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CHRONIC ORAL TOXICITY AND CARCINOGENICITY STUDY OF STEVIOSIDE IN RATS

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Abstract—Groups of 45 male and 45 female inbred Wistar rats were given diets containing stevioside (85% pure) at 0, 0.2, 0.6 or 1.2% for 2 yr. After 6, 12 and 24 months, five rats from each group were killed for haematological and clinical biochemical tests. Growth, food utilization and consumption, general appearance and mortality were similar in treated and control groups. The mean lifespan of rats given stevioside was not significantly different from that of the controls. No treatment-related changes were observed in haematological, urinary or clinical biochemical values at any stage of the study. The incidence and severity of non-neoplastic and neoplastic changes were unrelated to the level of stevioside in the diet. The maximum no-observed-effect level of stevioside was 1.2%, and an acceptable daily intake of stevioside for humans of 7.938 mg/kg body weight/day is suggested.

INTRODUCTION

Stevioside is a sweet component of the wild shrub *Stevia rebaudiana*, which is native to Paraguay, where its leaves have traditionally been used as a sweetener.

Bridel and Lavielle (1931) studied the chemical structure of the sweet matter and considered it to be a glycoside. In 1931, they extracted a pure crystalline compound and called it stevioside; it was 300 times sweeter than sucrose. Stevioside (Fig. 1) is a glycoside with an aglycone group (steviol) and under enzymatic hydrolysis it yields three moles of D-glucose and one mole of steviol (Ahmed and Doberstein, 1982; Hiroshi and Kazuo, 1975; Mosettig and Nes, 1955; Wood *et al.*, 1955). Besides stevioside, rebaudiosides A–E and dulcoside A and dulcoside B are other sweet components of *S. rebaudiana*. Stevioside and rebaudiosides A and C are the chief and sweetest constituents.

As early as 1945, Gattoni suggested that stevioside might be an advantageous substitute for saccharin. Masaaki *et al.* (1977) found that stevioside did not support the growth *in vitro* of several organisms responsible for the formation of dental caries (*Lactobacillus plantarum*, *L. casei* and *Streptococcus mutans*). At 10^{-2} M, it inhibited the activity of dextran sucrase derived from *S. mutans*, indicating that it might prove beneficial in the prevention of dental caries. Boeckh (1981) prepared a tea from the leaves of *S. rebaudiana* and gave it daily for 30 days to 18 healthy human volunteers. A lowering of blood pressure was noted, and, at a dose level of 9 mg/kg body weight, stevioside was shown to remove experimentally induced arrhythmias within a few minutes (Humbold *et al.*, 1977). In a study of 25 healthy human volunteers, Oveido (1970) reported an average of 35.2% fall in normal blood sugar levels 8 hr after they had drunk an aqueous extract of *S. rebaudiana* leaves. Hiroo *et al.* (1977) studied the effect of feeding rats a high carbohydrate diet containing 0.5% stevioside; after 2 wk there was a significant decrease in liver glycogen and after 4 wk there was a significant decrease in blood glucose (from 1100 to 1000 mg/litre).

The sweetening and potential pharmaceutical effects of stevioside have received worldwide attention. In Japan, there has been wide cultivation of *S. rebaudiana*, and stevioside has been used commercially as a sweetener since 1975. Cultivation is also being carried out in other countries including Malaysia, Indonesia and Thailand. In China, *S. rebaudiana* has been successfully planted in 25 provinces, and since the 1980s the Institute of Polymer Chemistry of Nankai University has manufactured stevioside of good quality using new resin techniques. The safety of the long-term consumption of stevioside has been investigated by Yamada *et al.* (1985), who reported a maximum no-effect-level as high as 550 mg/kg body weight/day in a 2-yr chronic toxicity and carcinogenicity study in F344 rats. In the present study, we have investigated the chronic toxicity and carcinogenic potential of stevioside fed to male and female Wistar rats for up to 2 yr. The stevioside was produced by the Stevia Sugar Plant of Nankai University.

MATERIALS AND METHODS

Materials and animals. Stevioside (Fig. 1) manufactured by the Institute of Polymer Chemistry, Nankai University was used throughout the experiments. It was a fine, white powder of 85% purity and 0.12% absorbency. This stevioside is 200 times sweeter than sucrose.

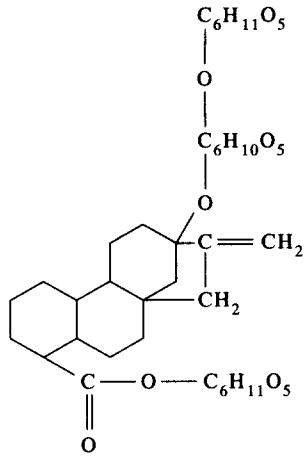

Fig. 1. Chemical structure of stevioside.

