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Safety evaluation of *Angelica gigas*: Genotoxicity and 13-weeks oral subchronic toxicity in rats

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ABSTRACT

As a well-known traditional medicine, *Angelica gigas* (AG) and its active constituents, including decursin and decursinol, have been shown to possess several health beneficial properties such as anti-bacterial, immunostimulating, anti-tumor, neuroprotective, anti-nociceptive and anti-amnestic activities. However, there is lack of toxicity studies to assess potential toxicological concerns, especially long-term toxicity and genotoxicity, regarding the AG extract. Therefore, the safety of AG extract was assessed in subchronic toxicity and genotoxicity assays in accordance with the test guidelines published by the Organization for Economic Cooperation and Development. In a subchronic toxicity study for 13 weeks (125, 250, 500, 1000 and 2000 mg/kg body weight, delivered by gavage), data revealed no significant adverse effects of the AG extract in food consumption, body weight, mortality, hematology, biochemistry, necropsy, organ weight and histopathology throughout the study in male and female rats. These results suggest that no observed adverse effect level of the AG extract administered orally was determined to be greater than 2000 mg/kg/day, the highest dose tested. In addition, a battery of tests including Ames test, *in vitro* chromosome aberration assay and *in vivo* micronucleus assay suggested that the AG extract was not genotoxic. In conclusion, the AG extract appears to be safe as a traditional medicine for oral consumption.

1. Introduction

Angelica gigas (AG) belongs to the Umbelliferae family. Based on its area of distribution, three common species of *Angelica* roots can be found in Asia: *A. gigas* from Korea, *A. sinensis* from China, and *A. acutiloba* from Japan (Lv et al., 2007). In particular, AG, known by the Korean name 'Cham-dang-gui', grows naturally and is cultivated in the alpine region of Korea (Hwang and Yang, 1997). The dried roots of AG have long been used as a traditional folk medicine for various pharmacologic effects including anti-bacterial (Lee et al., 2003b), immunostimulating (Han et al., 1998), anti-tumor (Lee et al., 2003a), neuroprotective (Kang et al., 2005), anti-nociceptive (Choi et al., 2003a) and anti-amnestic (Kang et al., 2003) activities. AG is also known for the improvement of hypercholesterolemia and prevention of atherosclerosis (Jang et al., 2014). In addition, AG exerted anti-inflammatory activities against carrageenan-induced inflammation (Shin et al., 2009), croton oil-induced ear inflammation (Shin et al., 2010), dinitrofluorobenzene-induced allergic dermatitis models (Joo et al., 2010), and thermal burn (Shin et al., 2008).

Abbreviations: AG, *Angelica gigas*; OECD, Organization for Economic Co-operation and Development; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; TC, total cholesterol; TP, total protein; TB, total bilirubin; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; γGT, γ-glutamyl transferase; TG, triglyceride; CHL, Chinese hamster lung; MNPCEs, micronucleated polychromatic erythrocytes; NCEs, normochromatic erythrocytes; HPLC, high-performance liquid chromatography.

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As there is considerable concern about the adverse effects of synthetic chemical drugs, there is increasing interest in natural products since traditional medicines are widely perceived as natural, safe, and free from side effects (Markman, 2002; Shin et al., 2013). In particular, traditional medicines can be less toxic when prescribed strictly with attention to plant origin, method of preparation, dose, and treatment duration (Liu et al., 2011). In other words, the popularity of traditional medicines demands a comprehensive analysis of safety issues. In fact, we recently found that various beneficial herbs have toxic side effects (Che et al., 2014, 2015; Yun et al., 2015). Although AG is currently available on the market as an herbal medicine in East Asian countries, there is lack of toxicity studies that have been carried out for the toxicity of the AG extract, and their safety is not guaranteed. In this study, we report the results from subchronic toxicity studies of orally administered AG extract and from genotoxicity studies including a bacterial reverse mutation assay (Ames test), *in vitro* chromosome aberration assay and a micronucleus assay in mice.

2. Materials and methods

2.1. Test substance and animals

A hot water AG extract was provided by the National Institute of Food and Drug Safety Evaluation (Osong, Korea). AG roots were purchased from an Oriental medicine market in Korea, and an extract of AG was obtained according to a method described previously (Yun et al., 2015). In brief, dried AG roots were ground by a mixer, and incubated with distilled water (DW) at 110 °C. After filtration through filter paper, the filtrate was freeze-dried and dissolved in DW for oral administration. The extraction yield of the hot water AG extract was 0.216 g of freeze-dried AG extract/g of dried AG root. Analysis of decursin and decursinol obtained from the AG extract was performed using the high performance liquid chromatography (HPLC) equipment Shimadzu SCL-10A_{VP} (Kyoto, Japan). The analysis was carried out using a YMC-Pack ODS-A column (150 × 6 mm, 5 μm particle size) and methanol-based mobile phase composed of methanol and 1% acetic acid water with gradient elution as follows: 0–10 min, methanol 55%; 10–11 min, methanol 55–80%; 11–25 min, methanol 80%; 25–26 min, methanol 80–100%; 26–40 min, methanol 100%. The detection was carried out using a diode array detector.

