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Research Paper

Subchronic oral toxicity of evodia fruit powder in rats

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ABSTRACT

Ethnopharmacological relevance: Evodia, a fruit from *Evodia rutaecarpa*, has been used in oriental medicine, and since its various pharmaceutical actions, including anti-cancer activity, have become known, evodia has been widely used as a dietary supplement. However, information regarding its toxicity is limited.

Materials and methods: Evodia fruit from *Evodia rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang (0, 25, 74, 222, 667, and 2000 mg/kg) was administered orally five times per week for 13 weeks. Clinical signs, body weight, food consumption, hematology, serum chemistry, urinalysis, vaginal cytology, sperm morphology, organ weight, and gross and histopathological findings were evaluated.

Results: Urinary ketone body excretion was detected in males at 667 and 2000 mg/kg and in females at 2000 mg/kg. An increase in absolute/relative liver weight was observed in both sexes at 2000 mg/kg. Although levels of serum alanine aminotransferase, glucose, total cholesterol, and triglycerides were significantly reduced in males and/or females at 200 and/or 667 and 2000 mg/kg, all values were within normal ranges and were considered non-adverse. In addition, no treatment-related differences in body weight, food consumption, hematology, vaginal cytology, sperm morphology, or gross and histopathological examination were detected.

Conclusions: The subchronic no-observable-adverse-effect level for evodia fruit powder following oral administration in rats is greater than 2000 mg/kg.

1. Introduction

Evodia, named “Wu-Chu-Yu” in China, is an immature fruit from *Evodia rutaecarpa*. Evodia has been used in oriental medicine to relieve headaches, stomachaches induced by gastrointestinal disorders, and postpartum bleeding (Sheu, 1999). Currently, evodia and its alkaloids has been shown to improves testosterone secretion (Lin et al., 1999) and exhibits anti-inflammatory (Chiou et al., 1997), anti-dementia (Park et al., 1996), anti-obesity (Kobayashi et al., 2001), and thermoregulatory (Tsai et al., 1995) effects. Many recent studies have focused on the pharmaceutical potential of the alkaloids in evodia, such as the major alkaloid evodiamine, which inhibits the growth of human colon carcinoma cells (Ogasawara et al., 2001) and hepatoblastoma cell lines (Xu et al., 2006). These studies demonstrated that the anti-tumor actions of evodiamine are associated with the inhibition of proliferation, invasion, and metastasis, as well as with the induction of apoptosis. Rutaecarpin, another alkaloid in evodia, also exhibits prospective pharmaceutical activity, such as vasodilation for treatment in cardiovascular disorders (Sheu, 1999). A recent study investigating the effects of the total alkaloids of evodia found that their antioxidant activity is related to the inhibition of 2,2′-diphenyl-1-picrylhydrazyl free radical formation and lipid peroxidation (Tan et al., 2012).

Dietary supplements containing evodia are becoming more popular as news of their pharmaceutical potential spreads (Haller

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et al., 2008). In addition, there is growing interest in the possible medical uses of the alkaloids in evodia. However, very little is known about the safety of evodia. The results from one previous study suggested that evodia might terminate pregnancy in female mice (Kong et al., 1986). Another study conducted in humans reported palpitations, trembling, dizziness, nervousness, and gastrointestinal symptoms after administration of an evodia extract (Kim et al., 2008). The results from several studies indicate that evodia affects drug metabolism or detoxication of environmental pollutants through the inhibition of cytochrome P450 in liver, and evodia can intensify these effects or side effects (Engels et al., 2004; Iwata et al., 2005; Jan et al., 2005). With the exception of acute oral toxicity studies in mice and *Drosophila melanogaster*, and repeated oral toxicity study in mice for 30 days (Miyazawa et al., 2002; Yang et al., 2006; Yang, 2008; Zhou et al., 2011), no toxicological reports on evodia have been published. Therefore, the aim of this study was to evaluate the safety of the powder of evodia in rats by examining subchronic toxicity following a 13-week repeated oral dosing schedule.

2. Materials and methods

2.1. Test substance

Evodia powder from *Evodia rutaecarpa* (Juss.) Benth. var. *officinalis* (Dode) Huang was provided by the National Institute of Toxicological Research (NITR), Korea Food and Drug Administration. The plant material was identified by Professor Kihwan Bae, Chungnam National University, Korea and voucher specimens was deposited at the Herbarium of College of Pharmacy, Catholic University of Daegu. The evodia fruits were cleaned and extracted with water (80 kg evodia fruits add 800 kg of distilled water) at temperatures of 100 °C for 4 h. After cooling the extract was filtered twice and was condensed into 30 L at 40–50 °C. The extract was then lyophilized at −70 °C, at pressure of 0.06 mbar for 72 h. The whole lyophilized powder provided for the study was produced as the result of single operation procedure.

