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# Chronic Oral Toxicity and Carcinogenicity Study of Steviol, a Metabolite of Stevioside, in Hamsters

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***ABSTRACT*** *: The carcinogenic potential of steviol, a metabolite of stevioside (a compound that is used as a sweetener for food and drink), was examined in hamsters of both sexes. Groups of 55 male and 55 female hamsters were given diets containing steviol at 0, 100 and 500 mg/kg diet for 22 months in males and 18 months in females. After 6, 12 and 22 months in males and 18 months in females, hamsters from each group were sacrificed for hematological and biochemical tests. Growth, food utilization and consumption, general appearance and mortality were similar in treated and control groups. The mean life span of hamsters given steviol was not significantly different from that of the controls. No treatment-related changes were observed in hematological, urinary and biochemical values at any stage of the study. There was no significantly altered development of neoplastic or non-neoplastic lesions attributable to steviol treatment in any organ or tissue. The highest level of steviol in the diet which still causes no effects in hamsters was 500 mg/kg diet, under the experimental conditions used.*

***Key Words*** *: Stevioside, Steviol, Carcinogenicity, Long term*

## I. INTRODUCTION

Stevioside is a major sweet diterpene glycoside present in the leaves of *Stevia rebaudiana*. It is a white crystalline, odorless powder and approximately 300 times sweeter than sucrose. It is widely used as a non-caloric sweetener in a variety of foods including seafoods, pickled vegetables, dessert items, ice cream, soft drinks and confectionary in many countries (Kinghorn and Soejarto, 1991; Melis and Sainati, 1991). Stevioside is composed of steviol, a diterpenic carboxylic alcohol and under enzymatic hydrolysis it yields three moles of D-glucose and one mole of steviol (Hanson and De Oliveira, 1993). Stevioside has been subjected to various assessments for safety and no serious toxic effect has been reported. The safety of long-term consumption of stevioside has been studied by many investigators (Yamada *et al.*, 1985; Xili *et al.*, 1992; Toyada *et al.*, 1997). The results shown that there was no significantly altered development of neoplastic or non-neoplastic lesions attributable to stevioside treatment in any organ or tissue under the experimental conditions. However, stevioside has been demonstrated be converted to its aglycone, steviol by intestinal bacteria when given orally to rats, and then steviol is nearly completely absorbed in the gastrointestinal tract (Wingard *et al.*, 1980).

The mutagenicity of stevioside has been studied in many test systems and no mutagenic activity was noted by using those systems (Suttajit *et al.*, 1993; Matsui, 1996; Klongpanichpak *et al.*, 1997; Oh *et al.*, 1999). However, steviol, the aglycone of stevioside, was demonstrated to be mutagenic with metabolic activation in the forward mutation assay, *umu* test, chromosomal aberration test and gene mutation assay (Pezzuto *et al.*, 1985; Matsui *et al.*, 1996). It also showed negative results in the Ames test, bacterial reversion assay, rec-assay and chromosomal damage using micronucleus test (Suttajit *et al.*, 1993;

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List of abbreviations : ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BW, body weight; °C, degree celcius; h, hour; g, gram; kg, kilogram; pH, log concentration of $H^+$; mg, milligram; SEM, standard error of mean

Matsui *et al.*, 1996; Temcharoen *et al.*, 2000). From these observations, the potential carcinogenicity of stevioside may be suggested through the mutagenicity of steviol when the compound is administered orally to animals. However, the long-term consumption of steviol has not been reported. In the present study, we therefore investigated the long-term effects of steviol administration to male and female hamsters. Hamsters were selected since they are more susceptible to the toxicity of steviol than rats and mice (Toskulkao *et al.*, 1997).

