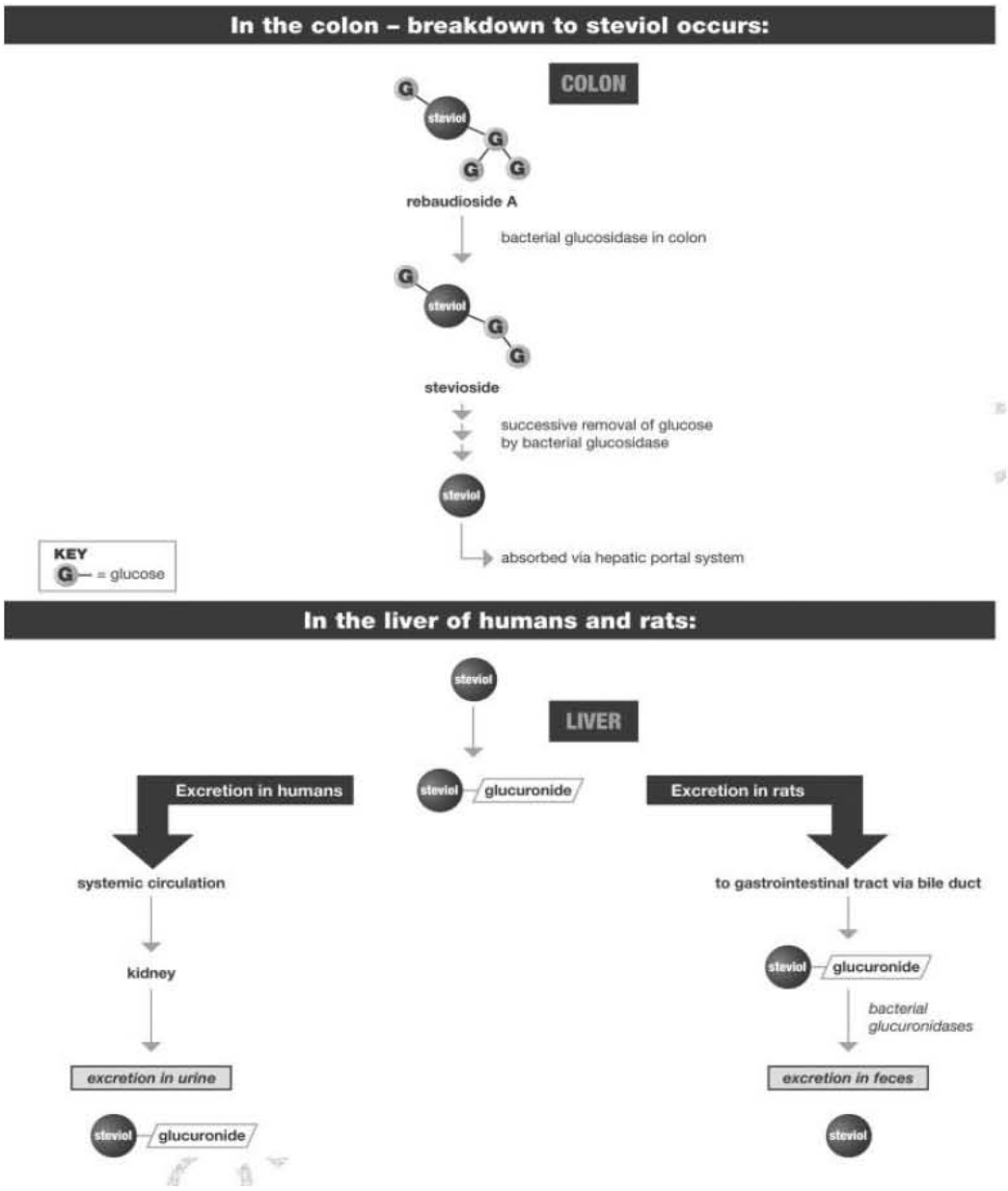

Fig. 2: Rebaudioside A metabolism

have therapeutic values as a contraceptive [39] and to have cardiovascular [40,16,41] and metabolic effects as well as effects on glucose absorption [42]. Changes in renal, renal blood flow, natriuresis and diuresis have been observed after intravenous stevioside administration.