Weanling inbred Wistar rats were obtained from the Division of Cancer, Tianjin Medical College.

The composition of the basal rat chow was as follows (as % of diet): wheat flour, 22.4; sorghum flour, 8.6; cornflour, 12.2; rice flour, 8.6; soya-bean flour, 21.6; bran, 7.2; fish powder, 4.3; yeast powder, 0.6; bonemeal 6.7; egg, 4.3; cod-liver oil, 0.9; peanut oil, 1.7; salt, 0.9; riboflavin, 40 mg/kg. The total protein content was 20.2–21.0%. The diets were prepared by thoroughly blending the ingredients and were made freshly once a week.

90-day study. In order to select doses for the carcinogenicity study, a 90-day feeding test was carried out. Three groups of 10 male and 10 female weanling rats, weighing 80–90 g, were housed individually and given feed and water *ad lib.* One group was given basal diet, and the other groups were given basal chow containing stevioside at 3 or 5%. Food consumption was recorded daily. The rats were weighed once a week, and their condition was checked daily. The doses of stevioside given to the rats in the long-term study were chosen on the basis of the results of this 90-day test: stevioside at 3 or 5% had no significant (analysis of covariance) effect on body weight gain (Fig. 2) or food conversion efficiency, and no signs of toxicity or abnormal behaviour were observed. It was concluded that in the long-term study the doses of stevioside could be decreased, since to use stevioside at the levels used in the preliminary study would have been prohibitively expensive, and a lower level would allow us to repeat in a different strain of rats the study by Yamada *et al.* (1985).

Chronic toxicity and carcinogenicity study. 360 weanling Wistar rats, weighing about 60 g were randomly allocated to four groups each of 45 males and 45 females. They were housed two to a cage and kept under controlled conditions of temperature ($23 \pm 2°C$) and relative humidity ($60 \pm 10\%$). The rats were given food and water *ad lib.* The doses of stevioside administered were 0 (control), 0.2, 0.6 and 1.2% in the diet for groups 1, 2, 3 and 4, respectively.

Dosing was continued for up to 24 months. All rats were checked daily, and their condition, and the occurrence of any diseases or tumours, were

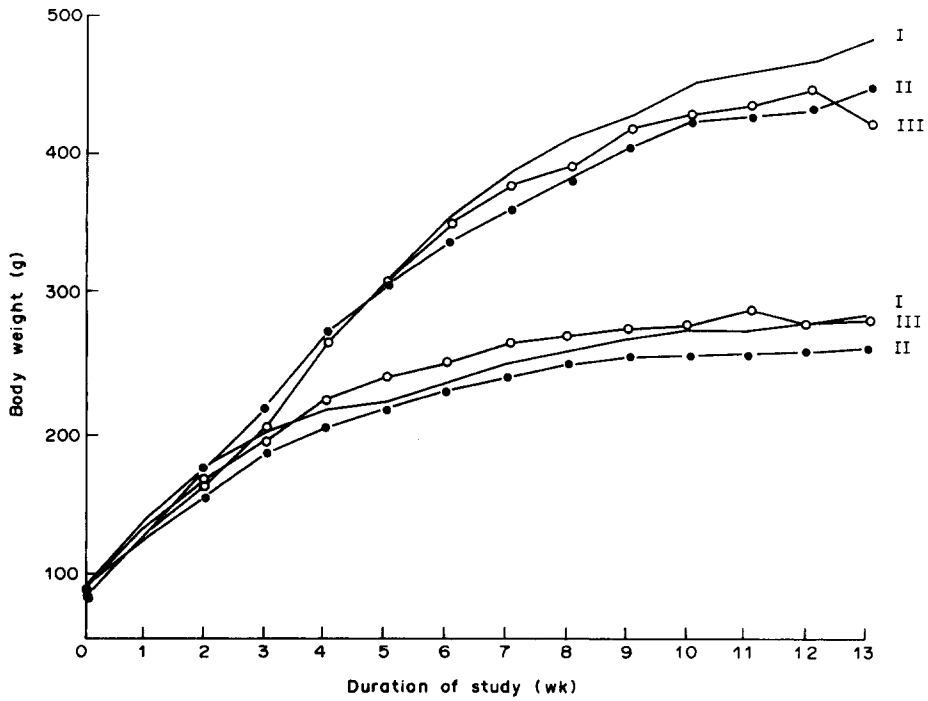

Fig. 2. Growth curves of male and female Wistar rats fed stevioside at dietary levels of 0% (group I), 3% (group II) or 5% (group III) for 90 days. Values are means for groups of 10 rats.

