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Toxicity assessment of Leonuri Herba aqueous extract orally administered to rats for 13 consecutive weeks

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ABSTRACT

Ethnopharmacological relevance: The Leonuri Herba has been traditionally used for women's disease in Asian countries.
Aim of the study: The objective of the present study was to evaluate the subchronic toxicity of Leonuri Herba aqueous extract in male and female F344 rats.
Material and methods: Leonuri Herba aqueous extract was administered orally once daily at dose levels of 0, 125, 250, 500, 1000 and 2000 mg/kg/day for 13 weeks. Toxicological assessment was performed including mortality, clinical signs, body and organ weights, food consumption, ophthalmology, urinalysis, hematology, serum chemistry, gross findings and histopathologic examination.
Results: There were no treatment related differences in clinical signs, urinalysis, hematology and serum chemistry, except for a histopathologic examination. The squamous cell hyperplasia in the forestomach was observed in both sexes of rats given 2000 mg/kg/day of Leonuri Herba aqueous extract.
Conclusion: In conclusion, the NOAEL (No Observed Adverse Effect Level) for Leonuri Herba aqueous extract was determined as 1000 mg/kg/day in both sexes of rats under the present experimental conditions. And the acceptable daily intake value for Leonuri Herba aqueous extract was calculated to be 10 mg/kg body weight per day using a safety factor of 100 to the NOAEL.

1. Introduction

Herbal medicine has been used in Asian countries as a traditional remedy for a variety of diseases. The use of herbal medicine as an alternative medicine has recently become widespread in western countries. Herbs used traditionally throughout history are considered safe for humans (Jordan et al., 2010). While herbal medicines are considered low risk in comparison with synthetic drugs, they are not completely free of toxic or other adverse effects (Smet, 2004). Recently, the safety of herbal products has been called into a question.

Leonuri Herba (*Leonurus japonicus*, *Leonurus. sibiricus*, *Leonurus heterophyllus or Leonurus mansharicus)*, also known as Motherwort, is widely distributed in Asian countries (Hong et al., 2001), where it is traditionally used to treat women's diseases, such as abnormal uterine bleeding after medical abortion, postpartum hemorrhage, dysmenorrheal, menorrhagia, menostasia and other irregular menstruation issues (Lin et al., 2007 and Li et al., 2011). Recently, antioxidant, as well as cardiac protective effects of Leonuri Herba have been reported (Sun et al., 2005, Liu et al., 2010 and 2012). However, no information exists regarding the safety of Leonuri Herba.

Adverse effects associated with herbal medicines typically result from long-term use (Ergil et al., 2002). Therefore, the objective of the present study was to evaluate the toxicity of Leonuri Herba aqueous extract (LHAE) orally administered to male and female F344 rats for 13 weeks. The present study was performed in compliance with the Good Laboratory Practice (GLP) of the Organization for Economic Cooperation and Development (OECD, 1997) and the Korea Food and Drug Administration (KFDA, 2009).

2. Materials and method

2.1. Preparation of LHAE and HPLC analysis

Freeze-dried LHAE powder was extracted from *Leonurus japonicus* Houtt collected in Korea and authenticated by Professor

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Byung-Sun Min of the College of Pharmacy at Catholic University of Daegu in the Republic of Korea. Botanical identification and high performance liquid chromatography (HPLC) analysis was performed and the voucher specimen CUD-2604-1 was deposited at the Herbarium of the College of Pharmacy at Catholic University of Daegu in Korea.

Leonuri Herba was chosen and tested by Prof. Byung-Sun Min and found to be safe with respect to pesticide contamination by assessing total dichlorodiphenyltrichloroethane, total hexachlorocyclohexane, aldrin, endrin and dieldrin, heavy metal (i.e., As, Cd, Hg and Pb) and aflatoxin B1 content. Total dichlorodiphenyltrichloroethane and hexachlorocyclohexane concentrations were less than 0.1 and 0.2 mg/kg, respectively. Aldrin, endrin, dieldrin and aflatoxin B1 concentrations were lower than 0.01 mg/kg. As, Cd, Hg and PB levels were found to be lower than 3.0, 1.0, 0.2 and 5.0 mg/kg, respectively.

Leonuri Herba was extracted according to the standard hot water extraction method of the Korea Pharmacopoeia and freeze-dried. Active components were measured using an established HPLC method. The concentration of rutin, the marker compound of Leonuri Herba, was 2.12 ± 0.07 mg/g (Bae et al., 2012). LHAE was stable at room temperature and 5°C for 6 months (Bae et al., 2012). The appropriate amount of freeze-dried LHAE powder was measured and suspended in distilled water for the highest dose group and this suspension was further diluted to prepare lower dose suspension.