The incubation of stevioside with intestinal bacterial microflora obtained from rats *in vitro* showed the complete conversion of stevioside to steviol within an incubation period of 2 to 4 days [38]. Steviol administered either intracecally or orally was nearby completely absorbed in the lower bowel of the rat [9]. Steviol has been reported to be a toxic substance with mutagenic and bactericidal activities in *Salmonella typhimurium* TM677 [43]. The inhibition of glucose absorption in hamster intestine was also observed after steviol treatment [44].

In contrast, steviol and stevioside have also been reported to have therapeutic to have therapeutic value as diuretic drugs [45] and also as diabetic drugs by stimulating insulin secretion from pancreas [15]. However detailed information concerning the toxicity of stevioside and steviol is required before thesis commercial use as a food additive or drug would be granted.

Investigations on the systemic effects of steviol have been performed. The intravenous infusion of steviol in to rats has also been found to affect kidney function and it induced diuresis and natriuresis with no significant change in glomerular filtration rate and renal plasma flow [45]. However, the experimental model used in the previous studies did not permit clear differentiation between the vascular and the renal tubular effects of steviol.

The renal proximal tubules serve an important function in the elimination of a wide range of xenobiotics via the organic anion and cation secretory systems. At present four mammal organic anion transporter isoforms (OAT1, OAT2, OAT3 and OAT4) and a fish isoform (FROAT) have been cloned and identified. In addition, the renal specific transporter and unknown putative transporter (UST1) have been sequenced and are candidates for the OAT family [46]. Among these transporters in the renal proximal tubule, the p-aminohippurate (PAH) transporter (OAT1) is regarded as major organic anion transporter that contributes to elimination of xenobiotics with diverse chemical structure. Because of the functional importance of this secretory transporter systems, interfering with or inhibiting its function could lead to an accumulation of potentially toxic compounds in the body. Steviol has been shown to inhibit the accumulation of the prototypical organic anion, p-aminohippurate (PAH), in rat renal cortical slices [42].

Comparative Metabolism of Rebaudioside A, Stevioside and Steviol: Roberts and Renwick [47], investigated the metabolism of stevioside, rebaudioside A and steviol in Spragoe-Dawley rats in order to determine the toxicokinetic and metabolic similarities between stevioside and rebaudioside A. The three compounds were radioleveled with C^{14} in the $=CH_2$ group of the steviol moiety. The rats were given a single oral dose of 5.0 mg kg^{-1} of rebaudioside A, 4.2 mg kg^{-1} of stevioside and 1.6 mg kg^{-1} of steviol. Even though the investigators concluded that the pharmacokinetics of stevioside and rebaudioside A in rats are similar, while that of steviol is different, it appears that most of the pharmacokinetic parameters are quite different for all three compounds in rats of the same sex. Steviol glucoramide and two unidentified metabolites were found in the plasma in lower concentration than steviol. The absorption through the gut from rebaudioside A treatment was 71% of males, 82% of females; from stevioside treatment was 78% of males and 81% of females; from steviol treatment was 97% of males and 99% of females. The investigator propose that because of the pharmacokinetic similarities between stevioside and rebaudioside A, information from stevioside safety studies can be used to extrapolates safety data on rebaudioside A. However, the pharmacokinetic parameters in rats are different enough that toxicity data from stevioside may not be reliably extraploted to rebaudioside A.

Stevia as Antimicrobial and Antitumor Agent: The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. These compounds protect the plant from microbial infection and deterioration [48]. *Stevia rebaudiana* is rich in terpenes and flavanoids, the phytochemical present in stevia are austroinullin, β-carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside and thiamin [49]. To the best of our knowledge, there is no previous reported work on the antimicrobial and antitumor activity of stevia, except that of Tadhani and Subhash [50].

The antibacterial activity of the acetone extract of stevia leaves was higher than that of the other extracts. The acetone extract showed greater activity against Gram positive organism than against Gram negative organism. The higher antibacterial activity of the acetone and ethyl alcohol extracts may be due to the greater solubility of the extract in these organic solvents [51]. The inhibitory activity of chloroform extract was not pronounced against *Bacillus subtulis* (8 mm), *Salmonella typhi* (7 mm), *Escherichia coli* (6 mm), respectively and non-existent against *Staphylococcus aurens*. The water extract of stevia leaves was practically ineffective against the test organisms [52-55]. It should be noted however, that growth media also seems to play an important role in the determination of antibacterial activity [56]. All the extracts were active against Epidermophyton species and *Candida albicans*. The ethyl acetate extracts showed high activity against Trichophyton mentagrophytes and Epidermophyton species and this may be due to the greater stability of the active principles in the solvents over a longer period of time.