recorded. For the first 14 wk of the study the rats were weighed weekly. During this period 10 male and 10 female rats from each group were housed individually and food consumption was measured daily. The rats were weighed once every 2 wk from 4 to 6 months, and then every month until the end of the study. After 6, 12 and 24 months, five male and five female rats were selected from each dose group. Blood was obtained from the tail vein, and erythrocyte and total and differential leucocyte counts, haemoglobin and haematocrit values were determined. One week later, the rats were housed in metabolism cages and 24-hr urine samples were collected. The rats were weighed and then killed by exsanguination from the femoral vein and blood samples were taken for biochemical analysis using a Vitatron ISP-M Biochemical Analyser. A careful macroscopic examination was carried out, including examination of the nasal, oral, abdominal and thoracic cavities, the fur, skin, eye, ear, vagina, genitalia, limbs, subcutaneous fat, and all organs. The liver, kidney, spleen, brain and testes were weighed. The following tissues and organs were fixed in buffered 10% formalin, sectioned by routine methods, stained with haematoxylin and eosin and examined histopathologically: liver, oesophagus, stomach, pancreas, lung, trachea, heart, bone marrow, spleen, lymph node, kidney, brain, pituitary, thyroid, thymus, adrenal gland, testis, mammary gland, uterus, ovary and urinary bladder. The same macroscopic and histopathological examinations were also carried out on all rats that died, or were killed when moribund, during the study and on those that were killed at 24 months.

Statistical analysis. Statistically significant differences ($P < 0.05$) between groups were determined using analysis of covariance and the chi-square test.

RESULTS

Chronic toxicity and carcinogenicity study

Body weight and food and stevioside consumption. Over the first 3 months of the study, for both male and female rats there were no significant differences between the controls and treated groups in body weight gain or food consumption or utilization (Table 1). Growth curves for this period are shown in Fig. 3. In the growth curves for 24 months (Fig. 4), a transient growth retardation was observed at month 13 in all groups. After month 20, body weights tended to decrease in all groups, including the controls. This appeared to be related to ageing.

The average daily consumption of stevioside (Table 2) was calculated from the food consumption data collected during the first 3 months of the study. During this period the average daily feed consumption by the growing weanling rats would have been higher than that of adult rats, and hence their consumption of stevioside would also have been higher. On the basis of these calculations, the consumption of stevioside by rats in the highest (1.2%) dose group was 838.9 and 748.6 mg/kg/day for females and males, respectively.

General appearance and mortality. No specific signs of toxicity were observed over the 2 yr of the experiment. After 1 yr, during the winter time, wheezing and coughing occurred, but with almost equal frequency in all groups. These signs were not related to the ingestion of stevioside. There were no significant differences between the groups in the incidences of deaths during the study or in the mean lifespan of the rats (Table 3).

Laboratory investigations. There were few significant differences between groups in haematological or clinical biochemical parameters. The erythrocyte counts of the males fed 0.6% stevioside were significantly (analysis of covariance) lower than those of the controls at 6 months, and the leucocyte counts of the males fed the highest dose and of the females fed 0.6% stevioside were significantly higher than control values at 12 months. However, these values were still within the normal range and at 24 months there were no significant differences between groups. Mean corpuscular haemoglobin and mean corpuscular volume for males in group 3 (0.6% stevioside) were significantly lower than control values at 24 months, but were still within the normal range. For other haematological parameters, there were no other statistically significant differences between the treated groups and the controls.

At 6 months, blood urea nitrogen was significantly lowered in females fed 1.2% stevioside, and levels of glutamic–pyruvic transaminase and glutamic–oxaloacetic transaminase were significantly lower in the group 3 females. Serum protein levels of high-dose males were significantly lower than controls at 12 months. Serum calcium was significantly lower at

Table 1. Food consumption, body weight gain and food conversion efficiency of rats fed stevioside (data for months 1–3 of the chronic toxicity study)

Sex	Stevioside (% in diet)	Body weight gain (g)	Total food consumption (g)	Food conversion efficiency (%)
Male	0 (control)	253.4 ± 23.2	1900.8 ± 147.0	13.33 ± 0.6
	0.2	258.3 ± 16.9	2061.7 ± 79.1	12.55 ± 0.5
	0.6	263.4 ± 18.7	1981.7 ± 127.0	13.29 ± 0.8
	1.2	257.1 ± 18.9	1994.3 ± 180.3	12.89 ± 0.7
Female	0 (control)	189.0 ± 31.4	1757.6 ± 134.9	10.75 ± 1.1
	0.2	186.5 ± 17.3	1805.0 ± 91.5	10.33 ± 0.6
	0.6	188.9 ± 26.9	1727.3 ± 113.0	10.94 ± 1.1
	1.2	189.4 ± 30.1	1744.2 ± 102.8	10.86 ± 1.4

Values are means ± SD for groups of 10 rats.

