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Assessment of stevia *(Stevia rebaudiana)*-natural sweetener: A review

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Stevia *(Stevia rebaudiana)* is the sweetener of the future. Steviosides and rebaudiosides are the sweetest form of stevia. Stevioside is commonly used as a non-caloric sugar substitute in Japan, Korea, Brazil, China and Paraguay. Pure stevia extract does not affect blood glucose level and may be used freely by diabetics. Stevia also has important role in medical research for treating diabetes, obesity, high blood pressure, tooth cavity and skin problems. The safety of stevia extracts has been confirmed by various toxicity, mutagenicity and other studies. Although more recent studies appear to establish the safety of stevia, government agencies have expressed concerns over toxicity, citing lack of sufficient conclusive research.

Keywords: *Stevia rebaudiana*, Stevioside, Steviol, Toxicity, Sweetener

Stevia (*Stevia rebaudiana)* is a small shrub native to subtropical and tropical South America and Central America (North to Mexico, Paraguay and Brazil). Native Indians of the Guarani Tribe appear to have used the leaves of this herb as a sweetener since pre-Columbian times. It is also called as sweet leaf or sugar leaf and is a genus of about 150 species of herbs and shrubs. It grows well in the sandy soil of elevated land and may grow to a height of 80 cm when it is fully mature. Stevia does produce seeds, but only a small percentage of them germinate. Planting cloned stevia is a much more effective method of reproduction. In 1887, a South American natural scientist named Antonio Bertoni first discovered it. First Bertoni nominated this plant as *Eupatorium rebaudianum*, but later shifted it to the genus *Stevia*. Bertoni named the "new" variety of the stevia genus in honor of a Paraguayan chemist named Rebaudi, who became the first to extract the plant's sweet constituent.

Different glycosides extracted from the stevia (Rebaudi 1900, Korbert 1915, Pomaret and Lavieille 1931) were named steviosides, rebaudiosides and dulcoside. Besides the intensely sweet glycosides, stevia leaf contains proteins, fiber, Fe, P, Ca, K, Na, Mg, Zn, rutin (a flavonoid), vitamin A, vitamin C and oil, which contains 53 other constituents. Processed forms of stevia are 250-300 times sweeter than sugar. Stevia leaf is about 5 cm long and 2 cm wide and leaves arrangement is crosswise, facing each other. Environmental factors like soil, irrigation methods, sunlight, air purity, cleanliness, farming practices, processing and storage affect stevia quality. Quality of stevia should be compared on the basis of aroma, taste, appearance and sweetness. In a 62-year-old sample from stevia leaf herbarium, the intense sweetness of stevia was conserved, indicating the stability of stevioside to drying, preservation and storage (Soejarto et al 1982, Hanson and De Oliveira 1993).

Stevioside

Stevia leaves contain a complex mixture of sweet diterpene glycosides, including stevioside, steviolbioside, rebaudiosides (A, B, C, D, E, F) and dulcoside A (Kennelly 2002, Starrat et al 2002). Stevioside is isolated and purified from *Stevia rebaudiana* Bertoni leaves after multiple and selective extractions followed by recrystallisation, resulting in a stevioside purity >95% and with rebaudioside A as the main impurity (≤2). Refined steviosides and rebaudiosides are the sweetest forms of stevia. Stevioside constitutes 5-15% of the dried leaves of stevia (Lima Filho and Malavolta 1997). The biochemical pathway for the formation of steviol is partly known (Kim et al 1996) and a simple and efficient method for the extraction of steviol glycosides has been described (Liu et al 1997). The chemical structure of steviosides (Nanayakkara et al 1987, Suttajit et al 1993), rebaudioside A, steviol (metabolite of stevioside) is shown in Fig. 1. The EC_{50} of stevioside is 650 mg/kg, steviol is more active with EC_{50} of 150 mg/kg. Steviol loses its deterrent activity after acetylation or glycosylation of the C-13 tertiary hydroxy group or methylation of the C-19 carboxylic acid substituent, but the activity of steviol is not greatly affected by modification of either the C-16 exomethylene group or its stereochemistry (Nanayakkara et al 1987).

Benefits of stevia (not approved or confirmed by FDA).