F344 rats (SLC, Hamamatsu, Japan) and ICR mice (Orient Bio, Seongnam, Korea) were used after a week of quarantine and acclimatization. During the studies, the animal facility was maintained under standard conditions (22 ± 2 °C, 40–60% humidity, and 12 h light/dark cycle). The animals were fed a rodent diet (LabDiet 5002 Certified Rodent Diet, PMI Nutrition International, St. Louis, MO, USA) and tap water *ad libitum*. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of the Biomedical Research Institute at the Seoul National University Hospital, and this study was performed in compliance with the guidelines published by the Organization for Economic Cooperation and Development (OECD) as well as the guidance for Good Laboratory Practices for toxicity tests issued by the Ministry of Food and Drug Safety (MFDS, 2005).

2.2. Experimental design for the oral toxicity study

For the 14-day repeat-dose toxicity study, the hot water AG extract was administered to F344 rats (5/sex/group) by oral gavage at doses of 125, 250, 500, 1000, and 2000 mg/kg of body weight/10 ml DW once daily for 14 days. For the 13-week repeat-dose toxicity study, the hot water AG extract was administered to F344 rats (10/sex/group) by oral gavage at doses of 125, 250, 500, 1000, and 2000 mg/kg of body weight/10 ml DW once daily for 13 weeks in accordance with OECD guideline 408 (OECD, 1998) and the US National Toxicology Program (NTP) protocol (https://ntp.niehs.nih.gov/testing/types/cartox/protocols/13week/index.html). During the administration period, the rats were observed for general appearance daily, and body weights, food intake, and water consumption were recorded weekly. The rats were anesthetized with isoflurane one day after the final gavage.

2.3. Hematology and serum biochemistry

Blood samples were collected *via* the posterior vena cava. The hematology parameters were measured using an automatic hematology analyzer MS9-5 Hematology Counter (Melet Schloesing Laboratories, Osny, France) for the following parameters: total white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential WBC. And, the standard serum biochemistry parameters were analyzed with an automatic chemistry analyzer 7070 (Hitachi, Tokyo, Japan) to evaluate the following serum biochemistry parameters: blood urea nitrogen (BUN), total cholesterol (TC), total protein (TP), albumin, total bilirubin (TB), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γGT), creatinine, creatinine kinase, triglyceride (TG), and glucose.

2.4. Gross findings, organ weights, and histopathological assessments

At the end of the treatment period, animals were exsanguinated, and organs and tissues were observed macroscopically. Organ weights were obtained for the liver, kidney, testis, thymus, heart, and lung. The eyes with the Harderian glands were fixed in Davidson solution (30 ml 95% ethyl alcohol + 20 ml formalin + 10 ml glacial acetic acid + 30 ml DW). The testis and epididymis were fixed in Bouin's solution. Other organs including the liver, kidney, adrenal gland, urinary bladder, spleen, pancreas, thymus, thyroid gland, parathyroid gland, trachea, esophagus, lung, heart, salivary gland, lymph node, stomach, duodenum, jejunum, ileum, colon, rectum, preputial gland, clitoral gland, skin, brain, pituitary gland, bone marrow, prostate, seminal vesicle, ovary, uterus, and vagina were fixed in 10% neutral buffered formalin. The nasal cavity and femora were treated with a decalcification solution for up to 3 weeks. Tissue samples were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E). After staining, the histological preparations from animals in the control, 1000, and 2000 mg/kg groups were initially examined *via* light microscopy. With respect to the organs and tissues showing significant histological changes, preparations of all rats in the other groups were examined microscopically.

2.5. Genotoxicity study

Five characterized histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537; MFDS, Osong, Korea) were utilized for bacterial reverse mutation assay (Ames test) in accordance with OECD guideline 471 (OECD, 1997a). *S. typhimurium* strains were incubated with the AG extract with or without an S9 mix in the dark at 37 °C for 48 h. The standard mutagens (2-nitrofluorene, sodium azide, mitomycin C, 9-aminoacridine, and 2-aminoanthracene; Sigma–Aldrich, St. Louis, MO, USA) were used as positive controls. The extract was considered to be positive if there was a twofold increase relative to negative control or a dose-dependent increase in the number of revertant colonies.