12 months in the females given 1.2% stevioside in the diet than in controls, but was higher than in controls at 24 months, and all fluctuations were within normal ranges. At the end of the study alkaline phosphatase levels were much higher than at 6 and 12 months in all groups, including the controls, and this is considered to be related to ageing. No significant differences were found between groups in serum albumin, albumin globulin, alkaline phosphatase, glucose, cholesterol, chlorine, or phosphorus.

Relative organ weights. No significant differences (analysis of covariance) in relative organ weights were found between the treated rats and the controls. The slight decrease in relative weight of the testis that occurred in all groups at 24 months was considered to be a normal physical phenomenon, relating to slight testicular atrophy in ageing males.

Histopathological findings. Non-neoplastic changes are listed in Table 4. The most common changes were inflammatory lesions and abscesses in the lungs. The distribution and frequency of the pathological lesions were similar in the stevioside-treated rats and the controls and there were no dose–effect relationships. We do not consider any of these lesions to be related to the administration of stevioside.

Incidence of tumours. The number of rats surviving when the first tumour emerged is termed the 'efficient number of animals'. For both sexes, in all four groups the efficient number was 45, since no rats died before the first tumour was observed. No significant differences (chi-square analysis) were observed between treated and control groups of either sex in the incidences of total neoplasms or of benign or malignant tumours (Table 5). The tumours are classified in Table 6. Most (73%) of the tumours observed were benign, and the most frequent were adenofibromas of the mammary gland, of which 93% occurred in females. Other sites of neoplasms were the kidney, oral cavity, subcutis, mesentery, ovary, peritoneum, pituitary, colon and uterus. These types of tumours are frequently found in ageing rats of Wistar strains (Lin, 1987; Shi, 1989).

Estimation of an ADI for stevioside. The calculation of stevioside consumption was based on data from the first 3 months of the study, during which the food consumption was highest. Taking 1.2% stevioside as the maximum no-observed-effect level, the mean consumption of stevioside at this level by male and female rats is calculated to be 748.8 and 838.9 mg/kg body weight/day, respectively (mean, 793.8 mg/kg/day). Extrapolating from rats to humans, and allowing a 100-fold safety factor, the acceptable daily intake (ADI) would be 7.938 mg/kg body weight/day or 476.28 mg stevioside for a 60-kg human. If the

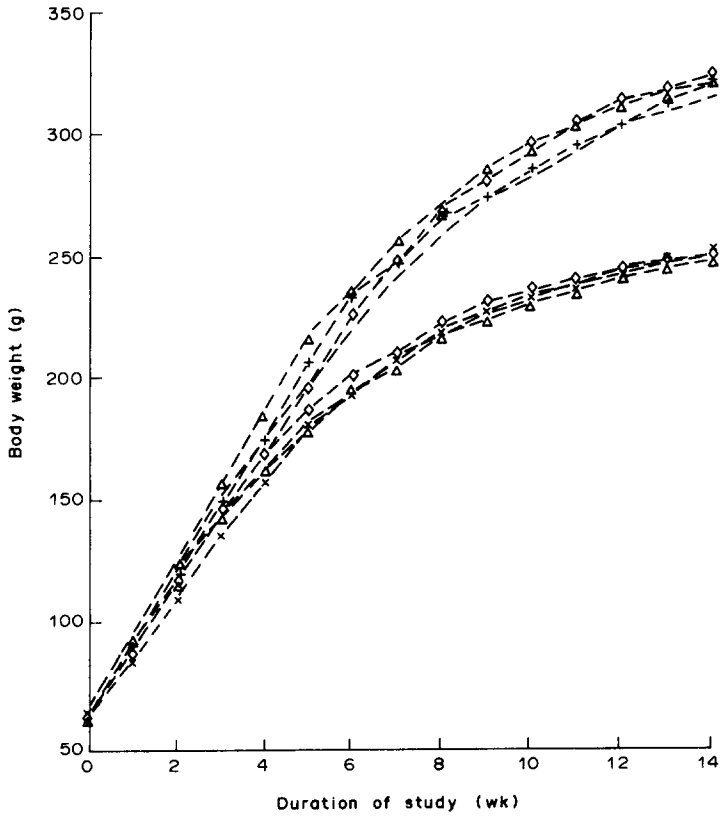

Fig. 3. Growth curves of male and female rats during the first 3 months of the chronic toxicity/carcinogenicity study: the rats were fed stevioside at dietary levels of 0% (×), 0.2% (□), 0.6% (△) or 1.2% (○). Values are means for groups of 10 rats.

average body weight is estimated to be 20 kg for children, who are the highest consumers of soft drinks, then they would be allowed a daily consumption of 160 mg stevioside.