No calories, will not affect blood sugar levels like common sugar, 100% natural, 250 to 300 times sweeter than sugar, heat stable up to 200°C, non-fermentable, flavour enhancer, prevents cavities, recommended for diabetics, non-toxic, leaves can be used in their natural state,

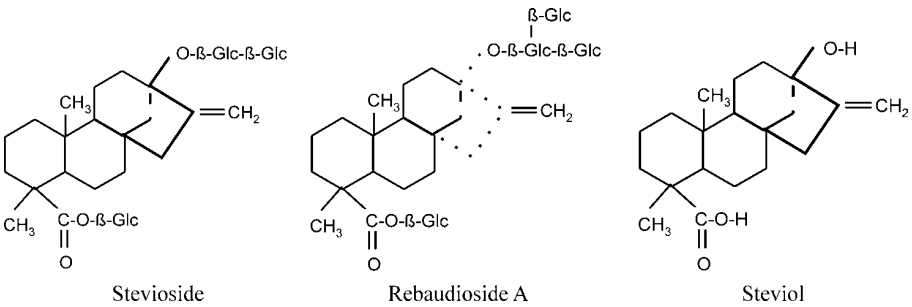

Fig. 1. Structure of stevioside, rebaudioside A and steviol

huge sweetening power and only small quantities are need to be used, leaves as well as the pure stevioside extract can be cooked, no bitterness and non-addictive sweetener for children.

Comparison between stevia and other artificial sweeteners

A study conducted by the Department of Food and Nutrition, FCF-UNESP in Araraquara, Brazil, compared the relative sweetness of stevia to that of aspartame, a cyclamate/saccharin combination and 10% sucrose concentration. The results were interesting. An equivalent dose of stevia, aspartame, cyclamate/saccharin combination and 10% sucrose concentration all had practically the same potency (Cardello et al 1999). Stevia has lower calorie and glycemic index compared to common sugar. Stevia has good source of protein, ash and crude fiber which are essential for good health (Savita et al 2004).

Health concerns

Human body does not metabolize the raw or processed form of sweet glycosides. They are excreted through the normal elimination channels. Human body does not obtain any calories from stevia. If stevia extracts are in their pure unadulterated form they do not adversely affect blood glucose levels and may be used freely by diabetics.

Diabetes: Stevia leaves have been used as herbal teas by diabetic patients in Asian countries. No side effects have been observed in these patients after many years of continued consumption (Suttajit et al 1993). Furthermore, studies have shown that stevia extract can actually improve blood sugar levels (Curi et al 1986). Stevioside helps in insulin secretion; it acts directly on pancreatic beta cells to secret insulin (Jeppesen et al 2000). Stevioside had no effect on gluconeogenesis or oxygen uptake in isolated Wistar rat renal cortical tubules at concentrations up to 3 mmol/l, equivalent to 2.4 mg/ml. Lack of activity was due to the inability of stevioside to penetrate the cell membrane (Yamamoto et al 1985).

Blood pressure: Three days of oral stevioside administration (750 mg/day) affected neither the systolic or diastolic blood pressure nor the fasting plasma glucose and insulin concentrations of healthy volunteers (Geuns et al 2007). One-time high dose of stevioside to rats resulted in reduction of blood pressure as well as an increased elimination of Na (Melis and Sainati 1991). A slight diuretic effect also occurred. The effect was additive when stevia was combined with verapamil (a medicine used to lower blood pressure in humans who have high blood pressure).

Melis (1995) administered extracts of stevia to rats for 20, 40, and 60 days. After 20 days, there were no changes in the stevia treated rats compared to the control group. But, after 40 or 60 days of administering the extract, there was a lowering of blood pressure; a diuretic effect was noted along with loss of sodium. The amount of blood going to the kidneys increased. Similar results were observed in human study. When normal human volunteers between the ages of 20 - 40 years were given tea prepared with stevia leaves, a lowering of blood pressure occurred (Boeckh 1981).

Teeth without cavities: There are several bacteria present in our oral cavity, particularly *Streptococci mutans*, which ferment various sugars to produce acids. These in turn damage the tooth enamel and form pockets or cavities. Stevioside and rebaudioside A, the two primary sweet constituents of the stevia plant were tested in a group of 60 rat pups (Das et al 1992). Rats were divided in 4 groups that is one control and 3 experimental (Stevioside, rebaudioside A and sugar) groups. There was no change in food and water intake and weight gain between the experimental and control group. However, the sugar fed group had significantly more cavities than the rest of the groups. It was concluded that neither stevioside nor rebaudioside A is cariogenic (cavity causing) under the experimental conditions. It shows that the chemicals within the stevia plant that impart sweetness are not fermentable, and thus does not cause tooth cavities.