## II. MATERIALS AND METHODS

### 1. Animals and Materials

Weanling male and female Syrian golden hamsters were supplied by the Animal Production Center, Faculty of Science, Mahidol University. They were maintained on a pelleted basal diet (C.P. Mice Feed, Pokphand Animal Feed Co., Ltd., Thailand) and tap water *ad libitum*. The animals were kept in stainless steel cages in an air-conditioned room at 25±2°C, 65% humidity with a 12 h light/12 h dark cycle. Steviol (approx. 90%) was obtained by oxidation of stevioside as described by Ogawa *et al.* (1980). The purity of steviol was determined by Dr. Duang Buddhasukh, Department of Chemistry, Faculty of Science, Chiang Mai University. Steviol was incorporated into the powdered diet at concentrations of 0, 100 and 500 mg/kg diet and stored in cold room at 4°C. The steviol diet was prepared freshly once a week for use within one week.

### 2. Determination of dose levels

In order to select doses for the carcinogenicity study, a 13-week feeding test was carried out. Steviol was incorporated into the powdered diet at concentrations of 0, 100 and 500 mg/kg diet, and given to 9 hamsters of each sex group *ad libitum*. The animals were weighed once a week, and their condition was checked daily. Steviol at a dose of 100 or 500 mg/kg diet had no significant effect on body weight gain or food conversion efficiency, and showed no observable signs of toxicity or abnormal behavior. On the basis of these results, the maximum tolerated dose of steviol given in the diet was estimated to be higher than 500 mg/kg diet for both sexes in hamsters. The dose levels chosen for the present carcinogenicity study were therefore 100 and 500 mg/kg diet, since the steviol consumption corresponds to a calculated stevioside consumption that was higher than the acceptable daily intake of stevioside in human (Xili *et al.*, 1992).

### 3. Chronic toxicity and carcinogenicity study

Weanling hamsters were randomly allocated to three groups, each consisting of 55 males and 55 females. Five animals were housed in each cage. Groups of hamsters were maintained on diets containing steviol at concentrations of 0, 100 or 500 mg/kg diet. After the 22 months treatment in males and 18 months treatment in females, all surviving animals were placed on the basal diet for an additional 2 week period. The duration of chronic toxicity and carcinogenicity tests of food additives in hamsters is usually 18 months, which is nearly their life span (Redman *et al.*, 1979).

Throughout the experiment, hamsters in all groups were given free access to both tap water and diet. All hamsters were observed daily and clinical signs and deaths were recorded. For the first 14 weeks of the study the hamsters were weighed weekly. All animals were weighed once every 2 weeks from 4 to 6 months, and then every month until the end of the study. After 6 and 12 months, five male and five female hamsters were randomly selected from each dose group and all surviving animals at the end of the study were housed in the metabolic cages and 24-hour urine samples were collected. The animals were weighed and then killed by exsanguination under ether anesthesia after overnight fasting, and autopsied. Blood samples were taken from the abdominal aorta, and examined for erythrocyte and total and differential leucocyte counts, hemoglobin and hematocrit values were determined, using a fully automated hematology analyser. For each plasma sample the following determinations were performed: protein, albumin and globulin, AST and ALT, alkaline phosphatase, urea nitrogen, glucose, cholesterol, calcium, sodium, potassium, chloride and bicarbonate. pH, specific gravity, glucose, protein and osmolarity of urine were determined. A careful macroscopic examination of various organs/

tissues was carried out. The liver, kidney, brain, heart, lung, spleen, stomach, pancreas, trachea, esophagus, adrenal gland, uterus, urinary bladder, testis, ovary and large intestine were excised and weighed. These organs/tissues were routinely fixed in 10% buffered formalin, sectioned, and stained with haematoxylin and eosin and microscopically studied for signs of neoplastic and non-neoplastic changes. The animals that died or were killed when moribund also underwent complete gross and microscopic examination. Those for which histopathological examinations could not be performed owing to advanced autolysis were excluded from the effective numbers.

### 4. Statistical analysis

Data for mortality rates among groups was compared by chi-square analysis, whereas other parametric data were statistically evaluated according to a one way analysis of variance (ANOVA) followed by a multiple range test (Duncan method). Statistical significance of differences between groups were taken at p values of less than 0.05.