DISCUSSION

Additives in food are incorporated into the human body throughout life. Many kinds of cancer have a long latency, appearing only in old age. Therefore, the duration of chronic toxicity and carcinogenicity tests of food additives in rats is usually 24 months (nearly all their lifespan).

In recent years efforts have been made worldwide to encourage a reduction in the consumption of dietary sugar. It is generally known that high sugar consumption is linked to dental caries, obesity and cardiovascular disease. One way of reducing sucrose

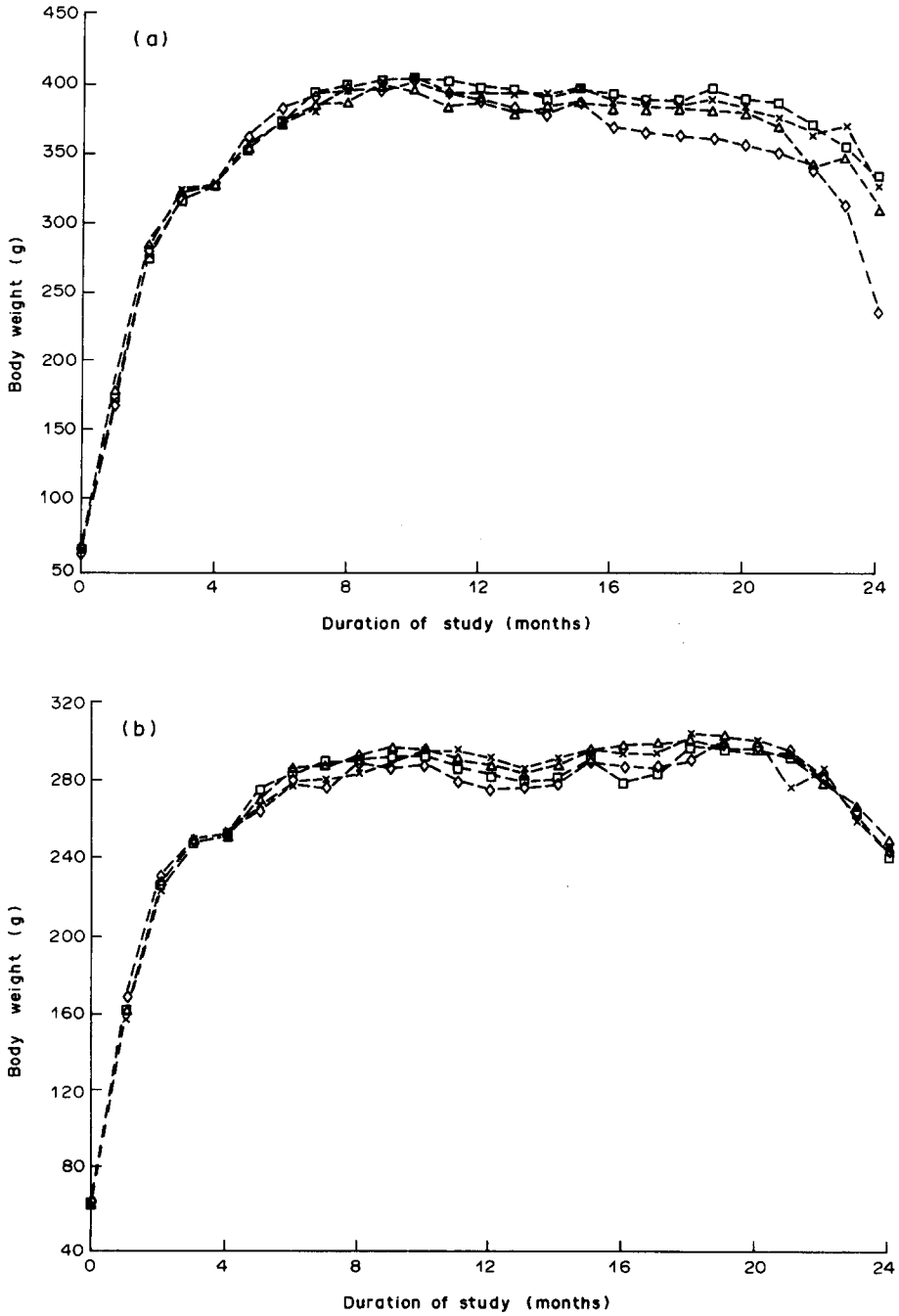

Fig. 4. Growth curves of (a) male and (b) female rats throughout the chronic toxicity/carcinogenicity study: the rats were fed stevioside at dietary levels of 0% (×), 0.2% (□), 0.6% (△) or 1.2% (○) for 2 yr.