Biological effects

Absorption, distribution and excretion: After oral application of radio-labeled stevioside, 1.5% of the radioactivity was excreted in the urine of intact rats, whereas in rats with a ligated bile duct 96% of the radio-activity was excreted in the urine. This indicates enterohepatic circulation of stevioside and/or its metabolites with an elimination half-life of 24 h. After oral administration of stevioside to the rat, a major part seems to be degraded by the gut flora to steviol (Nakayama et al 1986). In another study, labeled ^{131}I-stevioside was injected intravenously to male Wistar rats. Plasma level of radiolabel decreased fast. It shows rapid distribution in the body. The highest concentrations of radiolabel were found at 10 and 120 min after injection in the liver (45 and 5% of the injected dose, respectively) and the small intestine (18 and 66%). At 120 min after injection, the radiolabel eliminated in the bile represented 52% of the original dose. After 24 h of injection, the radiolabel eliminated 35% of the original dose in both faeces and urine (Cardoso et al 1996). It was concluded that this study was of limited value since introduction of a large ^{131}I atom into stevioside might significantly affect its absorption, distribution, metabolism, and excretion in bile or urine.

The renal excretion of stevioside and its effect on the renal excretion of several other substances were studied in groups of 10 male Wistar rats (Melis 1992). Stevioside was administered intravenously at doses of 4, 8, 12, or 16 mg/kg body weight (bw/h) for 30 min. It was observed that there was no major change in inulin clearance, but there was a considerable increase in *para*-aminohippuric acid clearance, fractional sodium excretion, urinary flow as percent of glomerular filtration rate, and glucose clearance when compared with controls at doses greater than 4 mg/kg bw/h. Stevioside clearance was greater than inulin clearance and smaller than *para*-amino-hippuric acid clearance at all doses tested. It was concluded that stevioside was secreted by the renal tubular epithelium and induces diuresis and natriuresis and a decrease in renal tubular reabsorption of glucose.

Of the administered labeled stevioside orally, 1.5% of the radioactivity was excreted in the urine of intact rats, whereas in rats with a ligated bile duct 96% of the radio-activity was excreted in the urine. This indicates enterohepatic circulation of stevioside and/or its metabolites with an elimination half-life of 24 h. After admin-

istration of stevioside to the rat, a major part was degraded by the gut flora to steviol. *In vitro* studies using the rat intestinal microflora have shown that the degradation, within 2 days of stevioside and rebaudioside A to steviol are approximately 100 and 65%, respectively. Stevioside was not absorbed, but steviol was readily absorbed and later excreted in the bile as conjugates, which subsequently were excreted in the faeces (Wingard et al 1980, Nakayama et al 1986). Degradation of stevioside was recently shown to occur by various digestive enzymes from the gastrointestinal tract of different animal species. The microflora of the human faeces metabolized stevioside to both steviol and steviol-epoxide. Thus, steviol and its epoxide may be formed and subsequently absorbed (Koyama et al 2003).

Effects on enzymes: Stevioside given to female RCR/Ha mice did not stimulate activity of glutathione *S*-transferase in liver or intestinal mucosa (Pezzuto et al 1986). Stevioside (0.8 mg/ml) inhibited oxidative phosphorylation and the activity of ATPase by 50%, succinate oxidase by 8% inhibition, and succinate dehydrogenase by 10% and no inhibition was observed in NADH-oxidase or L-glutamate dehydrogenase activity. The authors stated that stevioside acts as a weak uncoupler of oxidative phosphorylation (Kelmer-Bracht et al 1985). The stevioside administered to hamster at 0.8 and 4 mg/ml, and intestinal glucose absorption was examined in hamster jejunum. Glucose absorption was not inhibited (Toskulkao et al 1995a, b); 12 µg/ml of stevioside did not significantly alter the arginine-induced secretion of insulin or glucagon in the pancreas of male Wistar rats (Usami et al 1980).

Toxicology

Acute toxicity: Acute toxicity studies of the stevioside are summarized in Table 1. In these studies, no lethality was seen within 14 days after oral administration, and no clinical signs of toxicity or morphological or histopathological changes were found.

After intravenous administration of stevioside to pentobarbital-anaesthetized dogs at a dose of 32.5 µmol/l/kg bw (equivalent to 26 µg/kg bw), no significant changes were seen in any parameters of whole blood, plasma, or renal function and there was no significant alteration of the renal ultra structure. It was concluded that stevioside is totally devoid of acute extra renal effects (such as hypoxemia, which could contribute to nephrotoxicity) and direct renal effects during the 6 h period following intravenous administration (Krejci and Koechel 1992). After intraperitoneal injection of purified stevioside to mice and rats at a dose of 2.99 g/kg bw, no toxic effect was detected. The high dosages needed for the experiments did not permit to reach the real LD_{50} (Mitsuhashi 1976).