## III. RESULTS

### 1. Body weight and food and steviol consumption

There were no significant differences in body weight gain or food consumption or utilization between controls and treated groups for both male and female hamsters over the first 3 months of the study (Table 1). Figure 1 shows the growth curves for each group. The body weight was not significant differences between the controls and treated groups throughout the experiment. After month 19 in males and month 14 in females, body weights tended to decrease in all groups, including the controls. This appeared to be related to ageing.

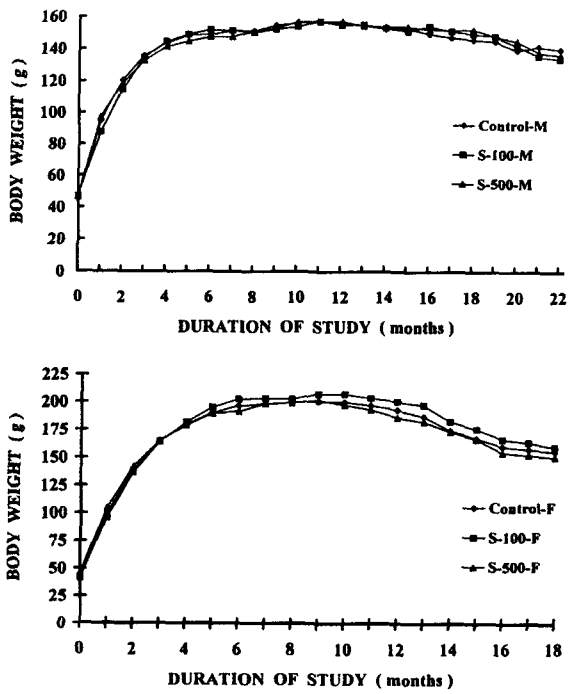


**Fig. 1.** Growth curves of male (M) and female (F) hamsters fed steviol at 0 (control), 100 and 500 'mg/kg diet throughout the chronic/carcinogenicity study.

The average daily consumption of steviol (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 hamsters would have been higher than that of adult hamsters, and hence their consumption of steviol would also have been higher. On the basis of these calculations, the consumption of steviol by hamsters in the higher (500 mg/kg diet) dose group was 199.8 and 183.1 mg/kg BW/day for males and females, respectively.

**Table 1.** Relative food consumption, body weight gain and food conversion efficiency of hamsters fed steviol during the first 3 months (98 days) of the chronic toxicity/carcinogenicity study

| Sex | Steviol (mg/kg diet) | Body weight gain (g) | Relative total food consumption (g) | Food conversion efficiency (%) |
|---|---|---|---|---|
| Male | 0 (control) | 89.9±5.3 | 613.1±30.8 | 14.7±0.9 |
| | 100 | 98.2±6.7 | 628.1±24.7 | 15.6±1.1 |
| | 500 | 87.5±4.8 | 580.5±19.5 | 15.1±0.8 |
| Female | 0 (control) | 129.4±5.0 | 681.7±18.5 | 18.9±0.7 |
| | 100 | 122.8±5.0 | 686.4±12.7 | 17.9±0.7 |
| | 500 | 120.2±4.3 | 683.6±14.6 | 17.6±0.6 |

Values are means±SEM of 9 hamsters/group.

**Table 2.** Amount of steviol ingested by hamsters during the first 3 months (98 days) of the chronic toxicity/carcinogenicity study

| Sex | Steviol (mg/kg diet) | Relative total food consumption (g) | Relative steviol consumption (g) | Mean body weight (g) | Steviol consumption (mg/kg/day) |
|---|---|---|---|---|---|
| Male | 0 (Control) | 5,517 | 0 | 139.2 | 0 |
| | 100 | 5,652 | 565 | 149.0 | 38.7 |
| | 500 | 5,224 | 2,612 | 133.4 | 199.8 |
| Female | 0 (Control) | 6,135 | 0 | 175.5 | 0 |
| | 100 | 6,177 | 617 | 168.5 | 37.4 |
| | 500 | 6,152 | 3,076 | 171.5 | 183.1 |

Values are means±SEM of 9 hamsters/group.