Table 2. Amount of stevioside ingested by Wistar rats during the first 3 months of the chronic toxicity/carcinogenicity study

Sex	Stevioside (% in diet)	Total food consumption (g)*	Total stevioside consumption (mg)*	Mean body weight (g)	Stevioside consumption (mg/kg/day)
Male	0 (control)	19,908	0	315.4	0
	0.2	20,617	41,234	327.4	128.5
	0.6	19,817	118,902	330.0	367.6
	1.2	19,943	239,316	326.1	748.8
Female	0 (control)	17,576	0	249.4	0
	0.2	18,050	36,100	251.8	146.3
	0.6	17,273	103,638	259.0	416.2
	1.2	17,442	209,304	254.6	838.9

*Over 98 days.
Values are means for groups of 10 rats.

consumption is to substitute other sweeteners, and stevioside is being investigated as such a sweetener.

The four dose levels used in the present study were determined with reference to Yamada *et al.* (1985) using the following estimations: (1) the mean level of ingestion of sucrose is 50 g/human/day; (2) 50% of ingested sucrose could be substituted by stevioside; (3) stevioside is 200 times sweeter than sucrose; (4) the mean body weight of an adult man is 60 kg. We can therefore calculate a likely maximum intake of stevioside by humans of 2 mg/kg body weight/day. If we add safety factors of 100-, 300- and 600-fold, the low-, mid- and high-dose groups must ingest 200, 600 and 1200 mg stevioside/kg/day. Taking the mean body weight of rats as 250 g, and an average food consumption of 25 g/day over the 2 yr of the study, the lowest dose to be used in this study must contain 50 mg stevioside/25 g of food or 0.2%. The mid- and high dose levels will be 0.6 and 1.2%, respectively.

During the 2-yr observation in this study no specific toxic effects of stevioside at these levels were observed. With the safety factors included, we can extrapolate these results and conclude that the ingestion of stevioside by humans at the anticipated levels will be safe.

The decrease in body weights that was observed in all rats after 20 months is related to ageing and the sharp increase in mortality after 18 months was related mainly to pulmonary inflammation, the swift development of tumours, and general poor health related to ageing. In haematological and biochemical investigations, although there were some differences between the treated rats and the controls, these either fluctuated within normal ranges or did not occur consistently throughout the study. At 24 months, serum alkaline phosphatase levels were increased to about twice the levels at 6 and 12 months, but these increases occurred in both treated and control groups. Similar changes were reported by Yamada *et al.* (1985), and we believe they were unrelated to the administration of stevioside.

Histopathological examination revealed a variety of changes in various organs, but in most cases the incidences of non-neoplastic lesions were similar in treated rats and controls, and most of the changes were lesions that are frequently found in ageing rats (Lin, 1987; Shi, 1989). Various types of neoplasms were observed in 11 organs and tissues in rats of both sexes. Again, almost all were of types that are frequently found in aged rats of the inbred Wistar strain (Lin, 1987; Shi, 1989). The majority (73%) of the tumours were benign; adenofibromas of the mammary gland were the most frequent, which is in agreement with reports by Lin and Dou (1987) and Shi (1989). Since an extensive range of organs and tissues from all of the rats were examined macroscopically and histopathologically, the present study should have uncovered any specific chronic toxicological effects of stevioside.

Over the last 10 yr, many studies on the toxicity of stevioside have been reported. Hisashi *et al.* (1982), using stevioside of 69% purity, determined an oral medial lethal dose of more than 16 g/kg body weight in mice, while an oral LD_{50} of 17.07 g/kg body weight in DDY-N mice was reported by Haruo and Yukio (1975). Stevioside administered at 3000 mg/kg body weight/day for 3 months produced no adverse effects in rats (Haruo and Yukio, 1975). These doses are

Table 3. Incidence of deaths and average lifespan of rats fed stevioside for up to 24 months

Sex	Stevioside (% in diet)	No. of deaths during months: 1–6	7–12	13–18	19–24	Total	Mortality (%)	Average lifespan (days)
Male	0 (control)	0	0	3	25	28	62.2	641.7
	0.2	0	1	5	22	28	62.2	619.8
	0.6	0	0	7	23	30	66.6	646.5
	1.2	0	3	3	21	27	60.0	613.8
Female	0 (control)	0	1	5	19	25	55.6	623.6
	0.2	0	0	6	16	22	48.9	632.9
	0.6	0	3	2	16	21	46.6	597.7
	1.2	0	1	6	20	27	60.0	617.4

There were 45 rats in each group at the start of the study. There were no significant differences between groups in mortality rate (chi-square analysis) or mean lifespan (analysis of covariance).

many times higher than the estimated maximum human intake of 2 mg/kg body weight/day. Yamada *et al.* (1985) fed stevia extracts (95.2% pure) to male and female F344 rats for 22–24 months, and found no significant dose-related effects on growth, general appearance, haematological or blood biochemical effects, histopathological findings or tumour incidence, even though at the highest dose the rats were receiving 550 mg stevioside/kg body weight/day.

