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Research article

Subacute oral toxicity and bacterial mutagenicity study of Korean Red Ginseng oil

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ABSTRACT

Background: Red ginseng oil (RGO) is produced by supercritical CO_2 extraction of secondary products derived from Korean Red Ginseng extract. As the use of RGO has increased, product safety concerns have become more important.
Methods: In the present study, the subacute oral toxicity and bacterial reverse mutagenicity of RGO were evaluated. Sprague–Dawley rats were orally administered with RGO for 28 d by gavage. Daily RGO dose concentrations were 0 mg/kg body weight (bw), 500 mg/kg bw, 1,000 mg/kg bw, or 2,000 mg/kg bw per day. Bacterial reverse mutation tests included five bacterial strains (*Escherichia coli* WP2 and *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537), which were used in the presence or absence of metabolic activation. The plated incorporation method for mutation test was used with RGO concentrations ranging from 312.5 μg to 5,000 μg per plate.
Results: The subacute oral toxicity test results did not reveal any marked changes in clinical characteristics. There were no toxicological changes related to RGO administration in hematological and serum biochemical characteristics in either control or treatment animals. Furthermore, no gross or histopathological changes related to RGO treatment were observed. The bacterial reverse mutation test results did not reveal, at any RGO concentration level and in all bacterial strains, any increase in the number of revertant colonies in the RGO treatment group compared to that in the negative control group.
Conclusion: The no-observed-adverse-effect level of RGO is greater than 2,000 mg/kg bw/d and RGO did not induce genotoxicity related to bacterial reverse mutations.

1. Introduction

Safety concerns related to the use of herbal products have increased as the worldwide use of herbal ingredients in medicine and dietary supplements has increased markedly [1,2]. Herbal products contain various compounds; thus, it can be more difficult to predict their toxicity than it is to predict that of a single compound used in general medicine [3]. Therefore, an accumulation of safety-related information on herbal products is important to reduce the safety risks associated with side effects.

Ginseng (*Panax ginseng* Meyer) is a widely used traditional herbal product that has been used as a medicinal treatment in many countries for several thousands of years [4]. In particular, Korean Red Ginseng, produced by steaming fresh *P. ginseng* in water vapor, is used as a medicine, cosmetic, and nutritional supplement in Asian countries including Korea. It has been demonstrated that Korean Red Ginseng has various effects including neurological improvement [5], blood pressure regulation [6], anti-inflammatory [7], anticancer [8], and liver protection effects [9]. Most of this efficacy is related to water-soluble saponin components, which are abundant when red ginseng is taken in the form of a hot-water extract, as is traditionally the case. However, studies evaluating the efficacy of lipid-soluble components have increased because of the higher bioability characteristics of such components [10,11]. In particular, several studies have investigated the efficacy of red ginseng oil (RGO), a lipophilic nonsaponin component of red

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ginseng. RGO is produced by performing supercritical CO_2 extraction of secondary products generated by water-based extraction of red ginseng. RGO contains various fatty acids, phospholipids, and phytosterols. Among these, the RGO phytosterol component has been reported to have various beneficial effects including anti-inflammatory, antioxidant, and hepatoprotective effects [12,13]. Phytosterols obtained from other plants are also reported to have anticancer and anti-inflammatory effects, and they have been used in the manufacture of nutrient supplements and cosmetics [14,15]. Despite the professed usefulness of RGO in these industries, little has been reported on the health-related safety of RGO. To date, there is only one report describing the results of a single-dose, oral administration toxicity test in rats [16]. Thus, additional safety data, such as data related to repeated-dose oral administration and the mutagenicity of RGO, are needed to elucidate the toxicity potential of RGO. In this study, the 28-d repeated-dose oral administration toxicity and bacterial reverse mutagenicity of RGO were evaluated in order to clarify the health effects of RGO.

2. Materials and methods

2.1. Test substance

RGO was obtained from Korea Ginseng Corporation (Korea), and the composition is shown in Table 1. The vehicle, corn oil, was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Subacute oral toxicity study

Subacute oral toxicity tests were based on the Organization for Economic Co-operation and Development (OECD) Guideline 407 [17].