### 2. General appearance and mortality

No other noteworthy changes in the animals' general condition resulted from administration of steviol. The incidence of deaths and average life span of hamsters fed with steviol is summarized in Table 3. There were no significant differences in the mean average life span and the mortality rate between the controls and steviol-treated groups. The percent mortality in steviol-treated group was slightly lower than the control group.

### 3. Laboratory investigations

There were few significant differences ($p<0.05$) between groups in hematological or biochemical parameters. The erythrocyte counts of the females fed steviol (500 mg/kg diet) were significantly lower than those of the controls at 12 months. However, these values were still within the normal range and at the end of the experiment there were no significant differences between groups. For other hematological parameters, there were no other statistically significant differences between treated groups and controls.

Plasma sodium and chloride were significantly lower in males fed steviol (500 mg/kg diet) at 6 months. At 12 months, plasma calcium was significantly higher in males fed steviol (100 mg/kg diet) than in controls. All fluctuations were within normal ranges. No significant differences were found between groups in plasma glucose, BUN, cholesterol, total protein, albumin, globulin, AST, ALT, alkaline phosphatase, inorganic phosphate, potassium or carbon dioxide.

The pH of urine of males fed steviol (500 mg/kg diet) at the end of the study and females fed steviol (100 mg/kg diet) at 12 months was significantly higher than those of the controls. However, these values were still within the normal range. No significant differences were found between groups in urine specific gravity, osmolarity, protein or sugar.

### 4. Relative organ weights

At the end of the study, eight important organs such as liver, kidney, brain, heart, lung, stomach and testis (ovary) were weighed and the relative organ weight was calculated. No significant differences in relative organ weights were found between the steviol-treated hamsters and the controls (data not shown).

**Table 3.** Incidence of deaths and average life span of hamsters fed steviol for up to 22 months (male) and 18 months (female)

| Sex | Steviol (mg/kg diet) | No. of deaths during months: | | | | | | Mortality (%) | Average Life span (days) |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-6 | 7-12 | 13-15 | 16-18 | 19-22 | Total | | |
| Male | 0 (Control) | 0 | 2 | 5 | 6 | 17 | 30 | 54.5 | 602 |
| | 100 | 0 | 3 | 3 | 4 | 18 | 28 | 50.9 | 609 |
| | 500 | 0 | 3 | 6 | 6 | 14 | 29 | 52.7 | 606 |
| Female | 0 (Control) | 0 | 4 | 8 | 21 | - | 33 | 60.0 | 499 |
| | 100 | 0 | 5 | 7 | 19 | - | 31 | 56.3 | 496 |
| | 500 | 0 | 4 | 8 | 19 | - | 31 | 56.3 | 506 |

There were 55 hamsters in each group at the start of the study. There were no significant differences between groups in mortality rate (chi-square analysis) or mean life span (analysis of covariance).

### 5. Histopathological findings

Non-neoplastic changes are listed in Table 4. Various types of non-neoplastic lesions in the tissues were observed in each group. Age-related chronic lesions such as amyloidosis and cystic lesion were found in all groups. The distribution and frequency of the pathological lesions were similar in steviol-treated hamsters and the controls for both sexes. In males, the severity of cystic lesion (liver), amyloidosis and calcinosis (kidney) and amyloidosis (spleen) in steviol-treated hamsters was significantly lower whereas the tracheitis was significantly higher than that in the controls. However, there were no dose-effect relationships.