The aqueous extracts of stevia showed no pronounced antitumor activity but the acetone and ethyl acetate extracts of stevia were more cytotoxic to HEP2 cells. Acetone extracts showed the highest cytotoxic activity followed by ethyl acetate and chloroform extracts.

Table 2: Sweetness Equivalence of Stevia in Comparison to Sucrose

Quantity of stevia (g)	Sucrose equivalent (g)	Perception (%)
1	10	14
1	15	14
1	20	58
1	25	14

Table 3: Nutrient Composition of Stevia 100 g^{-1} (dry weight basis)

Nutrient composition	100 g^{-1}
Proximate	
Moisture (g)	7.0
Energy (K cal)	270.0
Protein (g)	10.0
Fats (g)	3.0
Total carbohydrate (g)	52.0
Ash (g)	11.0
Crude fibre (g)	18.0
Minerals	
Calcium (mg)	464.4
Phosphorus (mg)	11.4
Iron (mg)	55.3
Sodium (mg)	190.0
Potassium (mg)	1800.0
Antinutritional factors	
Oxalic acid (mg)	2295.00
Tannins (mg)	0.01

Table 4: Functional Properties of Stevia Leaf Powder

Properties	Values
Bulk Density	0.443 gm l^{-1}
Water absorption capacity	4.7 ml g^{-1}
Fat Absorption capacity	4.5 ml g^{-1}
Emulsification value	5.0 ml g^{-1}
Swelling	5.01 g g^{-1}
Solubility	0.365 g g^{-1}
pH	5.95

Table 5: Glycemic Index of Stevia Bun in Diabetic and Normal Subjects

Test Products	Normal Subjects	Diabetics
Glucose	100.0	100.00
Control bun	60.4	72.00
Stevia bun	55.5	62.12

Stevia as Functional Component for Food Industry: *Stevia rebaudiana* is a natural sweet herb native of North-Eastern Paraguay, cultivated as a cash crop in number of countries. These appear to be no large scale mechanized production of stevia due to difficulties in producing the crop through seeds. Sweetness equivalence of stevia to sugar was carried out for threshold test. 1.0 g of stevia in 100 ml of water was asked to be m atched with 10 g, 15 g, 20 g and 25 g sugar in 100 ml water. Perception of duration of sweet stimules was measured in second in comparison to sweet stimules of sugar. Sweetness equivalence of stevia in comparison to sucrose is shown in Table 2.

Sweetness of 1.0 g of stevia in 100 ml water was equivalent to a sucrose solution containing 20 g of sucrose. Similar results were found by Cardello *et al*., [56] in which they observed that stevia leaf extracts were 152 times sweeter than 3.0% sucrose. Nutrient composition of stevia (Table 3) which was analyzed on dry weight basis indicated that energy value analyzed being 2.7 K cal g^{-1} which may be entitled as the status of low calorific sweetener due to its intense sweetness in comparison to other available low calorie sweeteners [57]. Intense sweetness includes acesulfame potassium (Calorie free), aspartame (4.0 Kcal g^{-1}), saccharin (calorie free) and sucralose (calorie free) [58]. Calorie contribution to the diet by commonly used sucrose being considered high as it gets utilized by the body more completely and has a potential to escalate towards overweight status. In this concern, the use of stevia as a low calorie sweetener could be of immense help in restricting the calorie intake in the diet of affluent and also where in calorie restricted diet are prescribed. The functional properties of stevia determined are depicted in Table 4.

Bulk density of stevia appears to be low compare to other protein rich pulses. Higher bulk densities are desirable as this is known to reduce the paste thickness. This is an important factor in child feeding where bulk is of concern. However, stevia appears to lack this property. It is known that proteins increase their water holding capacity, when their swelling ability is enhanced. It appears to have adequate fat absorption capacity. Fat absorption capacity has been attributed to the physical entrapment of oil. This is important since fat acts on flavor retainers and increases the mouth feel of foods. The ability of protein to aid the formation and stabilization of emulsion is critical for many applications such as cake, butters, coffee whitener, milks, frozen desserts etc, depending on the composition and stresses during processing under which it is subjected. Crammer and Ikan [49] expressed that since stevioside is stable at 95°C, it is a suitable sweet additive to cooked or baked foods. The mean scores of sensory evaluation for appearance, texture, flavour, test and overall acceptability showed no perceivable variation between the products. All the products developed were equally accepted. Surprisingly, the quantity of stevia required for each of these products was significantly small i.e. 0.25-1.0 g.