2.2.1. Test animals and environmental conditions

Five-wk-old male and female, specific pathogen-free Sprague–Dawley (SD) rats were purchased from ORIENT BIO Inc. (Korea) and acclimated for 7 d prior to starting the experiments. During the acclimation and experimental periods, the rats were housed in stainless mesh cages (1 rat per cage) in a room with controlled temperature (20.5–23.2°C) and humidity (36.2–56.3%), and a 12-h light/dark cycle. The rats were fed rodent chow (Harlan Teklad, USA) and filtered water *ad libitum*. This experiment was approved by the Institutional Animal Care and Use Committee of Biotoxtech (approval number, 100301). All procedures in this study have been performed in accordance with the provisions of Good Laboratory Practice.

Table 1
The fatty acids composition of red ginseng oil

Component	Proportion (%)
Linoleic acid	71.41
Palmitic acid	9.39
Linolenic acid	6.24
Oleic acid	5.02
cis-11,14-Eicosatyrienoic acid	1.34
Eucic acid	0.79
Stearic acid	0.76
cis-13,16-Docosadienoic acid	0.64
Pentadecanoic acid	0.6
Nervonic acid	0.52
Lignoceric acid	0.41
cis-11-Eicosenoic acid	0.4
Heptadecanoic acid	0.38
Palmitoleic acid	0.37
Arachidic acid	0.35
r-Linolenic acid	0.3
Arachidonic acid	0.23
Tricosanoic acid	0.23
Behenic acid	0.2
Myristic acid	0.17
Heneicosanoic acid	0.15
cis-10-Heptadecenoic acid	0.13
Total	100

2.2.2. Experimental group

At 6 wk, the rats were divided into four groups (5 rats in each group): vehicle control (corn oil), low-dose group (500 mg/kg/d), middle-dose group (1,000 mg/kg/d), and high-dose group (2,000 mg/kg/d). The rats were exposed to RGO following the toxicity test guidelines of the Korea Food and Drug Administration for Nonclinical Laboratory Studies applying Good Laboratory Practice. The dosing volume used was 5 mL/kg body weight (bw; oral administration). The maximum dose was determined according to the recommendations of the Korea Food and Drug Administration and OECD Guideline 423 [18].

2.2.3. Body weight changes

The body weight of each animal was measured at the initiation of administration, weekly thereafter, and on the day of scheduled sacrifice.

2.2.4. Food consumption

Food consumption was measured for 7 d in the 1st week and for 6 d in the 2nd week to the 4th weeks, then the daily average food intake was calculated.

2.2.5. Biochemistry and hematology

At the end of the 28-d experiment, the rats were 10 wk old. Prior to necropsy, food was withheld overnight. The rats were killed by exsanguinations following isoflurane inhalation after recording the terminal body weight. Blood samples were drawn from the descending aorta, collected in heparinized vacutainers, and analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, total protein, albumin (ALB), albumin/globulin ratio, total cholesterol, triglyceride, and glucose using a biochemical blood analyzer (Hitachi 7080; Hitachi, Japan). The blood was also analyzed for the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, and white blood cell count using a blood cell counter (ADVIA 120; Siemens, Erlangen, Germany).

2.2.6. Organ weights

After collecting the blood, brain, heart, liver, spleen, and kidneys were carefully removed and the absolute organ weights were recorded. Relative organ weights were calculated as the ratio between the absolute organ weight and the body weight of fasting.

2.2.7. Histopathology

The gross observation was recorded in all animals. The brain, heart, liver, spleen, kidneys, and lung were removed carefully and fixed in 10% neutral buffered formalin. Each organ was trimmed as described in previous guideline [19]. After paraffin infiltration by tissue processor, the organs were embedded in paraffin and cut into 4-μm sections. The sections were stained with hematoxylin and eosin, and examined under light microscopy.

2.3. Bacterial reverse mutation test

The bacterial reverse mutation test (also called Ames test) was carried out according to the OECD Guideline 471 for the testing of chemicals, “Bacterial reverse mutation test” [20]

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