Table 5 shows the incidence of tumors developing in the hamsters in the present study, lesions being observed in many organs or tissues of all groups, including controls. No significant differences were observed between treated and control groups of either sex in the incidences of total neoplasms or of benign or malignant tumors. The tumors are classified in

**Table 4.** Incidence of non-neoplastic tissue changes in hamsters fed steviol for up to 22 months (male) and 18 months (female)

| Site | Lesion | Incidence (no. of hamsters affected) among groups fed steviol at (mg/kg diet): | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | | 100 | | 500 | |
| | Sex ..... | M | F | M | F | M | F |
| | **Total no. of hamsters** | 45 | 47 | 46 | 48 | 45 | 47 |
| Liver | Cystic lesion | 20 | 9 | 8* | 9 | 6* | 14 |
| | Amyloidosis | 15 | 14 | 8 | 15 | 15 | 18 |
| | Slight fatty degeneration | 13 | 3 | 7 | 3 | 7 | 1 |
| | Inflammatory cell | 13 | 11 | 10 | 12 | 14 | 12 |
| Stomach | Calcinosis of epithelium | 4 | 1 | 5 | 4 | 1 | 4 |
| | Cystic lesion | 6 | 2 | 1 | 2 | 2 | 2 |
| | Amyloidosis | 1 | 0 | 0 | 1 | 0 | 0 |
| Colon | Colitis | 0 | 0 | 0 | 0 | 0 | 1 |
| | Amyloidosis | 0 | 0 | 0 | 0 | 1 | 0 |
| Heart | Thrombosis | 0 | 0 | 0 | 1 | 0 | 0 |
| | Vascular calcinosis | 3 | 5 | 7 | 3 | 4 | 2 |
| Lung | Bronchiolitis | 0 | 1 | 1 | 0 | 0 | 1 |
| | Interstitial pneumonia | 19 | 16 | 19 | 11 | 17 | 8 |
| | Lobular pneumonia | 0 | 2 | 3 | 0 | 0 | 4 |
| | Edema alveolar space congestion | 2 | 4 | 2 | 1 | 0 | 4 |
| | Calcinosis | 1 | 1 | 3 | 1 | 0 | 0 |
| Trachea | Tracheitis | 4 | 17 | 9* | 9 | 18* | 14 |
| | Squamous metaplasia | 1 | 1 | 0 | 0 | 0 | 1 |
| | Mucus gland hyperplasia | 3 | 0 | 3 | 1 | 1 | 4 |
| | Epithelial dysplasia | 1 | 0 | 0 | 0 | 1 | 0 |
| Thyroid gl | Amyloidosis | 2 | 3 | 1 | 0 | 2 | 6 |
| | Inflammatory cells in follicular lumen | 0 | 5 | 1 | 2 | 0 | 3 |
| Parathyroid | Hyperplasia | 0 | 3 | 0 | 3 | 4 | 2 |
| Pancreas | Cystic lesion | 1 | 0 | 0 | 0 | 0 | 0 |
| | Pancreatitis | 2 | 0 | 2 | 0 | 0 | 2 |
| Kidney | Amyloidosis | 27 | 17 | 7* | 16 | 11* | 17 |
| | Calcinosis | 19 | 3 | 9* | 3 | 8* | 0 |
| | Inflammatory cell infiltra-tion | 4 | 14 | 3 | 13 | 5 | 15 |
| Adrenal gl | Amyloidosis | 7 | 18 | 6 | 17 | 5 | 17 |
| Testis | Atrophy | 1 | 0 | 0 | 0 | 0 | 0 |
| | Hypospermatogenesis | 6 | 0 | 7 | 0 | 6 | 0 |
| Epididymis | Spermatocele | 1 | 0 | 1 | 0 | 0 | 0 |
| Spleen | Amyloidosis | 14 | 15 | 4* | 16 | 7* | 19 |
| Uterus | Cystic lesion | 0 | 1 | 0 | 1 | 0 | 0 |
| Ovary | Cystic lesion | 0 | 0 | 0 | 2 | 0 | 0 |

Incidences marked with asterisks differ significantly (chi-square test) from the corresponding control: *$p<0.05$, significant difference from control (male).