Identity and purity analyses were conducted by HPLC followed by electrochemical detection using an Alliance 2695 (Waters, Milford, MA) located in the Department of Herbal Medicine, Catholic University of Daegu (Li et al., 2012). Evodia powder was stored at 3.3–7.1 °C. Evodiamine (C19H17N8O, 99% purity) was used as the standard reference (Sigma Aldrich Co., Ltd., St. Louis, MO). The peak retention time for the test compound (20.905 min) was identical to that for the standard reference (20.904 min) (Fig. 1). Compound specificity was 91.20% and 90.98% at 1 and 400 mg/ml, respectively.

2.2. Experimental animals

Specific pathogen-free F344 rats (age 6 weeks) of both sexes were purchased from Japan SLC, Inc. (Shizuoka, Japan), and acclimated for 2 weeks prior to drug administration. The animals were housed in a good laboratory practice (GLP) facility with controlled temperature (22 ± 1.5 °C), humidity (52 ± 12.5%), ventilation (10–15 times/h), light (12:12 h, light:dark cycle), and illumination (150–300 lx). Food (certified rodent diet 5002; Labdiet, USA) and sterilized tap water were provided ad libitum. The animals were randomly allocated to one control group and five treatment groups, each consisting of 10 males and 10 females. The ethical committee at Biotoxtech Co., Ltd. approved our use of the animals and our study design (Approval no. 09102).

2.3. Administration

All procedures were carried out in accordance with the National Toxicology Program (NTP)'s guidelines for the "Descriptions of NTP Study Types, Toxicology/Carcinogenicity, 13-Week Toxicity Study" (as updated on January 18, 2006) and the study was conducted in compliance with the Good Laboratory Practice Regulations (No. 2005-79) issued by the Korea Food and Drug Administration, and the Principles of Good Laboratory Practice issued by the Organization for Economic Cooperation and Development.

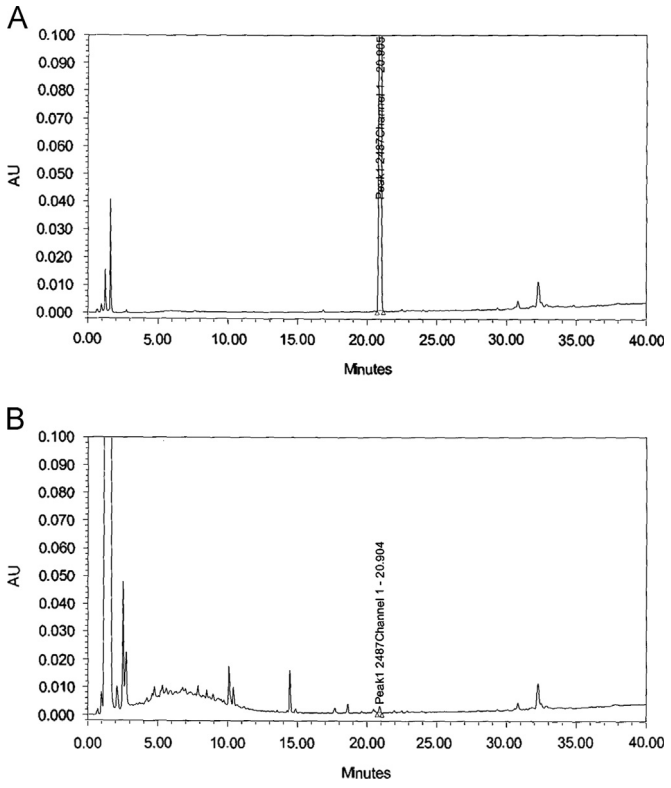

Fig. 1. HPLC of standard reference and test substance. Chromatograms of standard reference and test substance. (A) Evodiamine (retention time: 20.905 min) and (B) test substance (retention time: 20.904 min).

Evodia powder was obtained from the National Institute of Food and Drug Safety Evaluation (Osong, Korea). The test compound was suspended in water for injection (WFI: Choonwae Pharma Corp., Seoul, Korea) and was prepared once a week in accordance with the results from stability analyses assessed previously. The test substance was suspended in 5 ml of WFI and one dose (25, 74, 222, 667, or 2000 mg/kg body weight) per group per day was orally administered by gavage 5 days per week for 13 weeks; WFI (5 ml/kg/day) was administered to the controls. The treatment dose was determined by the previous 2-week repeated oral toxicity study. There were no adverse effects in 2000 mg/kg/day during the 2-week treated period (data not shown).

2.4. Body weight, food consumption, and toxicological signs

The animals were observed throughout the course of the study. On each day of dosing, animals were observed once before compound administration and once 6 h after administration. Body weight and food consumption were measured on day 0 and then weekly after treatments began.

2.5. Urinalysis

At the end of treatment, urinalyses with 3-h urine specimens were performed using either an autoanalyzer (Miditron Junior II; Roche Diagnostics, Mannheim, Germany, and 7080; Hitachi, Japan) or test kit (Combur 10 Test M stick; Roche Diagnostics, Mannheim, Germany). The 3-h urine specimens were analyzed for pH, protein,