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***Stevia rebaudiana* Bertoni does not produce female reproductive toxic effect : Study in Swiss albino mouse**

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SUMMARY

Sugars used for sweetening do contribute calories, which can lead to obesity, a risk factor for some chronic diseases. Hence, the craving for sweetness led to discover several forms of alternative sweeteners, which would offer consumers the sweet taste without calories. *Stevia rebaudiana* Bertoni is a natural herb with low calorie sweetener. Stevioside is one of the principal diterpene glycosides in this herb having sweetness 250-300 times more than sucrose. In search for an alternative natural calorie-free sweetener, stevioside would be suitable but it is essential that it is exonerated of toxicities. The objective of the present study was to evaluate the female reproductive toxicity of aqueous Stevia extract and Stevioside in a mammalian model system. This study reports that the oral intake of water-based sweet stevia extract and stevioside, at doses 500mg/kg body weight and 800mg/kg body weight, respectively, does not cause any significant female reproductive toxic effect in Swiss albino mouse.

Introduction

In early days, honey and fruits were used for their sweetness. It is only in the 14th century that sugar was refined and considered as a special food item. The main source of sugar has for long been cane sugar, with beet sugar contributing a small percentage. These sugars, along with sweetening qualities, also have been found to contribute calories, which can lead to obesity, a risk factor for some chronic diseases such as diabetes mellitus, hypertension, cardiovascular diseases, etc. Hence, the craving for sweetness led to discover several forms of alternative intense sweeteners without calories. Stevia (*Stevia rebaudiana* Bertoni) is a natural sweet herb native of northeastern Paraguay, cultivated as a cash crop in a number of countries. Stevioside is one of the principal diterpene glycosides having sweetness of 250-300 times that of sucrose (Kingham, 1987). Stevia leaves and herbal powder are 10-15 times sweeter than sucrose (Crammer and Ikan, 1986). Traditionally, the water decoction of the leaves is consumed by Guarani Indians in Paraguay for obesity, hypertension, heartburn and to help lower uric acid levels. Currently, many commercial products, containing this herb, have emerged in the market claiming antidiabetic effect. Although safety evaluations of Steviosides have been reported in a large body of literature (Oliveira-Filho et al., 1989; Toskulkao et al., 1997; Melis, 1999; Aritajat et al., 2001), in most of the studies the

material tested was poorly specified or of variable quality and no information is available on the other constituents or contaminants. Furthermore, the studies on the reproductive/developmental system, which implicated Stevia in reproductive and/or developmental toxicities, revealed several limitations (Planas and Kuc, 1968; Melis, 1999). While most of the putative toxicities in general are short-term, reproductive toxicity would produce long term chronic effects, and developmental toxicity would produce multigenerational effects. The objective of the present study was to evaluate if aqueous Stevia leaf extract and Stevioside would produce any female reproductive toxic effect in an animal model, Swiss albino mouse, adopting OECD (1983) guidelines.

Materials and Methods

Adult female Swiss albino mice (27±2g body weight) of regular estrous cycle (4-6days) were administered vehicle (saline) daily for a period of 45days, through oral route using a gavage, which formed the common control, and aqueous Stevia extract or Stevioside at doses 500mg/kg body weight and 800mg/kg body weight, respectively, formed the experimental (each, five animals). Food (pellet feed) and water were made available *ad libitum*. In the first phase of the study, the estrous cycle of both control and treated females was recorded. On the 46th day all the mice were sacrificed and the ovary and uterus

were subjected to histological analysis. The second phase was approached from two perspectives, i) pre-coital (pre-fertilization) when both control and treated female mice were mated with untreated male mice of proven fertility and ii) post-coital (post-fertilization) when females of day zero pregnancy were separated and administered with stevia extract, Stevioside or vehicle for a period of 15 days. On the 16th day of pregnancy, the females (dams) were sacrificed and the number of implantations and resorptions were noted. Fetuses were analyzed histologically (hematoxylin and eosin staining) for developmental toxicity. All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the level of significance between the control and treatment in different groups. A difference was considered significant if $P < 0.05$.

Results

The leaf extract and Stevioside did not alter the stages or duration of the estrous cycle of Swiss albino mice. There was no significant change in the histological features of uterus and ovary of Stevia extract and Stevioside treated mice when compared to control (Fig. 1-6). Mice in all groups proceeded towards successful mating and pregnancy (Table 1).

None of the mice exhibited significant foetal resorptions, indicating that the herb was non-toxic to the fetuses. No gross anatomical or histopathological changes

were observed in day 16-day embryos from both control and treatment groups (not shown). Statistically, no test agent-related changes in the maternal body weight, number of implantations and fetuses were observed.