**Table 5.** Incidence of neoplasms in hamsters fed steviol for up to 22 months (male) and 18 months (female)

| Sex | Steviol (mg/kg diet) | No. of hamsters examined | No. (%) of hamsters with | | Total no. (%) of hamsters with tumors |
|---|---|---|---|---|---|
| | | | Benign tumors | Malignant tumors | |
| Male | 0 (Control) | 45 | 2 (4.4) | 0 (0) | 2 (4.4) |
| | 100 | 46 | 1 (2.2) | 0 (0) | 1 (2.2) |
| | 500 | 45 | 1 (2.2) | 1 (2.2) | 2 (4.4) |
| Female | 0 (Control) | 47 | 1 (2.1) | 0 (0) | 1 (2.1) |
| | 100 | 48 | 1 (2.1) | 1 (2.1) | 1 (2.1) |
| | 500 | 47 | 0 (0) | 0 (0) | 0 (0) |

There were no significant differences between groups (chi-square test).

**Table 6.** Classification of tumors found in hamsters fed steviol for up to 22 months (male) and 18 months (females)

| Site | Tumor | Steviol (mg/kg diet) | Incidence (no. of hamsters affected)[a] | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | Males | | | Females | | | |
| | | | 0 | 100 | 500 | 0 | 100 | 500 | |
| | **Benign tumors** | | | | | | | | |
| Liver | Hemangioma | | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Parathyroid | Adenoma | | 0 | 0 | 1 | 1 | 1 | 0 | 3 |
| Spleen | Hemangioma | | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| | Total | | 2 | 1 | 1 | 1 | 1 | 0 | 6 |
| | **Malignant tumors** | | | | | | | | |
| Adrenal gl | Pheochromocytoma | | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Lung | Pheochromocytoma | | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | Total | | 0 | 0 | 1* | 0 | 0 | 0 | 1* |

[a]There were 45-48 hamsters in each group.
*The incidence of pheochromocytoma in adrenal gland and lung occurs in the same animal.

Table 6. None of the steviol-treated groups demonstrated a significant increase in the incidence of any specific tumor over that in the corresponding control group. All tumors observed in this study were similar to those occurring spontaneously in this strain of hamster (Van Hoosier and Trentin, 1979).

## IV. DISCUSSION

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 consumption is to substitute it with other non-caloric sweeteners, and stevioside is being investigated as such a sweetener. Up to now, it is likely that the natural sweetener, stevioside, is not carcinogenic to the rats (Yamada *et al.*, 1985; Xili *et al.*, 1992; Toyada *et al.*, 1997) although steviol, its metabolic product is demonstrated to be mutagenic in the forward mutation assay, *umu* test, chromosomal aberration test and gene mutation assay (Pezzuto *et al.*, 1985; Matsui *et al.*, 1996). Therefore, it is important to carry out carcinogenic test of steviol in more susceptible animal species such as hamsters (Toskulkao *et al.*, 1997). To investigate the effects of steviol on chronic/carcinogenic study in the hamsters, the duration of exposure should be nearly their lifespan which is usually 18 months (Redman *et al.*, 1979).

The three dose levels of steviol used in the present study were determined on the basis of the average of stevioside concentrations in Japanese food products such as ice cream (5 mg%), orange juice (7 mg%) and carbonated drinks (7 mg%) (Fugita and Edahiro, 1979). We selected the doses of steviol to cover the concentration of stevioside in Japanese food products by feeding the hamsters at dose-levels of 0, 100 and 500 mg/kg diet or equivalent to 0, 250 (25 mg%) and 1,250 (125 mg%) mg/kg diet of stevioside in the case that stevioside completely converts to steviol in animals. In fact, it is not known whether stevioside can completely convert to steviol *in vivo*. However, if the stevioside completely converts to steviol in the hamster, the doses of converted stevioside in diet in this study were higher than the concentration of stevi-

oside in Japanese food products by 5 and 25 folds.

The calculated steviol intakes by these animals were approximately 38.7 and 199.8 mg/kg BW/day in 100 and 500 mg/kg diet in the males, respectively, as well as 37.4 and 183.1 mg/kg BW/day in 100 and 500 mg/kg diet in the females, respectively. The estimate of steviol consumption fed with steviol 100 and 500 mg/kg diet represents intake of stevioside higher than 12 and 60 folds of an acceptable daily intake (ADI) of stevioside in humans (7.938 mg/kg BW/day) (Xili *et al.*, 1992) in the case that stevioside is completely converted to steviol. The steviol consumption was calculated based on the first 3 months because in this period, growing hamsters had higher average daily consumption than adult hamsters.