Table 1. Acute toxicity of stevioside given orally to rodents

Species	Sex	LD_{50}, g/kg bw	Purity, %	Reference
Mouse	M and F	>15	96	Toskulkao et al (1997)
Mouse	M	>2	96	Medon et al (1982)
Rat	M and F	>15	96	Toskulkao et al (1997)
Hamster	M and F	>15	96	Toskulkao et al (1997)
Mouse	M and F	>17	20	Asaki and Yokoyama (1975)
Mouse	M and F	>15	93.5	Asaki and Yokoyama (1975)

Subacute toxicity: A subacute toxicity study was carried out on rats using an aqueous extract of stevia containing about 50% w/w stevioside. Rats were divided into control, 0.25 and 0.5 g of stevioside. Animals were fed the experimental diets for 56 days. There were no abnormalities in stevia fed groups. But researchers could find a significant decrease in serum lactic dehydrogenase levels. (Asaki and Yokoyama 1975).

Subchronic toxicity: A subchronic oral toxicity study of stevioside was carried out in F344 rats at dose levels of 0, 0.31, 0.62, 1.25, 2.5 and 5% in diet. The rats were randomly distributed into 6 groups, each consisting of 10 males and 10 females. Between the control and treated groups, there were no differences in body weight gain during the administration period and in food consumption in the later period of the study. During biochemical and histopathological investigation, researchers found increased level of LDH and single cell necrosis in the liver in all male treated groups. But these were not considered as specific changes, because of the lack of any clear dose response, the relatively low severity and the limitation to males. Other parameters that were found to demonstrate significant differences on hematological and biochemical investigations were of minor toxicological significance. From these results, a concentration of 5% in diet was concluded to be a suitable maximum tolerable dose of stevioside for 2 years carcinogenicity study in rats (Aze et al 1991).

Long term toxicity and carcinogenicity: Stevia extracts were tested for 2 years, on 500 male and female F344 rats. Rats received stevia extract at 1% of their feed. It was concluded that no significant dose-related changes were found in the growth, general appearance, hematological and blood biochemical findings, organ weights, and macroscopic or microscopic observations. The results obtained are supportive of the safety of stevia extracts, stevioside and rebaudioside A (Yamada et al 1985).

A carcinogenicity study was performed in F344 rats, using a stevioside extract (95.6% purity). The doses were 155, 310, 625, 1250 and 2500 mg/kg bw/day, each group consisted of 50 males and 50 females. It was concluded that stevioside was not carcinogenic in F344 rats under these experimental conditions. However, almost all male rats including controls developed interstitial cell tumors in the testis. It was suggested that a chronic oral toxicity and carcinogenicity study should be performed in another rat species than F344 (Xili et al 1992).

So researchers performed a 2 years study in Wistar rats, using a stevioside powder of 85% purity. Stevioside was given in the diet at 100, 300 and 600 mg/kg bw/day. Body weight, food consumption, general appearance, and mortality were similar in treated and control groups. The mean life span of treated rats was not significantly different from that of the controls. No changes were observed in hematological, urinary or clinical biochemical. The incidence and severity of

non-neoplastic and neoplastic changes were unrelated to the concentration of stevioside in the diet. The NOEL was 1.2%, equivalent to 600 mg/kg bw/day. Xili (1992) suggested that the acceptable daily intake of stevioside for humans was 7.9 mg/kg bw/day.

Stevioside (purity, 95.6%) was added to diet at 0, 2.5 and 5% levels. Study was conducted in 50 male and 50 female Fischer 344/DuCrj rats for 104 weeks. All surviving rats were sacrificed at 108th week. When the organs and tissues of the rats were microscopically examined, there was almost no difference between treated and control group. Also there was decreased incidence of breast tumors in treated females, and the males showed a lesser incidence of kidney damage. It was concluded that stevioside was not carcinogenic in rats under the experimental conditions. In histopathological examination, there was no significantly altered development of neoplastic or non-neoplastic changes, except for a decreased incidence of mammary adenomas in females and a reduced severity of chronic nephropathy in males. It was concluded that stevioside was not carcinogenic under the experimental conditions (Toyoda et al 1995, 1997).