Masoya *et al.* (1978) reported negative results in the Rec assay, reversion test, and Ames test of stevia extracts and crystals, and negative results were also obtained in a micronucleus test and a testis chromosomal aberration test (L. Gan, China University of Medicine, personal communication, 1983). No effects

Table 4. Incidence of non-neoplastic tissue changes in rats fed stevioside for 2 yr

			Incidence (no. of rats affected) among groups fed stevioside at (% in diet):							
			0 (control)		0.2		0.6		1.2	
Site	Lesion	Sex ...	M	F	M	F	M	F	M	F
	Total no. of rats ...		45	45	45	45	45	45	45	45
Liver	Microfocal lesion		1	0	1	0	0	0	0	0
	Congestion		3	1	1	0	0	1	2	0
	Slight fatty degeneration		5	8	4	6	3	2	5	7
	Abscess		0	0	1	0	0	1	0	1
Oesophagus	Erosion of mucosa		1	0	0	0	0	0	0	0
	Ectasia		1	2	1	1	1	0	1	1
	Microfocal lesion		0	0	1	0	0	0	0	0
	Abscess		0	1	0	0	0	0	0	1
	Epidermoid cyst		0	1	0	0	0	0	0	1
Stomach	Ulcer of the lesser curvature		0	0	0	0	0	1	0	0
	Haemorrhage		2	0	0	0	0	0	0	0
	Papilliform hyperplasia of mucosa		1	0	0	0	0	0	0	0
Pancreas	Microabscess		0	0	1	0	0	0	0	0
Lung	Chronic pleurisy		1	0	0	0	0	0	0	0
	Interstitial pneumonia		0	1	2	1	1	0	1	1
	Lobular pneumonia		6	4	5	4	5	6	6	4
	Abscess		10	9	12	13	12	11	14	10
	Oedema		0	0	0	0	0	0	1	0
	Emphysema		0	0	0	0	0	0	1	0
	Congestion		2	4	3	5	3	4	6	5
Trachea	Tracheitis		1	4	2	1	1	3	3	1
	Microabscess		0	0	1	0	0	0	0	0
	Abscess of soft tissue		0	1	0	0	0	0	0	0
Heart	Myocarditis		1	0	2	0	2	0	1	1
	External cardiac haematoma		0	0	0	1	1	0	0	0
Spleen	Acute infections		2	2	0	3	0	2	1	1
	Atrophy		0	0	0	1	0	0	0	0
Lymph node	Lymphadenitis		1	0	1	1	0	0	1	0
	Abscess		0	0	0	0	0	0	1	0
Kidney	Tubular degeneration and necrosis		2	0	0	0	1	0	2	0
	Abscess		0	0	0	0	2	0	0	0
	Congestion		1	0	0	1	1	0	2	0
	Hydronephrosis		0	0	0	1	0	0	0	0
Brain	Slight oedema of the cerebral surface		1	0	0	0	0	0	0	0
	Focal oedema		0	1	0	0	0	0	0	0
	Verrucoid hyperplasia (midst of pans)		0	0	0	1	0	0	0	0
	Pyencephalus		1	0	0	0	0	0	0	0
Pituitary	Acute purulent hypophysitis		0	1	0	0	2	0	0	1
	Abscess		1	0	0	1	1	0	2	0
	Congestion		1	4	2	3	1	4	3	3
Thyroid	Chronic lymphocytic thyroiditis		1	0	0	1	0	1	0	0
Adrenal gland	Medullary hyperplasia		0	0	0	0	0	1	0	0
	Congestion		1	1	1	1	1	2	0	2
	Abscess		0	1	0	2	0	0	1	0
	Medullary haemorrhage		0	0	0	1	1	0	0	0
Testis	Atrophy		1	0	1	0	1	0	1	0
	Congestion		0	0	1	0	0	0	0	0
Mammary gland	Chronic inflammatory granulation tissue		0	0	0	0	0	0	1	0
Uterus	Acute and chronic endometritis		0	3	0	1	0	2	0	3
	Chronic cervicitis		0	0	0	1	0	1	0	1
	Retention cyst of the cervix		0	0	0	1	0	0	0	0
	Purulent endometritis		0	1	0	0	0	0	0	0
	Pyometra		0	1	0	1	0	2	0	3
	Abscess		0	4	0	3	0	2	0	3
Ovary	Abscess		0	4	0	3	0	4	0	4
	Follicle cyst		0	2	0	1	0	1	0	1
	Simple cyst		0	0	0	0	0	3	0	0
	Congestion		0	1	0	1	0	0	0	0
Bladder	Abscess		0	2	1	0	3	0	1	0
	Oedema		0	0	0	0	0	0	1	0

