SAFETY EVALUATION OF AQUEOUS EXTRACTS FROM AEGLE MARMELOS AND STEVIA REBAUDIANA ON REPRODUCTION OF FEMALE RATS

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Abstract. The purpose of this study was to evaluate the safety of a Thai medicinal plant, Aegle marmelos, and a non-caloric sweetener, Stevia rebaudiana, on the reproduction of female rats. Female rats were treated orally with aqueous extract of A. marmelos (6%) and S. rebaudiana at various concentrations (0, 0.2, 1, or 10%) for 60 days (1 ml/day) before mating. The control rats received only distilled water. At the end of the treatment period, treated females were mated with untreated males and the effects on reproduction were examined at day 14 of pregnancy. No notable abnormalities were observed in any of the pregnant rats. The number of corpus lutea, implanted and dead fetuses, as well as the sizes of the fetuses in the treated rats were not significantly different from those of the controls. Based on these results, it may be concluded that aqueous extracts of A. marmelos and S. rebaudiana at the concentrations used in this study do not alter the reproduction of female rats.

INTRODUCTION

Stevia rebaudiana, so called “stevia”, is a shrub that grows worldwide, and is consumed in many countries as a non-caloric sweetener. The main leaf components are steviolosides, which are 300 times sweeter than sugar (Kirkland, 1999). Due to the sweetness and lack of calories, they are of great interest for food and pharmaceutical purposes. Today stevia is used worldwide, especially in diabetic patients and people with obesity problems. Nevertheless, the use of stevia in Thailand has been controversial. Although safety evaluations of steviolosides have been reported in a large body of literature (Oliveira-Filho et al, 1989; Toskulkao, 1997; Toyoda, 1997; Melis, 1999; Aritajat et al, 2001), research concerning the effects of stevia on female reproduction and teratogenic effect have been inadequate. Since stevia leaves are generally used in brewing herbal tea, the safety of stevia for female rat reproduction was evaluated in this study by mixing an aqueous extract of this plant with an extract from Aegle marmelos, a Thai medicinal plant popularly consumed as a herbal tea.

MATERIALS AND METHODS

Plant extraction

Fruits of Aegle marmelos and leaves of S. rebaudiana were sliced, dried at 50-60 °C and ground into a fine powder. The aqueous extracts of both plants were prepared by Soxhlet extraction and evaporated by rotary evaporator. The crude extract of each plant was dissolved in distilled water to the required concentrations.

Animal preparation

Male and female Wistar rats (Rattus norvegicus), approximately 8 weeks of age and weighing 200-250 g, were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. They were allowed to acclimate in the departmental animal facility for at least one week before the experiment. They had access to water and a standard pellet diet (C.P. No. 082). The study room was maintained at approximately 25 ± 2 °C. The photoperiod was 12-hours light and dark.

Experiment design

Female rats were randomized into 5 groups (8 each) and treated orally for 8 weeks with 1) distilled water 1 ml/day (control) , 2) A. marmelos extract (0.5 g/kg) and 3-5) mixture of extract from A. marmelos and S. rebaudiana at concentrations of 0.2, 1.0, and 10.0%, respectively. The rats’ body weights were recorded weekly. During gestation, females were observed closely for signs of abortion or mortality. At the end of the treatment period, treated females were caged with untreated males for overnight mating (1male : 2 females). The presence of copulation plug or sperm in the vaginal smears on the following morning was regarded as pregnancy day 1. All pregnant females were

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isolated and sacrificed at day 14 of pregnancy. During autopsy, the numbers of corpus lutea were recorded. Viable and dead fetuses, the number of implantations, and the sizes of the fetuses along the uterine horns were recorded. Live fetuses were removed from the uterus and examined for gross abnormalities. Half of the fetuses were preserved in Bouin’s solution and examined for abnormality using a routine histology technique (Brancroft and Cook, 1994).

Statistics
Means and standard deviations were calculated. Significant differences were analyzed by Student’s t-test.

RESULTS
There were no significant differences between treated and control for percentage of females mated and pregnant. Examination at day 14 of pregnancy revealed no differences between treated and control rats in numbers of corpus lutea, total implantation, or viable and dead fetuses (Fig 1).

No malformations or developmental variations indicative of a treatment effect were observed. The fetus sizes in all groups were equivalent (Fig 2). No tissue or skeletal abnormalities were related to A. marmelos and S. rebaudiana treatment.

Neither mortality nor abortion occurred among the treated females. The body weights of the treated rats were also not significantly different from the controls (Fig 3).
DISCUSSION

Much of the controversy surrounding the existing literature on stevia results from inadequate research aimed at reproduction of both laboratory animals and human beings. The results of the present study clearly indicate the non-toxic effect of *S. rebaudiana* on the reproduction of female rats.

After mating with untreated males, all treated females fell pregnant and produced a similar number of fetuses. This result indicated that the extracts of *A. marmelos* and *S. rebaudiana*, at the doses used in this study, did not impair reproduction in female rats. Also, the indifferent number of corpus lutea in treated and control groups could be used as an index for the female rat reproductive system. Dead fetuses were observed in all groups, even the control group. Nevertheless, < 4% of fetuses died, which could be accounted for by the normal percentage pre-birth death among rats (Zimnan, 1970). Generally, the fetuses implanted in the endometrium after 5 days of fertilization (Bodhanker *et al*, 1974). The fetuses sizes in the treated groups, which were not different from the controls, indicated a similar implantation period. Furthermore, the normal development of the fetuses, which was confirmed by histological investigations, revealed no teratogenic effects from the extracts. Toxicological information about *A. marmelos* and *S. rebaudiana* has been reported previously. The non-genotoxicity of *A. marmelos* has been proven in rats by micronucleus test (Aritajat *et al*, 2001). Dominant lethal testing also revealed that the aqueous extracts of these 2 plants had no toxic effects on male rats’ reproduction or progeny outcome (Aritajat *et al*, 2000). It is concluded that the aqueous extract of *A. marmelos* and *S. rebaudiana* at all doses used in this study had no adverse effects on female rat reproduction, and had no teratogenic effects.

REFERENCES