The effect of stevioside on urinary bladder carcinogenesis was carried out in F344 male rats; 0.01% of the nitrosamine was administered through drinking-water for 4 weeks and then 5% stevioside was given through diet for 32 weeks. All surviving rats were sacrificed after 36 weeks and examined histologically. Treatment with 5% stevioside did not affect the incidence or extent of papillary or nodular hyperplasia in nitrosamine-treated rats. No preneoplastic or neoplastic lesions of the urinary bladder were observed in rats treated with stevioside only. It was concluded that stevioside does not promote bladder carcinogenesis (Hagiwara et al 1984, Ito et al 1984).

Genotoxicity: In a genotoxicity study Wistar rats were treated with 4 mg/ml stevioside for 45 days (7 weeks). DNA damage was analyzed by Comet assay every week, and it was found that there was no significant difference between control and treated groups until 4th week. But from 5th week significant increase in DNA damage in control as well treated group was seen. It was suggested that this may be due to stress or statistical differences between both the groups. After 45 days, animals were sacrificed and liver, brain and spleen were collected for analysis. There was significant increase in DNA damage in treated groups. In addition, more DNA damage was observed in spleen. It was suggested for additional studies for a better understanding of the molecular stevioside action in metabolism (Nunes et al 2007). An *in vitro* and *in vivo* study of stevia extract showed negative response, stevia extract and steviol did not show DNA-damaging activity in cultured cells and mouse organs (Sekihashi et al 2002). Genotoxicity studies of the stevioside by reverse mutation, forward mutation, gene mutation and chromosomal aberration are summarized in Table 2, which shows that the results were found to be negative.

Reproductive toxicity

Hamster: A study conducted on hamster to see the effect of daily ingestion of stevioside and its effects on two subsequent generations showed no significant difference in the average growth of the first generation of hamsters in the groups receiving 500, 1000 and 2500 mg/kg bw of stevioside. Even the third generation of hamsters, at 120 days of age, showed no significant differences in body weight of all experimental groups. In all three generations mating performance was equal to the controls, irrespective of the dose of stevioside they received. Stevioside at 2.5 g/kg bw affected neither the growth nor reproduction in hamsters (Yodyingyuad and Bunyawong 1991).

Rat: Group of 11 male Wistar rats were given stevioside (purity, 96%) in the diet at 0, 150, 750, and 3000 mg/kg bw/day for 60 days before and during mating, and groups of 11 female Wistar rats received the same diet for 14 days before mating and for 7 days during gestation. At high dose slight decrease in body-weight gain was observed. There was no treatment-related effect on mating performance or fertility, and no deformities were seen in the fetuses. Stevioside had no adverse effect on fertility or on the development of fetuses (Mori et al 1981). They also noted a slight but not statistically significant increase in the number of dead or resorbed fetuses at the highest dose. But in another study 5 g dry stevia in 100 ml water was given orally to inbred, adult female rats for 18 days. Females were mated with untreated male rats during the last 6 days. Fertility was reduced to 21% of control rats and remained reduced (47%) even after 50-60 days recovery period (Mazzei-Planas and Kuc 1968).

Table 2. Genotoxicity of stevioside

Assay	Test object	Concentration	Results	Reference
Reverse mutation	*S. typhimurium* TA98, TA100	50 mg/plate[1]	Negative	Klongpanichpak et al (1997)
Reverse mutation	*S. typhimurium* TA98, TA100	50 mg/plate[1]	Negative[2]	Suttajit et al (1993)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100,	5 mg/plate[3]	Negative	Matsui et al (1996)
Forward mutation	*S. typhimurium* TM677	10 mg/plate[1]	Negative	Matsui et al (1996)
Forward mutation	*S. typhimurium* TM677	10 mg/plate[1]	Negative	Pezzuto et al (1985)
Forward mutation	*S. typhimurium* TM677	Not specified[1]	Negative	Medon et al (1982)
Gene mutation	*B. subtilis* H17 rec^+, M45 rec^-	10 mg/disc[1]	Negative	Matsui et al (1996)
Chromosomal aberration	Chinese hamster lung fibroblasts	8 mg/ml[3]; 12 mg/ml[4]	Negative	Matsui et al (1996)
Chromosomal aberration	Human lymphocytes	10 mg/ml	Negative	Suttajit et al (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	12 mg/ml[3]	Negative	Ishidate et al (1984)

[1]With and without metabolic activation, [2] A positive response towards TA98 was seen without metabolic activation at 50 mg/ml, but not at lower concentrations up to 20 mg/ml, [3]Without metabolic activation, [4]With metabolic activation

Teratogenicity study: Teratogenicity of stevioside (purity, 95.6%) was examined in rats. Stevioside dissolved in distilled water was given to pregnant Wistar rats by gavage once a day from days 6 to 15 of gestation at doses of 0, 250, 500 and 1000 mg/kg/day. The rats were sacrificed on 20th day of pregnancy and their fetuses were examined for malformation. Stevioside caused no increased incidences of fetal malformation, and no toxic signs in the pregnant rats and the fetuses. It was concluded that stevioside has no teratogenicity in rats when given by gavage (Takanaka et al 1991, Usami et al 1995).