Discussion

Much of the controversy surrounding the existing literature (Melis, 1999; Planas and Kuc, 1968) on Stevia results from inadequate research aimed at finding reproductive toxicity in both laboratory animals and human beings. The results of the present study clearly indicate that *S. rebaudiana* and its pure compound Stevioside are non-toxic to the aspects of reproduction in the female. This is adequately reflected in the outcome of mating of treated females with untreated males since all treated females fell pregnant and produced similar number of fetuses as the control mice. The duration of and stages of the estrous cycle, and the histological status of uterus and ovary of treated and control mice could be used as indices of the female reproductive status and there was no significant difference. Dead fetuses were observed in all groups, even the control group. Nevertheless, <4% of fetuses died, which could be accounted for the percentage pre-birth death among rats (Zimnan, 1970). Generally, the fetuses are implanted in the endometrium 5 days after fertilization (Bodhanker et al., 1974). The fetuses of the treated mice were not different from those of the control mice and, thus, indicated similar implantation periods. Furthermore, the normal development of the fetuses, which was confirmed

Table 1. Treatment modalities and reproductive outcomes. Values are Mean \pm SE.

Treatment for 45 days	Dose (Oral) 0.5ml/day	Total no of implantations	No of resorptions in uterine horns	Anomalies	Pregnancy (%)
Control	Saline	13.6 \pm 1.14 ^b	0.2 \pm 0.45 ^a	-ve	100%
Aqueous Stevia extract (Post-coital)	500 mg/kg	10.8 \pm 1.09 ^a	0.6 \pm 0.24 ^a	-ve	100%
Stevioside (Post-coital)	800 mg/kg	11.2 \pm 1.30 ^a	0.4 \pm 0.55 ^a	-ve	100%
Aqueous Stevia extract (Post-coital)	500 mg/kg	11.2 \pm 0.45 ^a	0.2 \pm 0.45 ^a	-ve	100%
Stevioside (Post-coital)	800 mg/kg	10.6 \pm 1.14 ^a	0.4 \pm 0.55 ^a	-ve	100%