Table 5 presents the glycemic response of bun with stevia on normal and diabetic subjects. The concept of glycemic index (GI) of various foods has emerged as a boon to diet therapy for diabetes mellitus indicating beneficial aspects of foods consumed both individually,

Table 6: Effect of Microbial Inoculation on Nitrogen and Phosphorus Content of Stevia

	Nitrogen content (mg plant^{-1})		Phosphorus content (mg plant^{-1})	
Treatments	Shoot	Root	Shoot	Root
Control (Uninoculated)	2.41	0.20	0.32	0.56
Glomus fasciculatum (GF)	4.61	0.37	0.72	1.35
Azotobactor chroococcum (AC)	7.33	0.38	0.99	1.21
Pseudomonas fluorescence (Pf)	4.80	0.38	0.81	1.06
Aspergillus awamuri (Aa)	4.33	0.40	0.65	0.95

supplemented or in mixed diet. The GI for many foods was reported by Jenkins *et al*., [59] for 62 commonly eaten foods and sugars. Dilawari *et al*., [60] obtained GI for legumes and cereals. There is need for creating awareness among the people about the availability/nutritional and therapeutic values of natural low calories sweetener "*Stevia rebaudiana*". The consumers demand for herbal foods may encourage stevia cultivation and production would help to enjoy the sweet taste with minimal calories for these who have to restrict carbohydrate/sugar in their diet.

Stevia to Biofertilizers: As the drug industries require large quantities of biomass for extraction of the sweetening compounds, there is a need to enhance its biomass through cultural techniques and application of manures and fertilizers including biofertilizers. Nitrogen and phosphorus are the two major plant nutrients responsible for influencing vegetative and reproductive phase of the plant growth respectively. In the present study, nitrogen content in the biomass of the inoculated plants was higher and it was significantly highest in the treatments inoculated with *Azotobacter chroococcum* (Ac) being a free living N_2 fixer might have supplemented more nitrogen to biomass during the growth. It has been reported increased N content of *Adhatoda vasica* plants inoculated with the consortia of all four organisms shown in Table 6. The dual and single inoculations resulted in significantly lower P content as compared to consortium. Hence, co-inoculations of N_2 fixer, P solubilizer, plant growth promoting rhizobacteria (PGPR) and arbascular mycorrhiza forms better combination in improving the growth and biomass of stevia. Hemavathi *et al*., [61] reported better response of *Ocimum basilicum* plants inoculated with consortia of *Glomus fasciculatum* (Gf), *Pseudomonas fluorescence* (Pf) and *Bacillus megaterium.*

Biofertilizers improve the plant growth, biomass and yield by supplementing plant nutrients and producing growth hormones. In addition to the improvement of a plant growth, the microorganisms also improve the soil fertility by aggregation and adding nutrients to soil. In the present experiment, biofertilizer application in consortia enhanced the growth and biomass of stevia plants.

Stevia and the FDA: America has drummed its figures in vain awaiting a natural and safe alternative to sugar and chemical sweetness. People around the world have enjoyed for centuries the healthful, sweet herb that is stevia. Yet for over twenty years, the United State FDA (Food and Drug Administration) has derived stevia as an unsafe food additive. Their vehement opposal to its importation almost suggests that stevia is a some sort of narcotic. The FDA has in the past even implemented seizure campaigns to stop the import of stevia in to the USA. Under legislation passed in 1994, stevia manufactures were eventually given the right to market stevia as a dietary supplement.

CONCLUSION

The information submitted on the plant products was insufficient with regard to specification and standardization of the commercial product and contains no safety studies. The data submitted or essentially concern with the leaf extracts of stevia, their toxicological and therapeutic aspects as a zero calorie natural sweetener. The different studies confirms the antimicrobial and antitumor activities of *Stevia rebaudiana* leaves extracted using various solvents, a potential drug that requires further studies and development.

In recent years stevia has been grown *in vitro* conditions in tissue culture laboratory at College of Biotechnology & Allied Sciences, Allahabad Agricultural Institute-Deemed University, Allahabad, India. The aim of this cultivation procedure of stevia is to develop therapeutic drugs through various methods of phytochemical screening of stevia leaves for prevention of chronic diseases rather than a zero calorie natural sweetener.

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