2.2. Animals and maintenance

Seventy male and female specific pathogen-free F344 rats were obtained from Orient Bio Co. (Seongnam-si, Republic of Korea) at 6 weeks of age. The animals were acclimated for 9 days and healthy animals were selected for the study. Sixty male and 60 female rats were randomly assigned to six groups (one control group and five treatment groups) using the Path/Tox system (Version 4.2.2, Xybion Medical Systems Corporation, USA). Each group consisted of 10 rats of each sex. The body weight range prior to the start of dosing ranged from 131.4 to 179.7 g for males and 104.6 to 124.8 g for females.

The animals were housed in polycarbonate cages with bedding (Laboratory animal Aspen bedding, ABEDD BALTIC LTD., Latvia) throughout the study period. Sterilized tap water and pellet food (PMI nutrition International, USA) was given to the animals ad libitum. The animal room was maintained at a temperature of 22 ± 3 °C, a relative humidity of ~30–70%, air ventilation of 10–20 times/h and light intensity of 150–300 l*x* with 12-h light-dark cycles. This study was approved by the Institutional Animal Care and Use Committee of the Korea Institute of Toxicology and performed in compliance with Testing Guidelines for Safety Evaluation of Drugs (Notification No. 2009-116 issued by the Korea Food and Drug Administration on August 24, 2009).

2.3. Treatment and toxicity assessment

Oral administration was chosen for this study because it is the intended clinical route of LHAE administration in humans and has been used in the previous non-clinical studies. The dosing volume of 10 mL/kg was calculated using the Path/Tox system and based on most recent body weight. A previous 2-week study by the Korea Institute of Toxicology indicated LHAE doses of 0 (vehicle), 125, 250, 500, 1000 and 2000 mg/kg/day were well tolerated (results not published). The same doses were selected for this 13-week repeated dose study. All measurement and examination records were collected using the Path/Tox system. The condition and behavior of all animals was checked once daily throughout the acclimation period. All animals were examined and clinical signs were recorded twice daily before and after dosing during the treatment period and once on the day of necropsy.

Animals were weighed prior to randomization on the day of arrival, before dosing on the first day of treatment and once weekly thereafter. A final weighing was performed on the day of necropsy. Cage food consumption was recorded once during the acclimation period and once weekly during the treatment period. Individual food consumption was calculated as g/rat/day. External eye examinations were performed on all animals during the acclimation period, while both external and fundus examination of animals in the vehicle control and highest dose (2000 mg/kg/day) groups were performed using a binocular indirect ophthalmoscope (IO-H, Neitz Instrument Co., Japan) on week 13 before necropsy. Prior to examination via binocular indirect ophthalmoscopy, a mydriatic compound (Midrin-P, Santen Pharmaceutical, Japan) was used in each eye.

Urine samples were collected overnight for ~16 h from animals housed in metabolism cages in the last week of treatment. Each animal was housed in an individual metabolism cage; food was withdrawn overnight for urine collection, but water was available. Urinalysis was performed using a urine automatic analyzer (Clini-Tek-500, Bayer, USA) and urine stick (Multistix 10 SG, Siemens, Germany) to evaluate the following parameters: urine volume, color, specific gravity, pH, and protein, ketone body, occult blood, glucose, bilirubin, nitrite and urobilinogen content. Microscopic examination of urine cast, epithelial cell, red blood cell and white blood cell content was also performed.

All animals were fasted overnight prior to necropsy and blood sampling. Blood samples were collected for hematology, coagulation and serum chemistry from the vena cava of all animals under isoflurane anesthesia at necropsy. Blood samples were collected

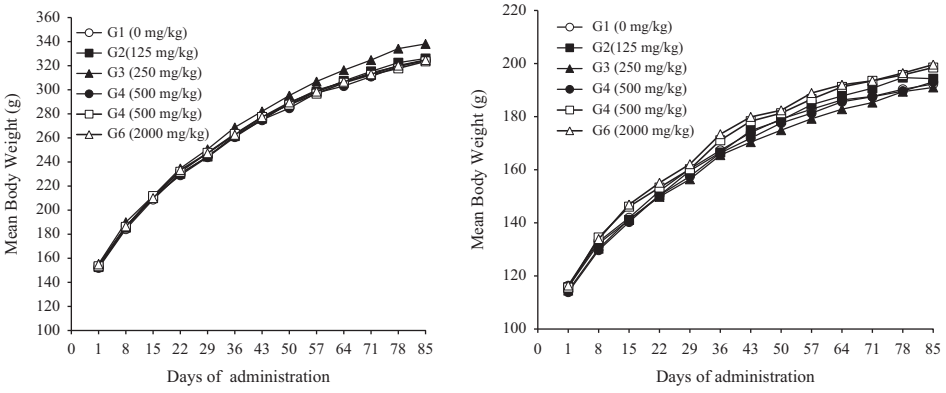

Fig. 1. An increase in body weight was observed in males (left) and females (right) treated with LHAE for 13 weeks.