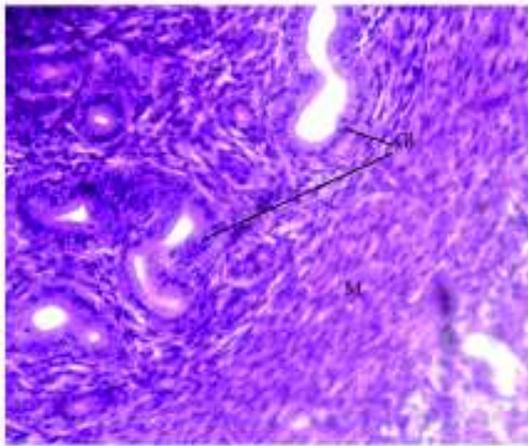


Fig. 1

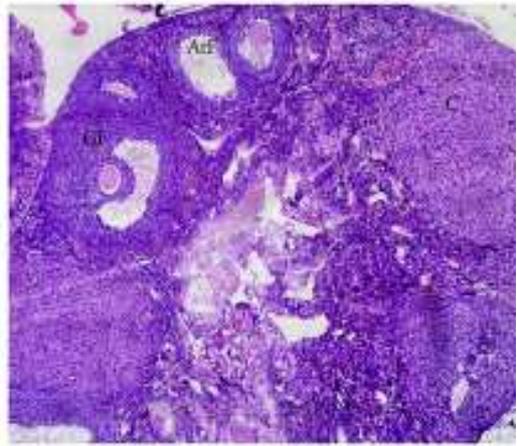


Fig. 4

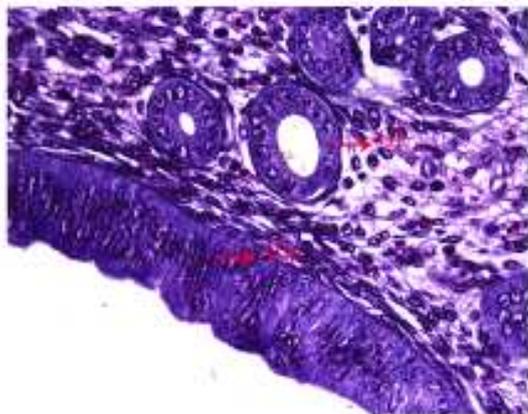


Fig. 2

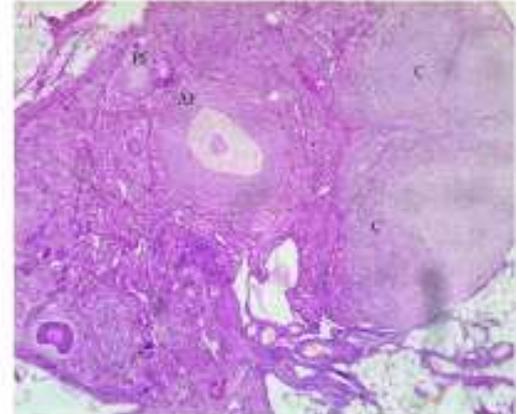


Fig. 5

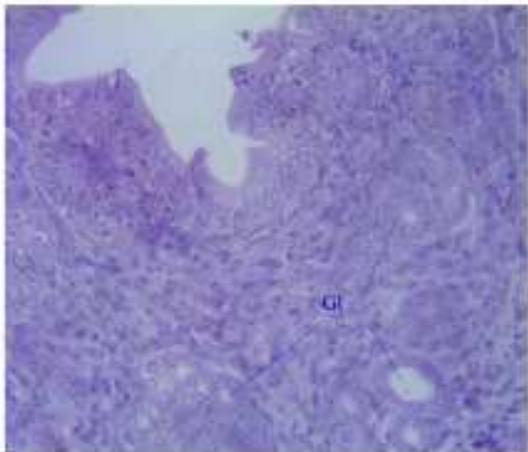


Fig. 3

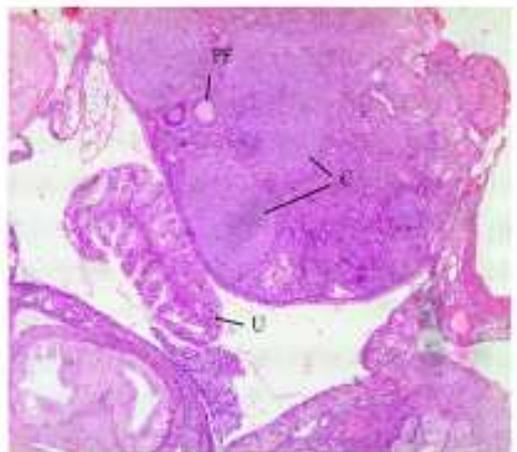


Fig. 6

Fig.1. Control uterus, Swiss albino mouse, H & E x 40; **Fig.2.** Stevia extract treated (500 mg/kg.) uterus, H& E x 40; **Fig. 3** Stevioside treated (800 mg /kg) uterus, H&E, x 40 Gl, Glandular epithelium, M, Myometrium.

Fig 4. Control ovary, Swiss albino mouse, H & E x 40; **Fig.5.** Stevia extract treated (500 mg/kg.) ovary, H & E x 40; **Fig. 6.** Stevioside treated (800 mg / kg.) ovary, H & E x 40. GF, Graafian follicle, C,Corpus luteum; PF, Primary follicle; U, Uterine wall.

by histological analysis, revealed that no teratogenic effect was produced by the treatments. Thus, the present findings indicate that the water-based extract of Stevia leaf and Stevioside do not pose any significant female reproductive toxicity or complication in pregnancy. In other words, this study exonerates Stevia and the sweetener Stevioside obtained from it of any female reproductive toxic or teratogenic effect.

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References

- Aritajat S, Kaweewat K, Manoruang W (2001) Toxocological study of some antifertility plant extracts by micronucleus test. *J Sci Fac CMU* 28:113-118.
- Bodhanker SL, Garg SK, Mathur VS (1974) Antifertility screening of plants, Part IX. Effect of five indigenous plants on early pregnancy in female albino rats. *Indian J Med Res* 62: 831-837.
- Cramer B, Ikan R (1986) Sweet glycosides from the Stevia plant. *Chemistry in Britain* 22:915-916.
- Kinghorn AD (1987) Biologically active compounds from plants with reputed medical and sweetening properties. *J Nat Prod* 50:1009-1024.
- Melis MS (1999) Effect of chronic administration of *Stevia rebaudiana* on fertility in rats. *J Ethnopharmacol* 67:157-161.
- OECD (1983) Test Guideline 415. One-generation reproduction toxicity study. In: *OECD Guideline for Testing of Chemicals*. Organization of Economic Co-operation and Development, Paris, France [Online].
(<http://browse.oecdbookshop.org/oecd/pdfs/browseit/9741501E.PDF>)
- Oliveira-Filho RM, Uehara OA, Minetti CA, *et al* (1989) Chronic administration of aqueous extract of *Stevia rebaudiana* Bertoni in rats: endocrine effects. *Gen Pharmacol* 20:187-191.
- Planas G, Kuc J (1968) Contraceptive properties of *Stevia rebaudiana*. *Science* 162: 1007.
- Toskulkao C, Chaturst L, Temcharoen P, *et al* (1997) Acute toxicity of Stevioside, a natural sweetener and its metabolite, Steviol, in several animal species. *Drug Chem Toxicol* 20:31-44.
- Zimnan D (1970) DDT linked to genetic damage. *DDT Newsletter* 1:13.197