The decrease in body weight of hamsters in all groups, that was observed in the females after 14 months and the males after 19 months, is related to aging. The sharp increase in mortality of the hamsters after 18 months was related mainly to pulmonary inflammation, the swift development of tumors, and/or general poor health related to ageing. The mortality rate of hamsters in this study was similar to the mean survival times of hamsters that was reported by many investigators (Redman *et al.*, 1979; Pour and Birt, 1979). Our results demonstrate that feeding hamsters with steviol at doses of 100 and 500 mg/kg diet for up to 22 months in males and 18 months in females had no detrimental effect on the survival of the animals.

Steviol at doses levels of 100 and 500 mg/kg diet could not affect the relative food intake, body weight gain and food conversion efficiency either in male and female hamsters during the first 3 months. No steviol-related changes were observed in the general condition of the animals or in the relative organ weight of the hamsters fed with steviol for their lifetime. Plasma biochemical and hematological parameters in steviol-treated hamsters indicated that steviol was harmless to the animals.

Histopathological examination revealed a variety of changes in various organs, but in most cases the incidences of non-neoplastic lesions were similar in steviol-treated hamsters and in controls. These non-neoplastic lesions are of types that are well known to occur spontaneously in this strain of hamster (Van Hoosier and Trentin, 1979; Van Hoosier and Ladiges, 1984). However, liver cystic lesion, spleen amyloidosis as well as kidney amyloidosis and calcinosis in male hamsters fed with steviol were significantly lower than those of the controls. A variety of tumors in various organs was detected in all groups. However, the distribution of tumors in various organs was similar to that occurring spontaneously in this strain of hamsters (Hoosier and Trentin, 1979). No evidence of carcinogenicity was observed in both sexes of the hamsters maintained on chronic steviol at dietary levels of 100 and 500 mg/kg diet. From this study, there were no significant increases in the incidences of non-neoplastic and neoplastic lesions in any organs/tissues that could be attributed to the treatment of steviol.

Chronic/carcinogenicity of stevioside have been reported over the last 15 years. No significant dose-related effects were observed on growth, general appearance, hematological or blood biochemical effects, histopathological findings or tumor incidence of animals treated with stevioside (Yamada *et al.*, 1985; Xili *et al.*, 1992; Toyada *et al.*, 1997). However, stevioside is swiftly transformed into steviol by the action of anaerobic micro-organisms in the intestine (Wingard *et al.*, 1980; Hutapea *et al.*, 1997). It has been reported that stevioside itself shows no mutagenic potential (Suttajit *et al.*, 1993; Matsui et al., 1996; Klongpanichpak *et al.*, 1997; Oh *et al.*, 1999), whereas the aglycone steviol causes mutations after metabolic activation in the forward mutation assay, *umu* test chromosomal aberration test, and gene mutation assay (Pezzuto *et al.*, 1985; Matsui *et al.*, 1996) but not in the reverse mutation test (Ames test) using *Salmonella typhimurium* TA 100, TA 98, TA 102 or TA 97 and also negative results were obtained in a micronucleus test (Suttajit *et al.*, 1993; Matsui *et al.*, 1996; Temcharoen *et al.*, 2000). As yet, stevioside metabolism has not been reported in humans. However, Toyada *et al.* (1997) demonstrated that steviol is indeed produced in rats treated with stevioside at the large intestine and orally administered of steviol is completely absorbed through the lower bowel (Wingard *et al.*, 1980). In this study, hamsters were fed with steviol in the diet, the results, therefore, indicate that steviol exerts no carcinogenic activity in hamsters when administered continuously in the diet at concentrations of 100 and 500 mg/kg diet for up to 22 months

in males and 18 months in females, presumably suggesting that any mutagenicity exerted by steviol is not significant for neoplasia under these circumstances.

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