Table 5. Incidence of neoplasms in rats fed stevioside for up to 24 months

Sex	Stevioside (% in diet)	Effective no. of rats	No. (%) of rats with: Benign tumours	Malignant tumours	Total no. (%) of rats with tumours
Male	0 (control)	45	3 (6.6)	0 (0)	3 (6.6)
	0.2	45	1 (2.2)	0 (0)	1 (2.2)
	0.6	45	2 (4.4)	0 (0)	2 (4.4)
	1.2	45	0 (0)	2 (4.4)	2 (4.4)
Female	0 (control)	45	4 (8.8)	1 (2.2)	5 (11.1)
	0.2	45	4 (8.8)	3 (6.6)	7 (15.5)
	0.6	45	6 (13.3)	1 (2.2)	7 (15.5)
	1.2	45	4 (8.8)	2 (4.4)	6 (13.3)

Table 6. Classification of tumours found in rats fed stevioside for up to 2 yr

Site	Tumour	Stevioside (% in diet) ...	Incidence (no. of rats affected)* Males 0	0.2	0.6	1.2	Females 0	0.2	0.6	1.2	Total
	Benign tumours										
Kidney	Lymphangioma		0	0	1	0	0	0	0	0	1
Stomach	Papilloma		1	0	0	0	0	0	0	0	1
Subcutis	Hard fibroma		1	1	1	0	0	0	0	0	3
Mammary gland	Adenofibroma		1	0	0	0	2	4	4	4	1
	Adenoma		0	0	0	0	1	0	0	0	1
Brain	Choroid papilloma of the third and fourth ventricle		0	0	0	0	1	0	0	0	1
Mesentery	Cavernous capillary haemangioma		0	0	0	0	0	0	1	0	1
Ovary	Fibroma		0	0	0	0	0	0	1	0	1
	Total		3	1	2	0	4	4	6	4	24
	Malignant tumours										
Peritoneum	Fusiform mesothelioma										
Pituitary	Acidophil adenocarcinoma		0	0	0	1	0	0	0	0	1
	Chromophobe adenocarcinoma		0	0	0	1	0	0	0	0	1
	Adenocarcinoma		0	0	0	0	0	0	1	0	1
Rectum	Adenocarcinoma		0	0	0	0	0	0	0	1	1
Uterus	Endocervical squamous carcinoma		0	0	0	0	0	0	0	1	1
	Polymorphic leiomyosarcoma		0	0	0	0	0	2	0	0	2
	Total		0	0	0	2	1	3	1	2	9

*There were 45 rats in each group.

on blood pressure, cardial rhythm electrocardiogram or blood sugar were observed when dogs were given stevioside orally at 5 g/kg body weight/day for 20 days (L. Gan, personal communication, 1983).

The sweetening properties and the potential pharmaceutical activities of stevioside have received worldwide attention. Wingard *et al.* (1980) suggested that stevioside is swiftly transformed into steviol by the action of anaerobic micro-organisms in the intestine. However, Pezzuto *et al.* (1985) carried out a comprehensive study in which stevioside was incubated for up to 3 months, at pH values ranging from 2 to 8 and temperatures ranging from 5 to 90°C, with various intestinal micro-organisms; no steviol production could be detected. Studies with steviol-17[^{14}C] showed that 96% of an oral dose was eliminated in the faeces of rats, and that the aglycone is completely absorbed through the lower bowel (Wingard, 1980). As yet, stevioside metabolism has not been investigated in humans.

We conclude that from the results of the present study that administration of stevioside at up to 1.2% in the diet for 24 months does not have any carcinogenic or other adverse effects in Wistar rats; an ADI of 7.938 mg/kg body weight/day was determined. These results, and those of other studies on stevioside, indicate that it is a safe, natural sweetener, of high sweetness and low calorific value. It is non-toxic, non-mutagenic and non-carcinogenic, and could be beneficial in the prevention of dental caries and obesity associated with high sucrose consumption. Stevioside may also have therapeutic value in the treatment of patients with diabetes-related obesity, hypertension or cardiac disease.

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