Special studies

a. Detection test made by Japan Food Sanitation Corporation in 1992: Arsenic, lead, cadmium, tin, or pathogenic bacteria were not detected.
b. Patch test made by the Association of Japan Hair Science in 1990: All negative. The fin chambers with stevia extract liquid applied were pasted tight on the elbow skin of 42 examinees for 48 h.
c. Live bacteria test made by Kitazato University Hygiene Science Center in 1991: All negative
d. Detection test on prohibited drugs made by Laboratory of Japan Racing Chemistry Foundation in 1991: All negative

Government actions

Food and Drug Administration of the United States labeled stevia as an "unsafe food additive" and restricted its import. The stated reason of FDA was "toxicological information on stevia is inadequate to demonstrate its safety." The Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of stevia leaves." The Committee also restated "its earlier opinion that the stevioside is not acceptable as a sweetener on the presently available data." The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed stevioside in 1998, but could not quantify an Acceptable Daily Intake (ADI) because of inadequate data on the composition and safety of stevioside. The 63rd meeting of the Joint FAO/WHO Expert Committee (2005) on Food Additives (JECFA) reviewed stevioside. The Committee concluded that stevioside and rebaudioside A are not genotoxic in *in vitro* or *in vivo* and genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*. The NOEL for stevioside was 970 mg/kg bw/day in a long-term study evaluated by the committee at its 51st meeting. The committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses of 12.5–25 mg/kg bw/day [equivalent to 5–10 mg/kg bw/day expressed as steviol (a metabolite of stevioside)].

The evidence available at present is inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g. those with hypotension or diabetes). The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol). The committee noted that this temporary ADI only applies to products complying with the specifications. The committee required additional information to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics. A toxicological monograph was prepared, incorporating summaries of the key toxicological data on the evaluation of stevioside conducted by the committee at its 51st meeting. New tentative specifications were prepared, accompanied by a chemical and technical assessment. In order to be able to remove the tentative designation from the specifications, further information for commercially available products was required by 2007 (WHO 2005).

Conclusion

People are using stevia from last 1500 years in Paraguay and 30 years in Japan, but so far no complaint has been reported. It is safe for human consumption and has several benefits. Stevioside is used as non-caloric substitute. Persons having diabetes, obesity, high blood pressure, tooth cavity and skin problems can use stevia. Stevioside has very low acute toxicity. Acute toxicity studies of the stevioside showed no lethality within 14 days and no clinical signs of toxicity or morphological or histopathological changes were found. In a subacute toxicity study on rats, no abnormalities were found, except a significant decrease in serum lactic dehydrogenase levels.

In a subchronic oral toxicity study of stevioside in F344 rats, increased level of LDH and single cell necrosis in the liver in all male treated groups were noticed. Stevioside is not carcinogenic in rats under the experimental conditions. *In vitro* and *in vivo* genotoxicity studies of stevia extract showed negative responses, stevia extract and steviol do not have DNA-damaging activity in cultured cells and mouse organs. A reproductive toxicity study conducted on hamster showed no significant difference in the average growth of the first generation and third generation of hamsters. In all three generations mating performance was equal to the controls. But in rat, slight decreased bodyweight gain was observed in high dose. There was no effect on mating performance or fertility, and no deformities were seen in the fetuses. But in females fertility was reduced. Teratogenicity of stevioside in rats, showed no increased incidences of fetal malformation, and no toxic signs in the pregnant rats and the fetuses.

Although not all of stevia's potential side effects may have been identified, its main known side effect is an itchy rash that individuals who are allergic get from handling the plants. Stevia should be avoided by pregnant and breast-feeding women due to a lack of information about its possible effects on developing babies and infants. Individuals with kidney conditions should also avoid taking stevia because some laboratory animals have suffered kidney damage when they were given high doses.

The government agencies like USFDA and WHO are not satisfied with the submitted documentation and have

concern about possible toxicity and hence still it is not acceptable as a sweetener based on the presently available data.

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