Journal of Ethnopharmacology 152 (2014) 457–463

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Subchronic toxicity study of Coptidis Rhizoma in rats

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form
26 December 2013
Accepted 14 January 2014
Available online 30 January 2014

Keywords:
Coptidis Rhizoma
Fischer 344 rat
Subchronic toxicity
NOAEL
GLP

ABSTRACT

Ethnopharmacological relevance: Coptidis Rhizoma (CR) is a medical herb from the family *Ranunculacease* that has been used to treat gastroenteritis, dysentery, diabetes mellitus, and severe skin diseases.
Aim of the study: To evaluate the no-observed-adverse-effect level (NOAEL) and the toxicity of CR, following repeat oral administration to rats for 13 weeks.
Materials and methods: CR was administered by oral gavage to groups of rats ($n=10$/group, each sex) at dose levels of 0 (control), 25, 74, 222, 667 or 2000 mg/kg/day 5 times per week for 13 weeks. Mortality, clinical signs, body weights, food consumption, hematology, serum chemistry, urinalysis, vaginal cytology and sperm morphology, organ weights, gross and histopathological findings were compared between control and CR groups.
Results: Urinalysis showed a significant increase in *N*-acety1-β-glucosaminidase in males in the 2000 mg/kg/day group ($P<0.01$). However, no mortality or remarkable clinical signs were observed during this 13-week study. No adverse effects on body weight, food consumption, hematology, serum chemistry, organ weights, gross lesion, histopathology, vaginal cytology, sperm motility, or deformity were observed in the males or female rats treated with CR.
Conclusions: On the basis of these results, the NOAEL of CR is determined to be 667 mg/kg/day for males and 2000 mg/kg/day for females.

1. Introduction

CR is a medical herb from the family *Ranunculacease*, including *Coptis chinensis* Franch (Tang et al., 2009). It has been used to treat gastroenteritis, dysentery, diabetes mellitus, and severe skin diseases in traditional oriental medicine (Tang et al., 2009; Kim et al., 2010). It contains alkaloids including berberine, coptisine, palmatine, jatrorrhizine, epiberberine, worernine, columbamine and magnoflorine, berberine as its primary constituent with a range of bioactivities (Chen et al., 2008; Tang et al., 2009). The pharmacological effects of CR have been reported to include antibacterial, antiviral, antiinflammatory, antineoplastic, antihypertensive, antioxidative, antihyperglycemic and cholesterol-lowering activities (Fukutake et al., 1998; Li et al., 2000; Sanae et al., 2001; Yokozawa et al., 2003, 2004; Choi et al., 2007; Kim et al., 2008;).

Herbal medicines have been widely believed to be safe and harmless because they were originated from nature and were used traditionally. Some reports, however, have pointed out that herbal

Abbreviations: ANOVA, analysis of variance; CR, Coptidis Rhizoma; NOAEL, no-observed-adverse-effect level; NTP, National Toxicology Program; GLP, good laboratory practice; MFDS, Ministry of Food and Drug Safety; HPLC, high performance liquid chromatography; NAG, *N*-acetyl-β-glucosaminidase; CRE, creatinine; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; RBC, total erythrocyte counts; HGB, hemoglobin concentration; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; PLT, platelet count; RBC, total leukocyte counts; NEU, neutrophils; EOS, eosinophils; Baso, basophils; Lym, lymphocytes; MONO, monocytes; RET, reticulocytes; PT, prothrombin time; APTT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; SDH, sorbitol dehydrogenase; BUN, blood urea nitrogen; T-BIL, total bilirubin; TP, total protein; ALB, albumin; A/G, albumin/globulin; T-CHO, total cholesterol; TG, triglycerides; GLU, glucose; Ca, calcium; Cl, chloride; Na, sodium; K, potassium

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http://dx.doi.org/10.1016/j.jep.2014.01.011

products could produce adverse effects in humans. High doses of barberry (*Berberis vulgaris*) can cause nausea, vomiting, convulsions, a sudden decrease in blood pressure, and depression of heart rate and respiration (Arayne et al., 2007). Goldenseal (*Hydrastis canadensis*) can induce neonatal jaundice, at high doses, gastrointestinal upset, hypertension, seizures, respiratory failure, cardiac spasms, anorexia, dermatomyositis, elevated serum iron, psychosis, swollen liver, damaged stomach lining, and even death (Elvin-Lewis, 2001).

It has been reported that CR caused adverse effects such as nausea, vomiting, enterocinetic sound, abdominal distortion, diarrhea, polyuria, and erythropenia in adult (Bao, 1983) and haemolytic anemia and nuclear jaundice in newborn infants (Yang and Gao, 1996). More serious adverse effects have also been reported including respiratory failure, extrapyramidal system reactions, severe arrhythmia, liver function injury, and even death in China (Li et al., 2008). Recently, the currently recommended doses of CR in humans were shown to be relatively safe in rats treated orally with CR for 90 days (Yi et al., 2013). However, this study was fairly limited and did not determine no-observed-adverse-effect level (NOAEL). The present study was conducted to evaluate the NOAEL and the toxicity of 13-week repeated oral administration of CR to rats.

2. Materials and methods

2.1. Test material

Coptidis Rhizoma dry powder (*Coptis chinensis* Franch) was supplied from the Ministry of Food and Drug Safety (MFDS). The powder was extracted as previously described (Ma et al., 2010) and reconstituted in distilled water to the final concentration indicated for this study. Distilled water served as the vehicle control.

Determination of CR contents was performed using high performance liquid chromatography (HPLC) (Agilent 1100 Series: Agilent Technologies Ltd., USA), using an XDB-C18 column (4.6 mm × 15.0 mm; particle size 5 μm), with gradient elution in a mixture of hexanesulfonic acid-Na and acetonitrile at column temperature of 30 °C, flow rate 1.0 mL/min and UV detection (wave length: 254 nm). Berberine was used as the reference standard to identify and quantify the major component of CR. The quantified ingredient was detected at the same retention time (22.52 min) observed for the reference standard.

2.2. Animals and experimental design

Five-week-old specific pathogen free male and female Fisher 344 rats were purchased from Japan SLC Inc. (Shizuoka, Japan) and acclimated for 1 week. The rat body ranged from 123 to 147 g for males and 97 to 112 g for females. The rats were housed singly in polycabonate cages (260 mm × 420 mm × 180 mm) and supplied with commercial rodent diet (LabDiet®, USA) and filtered tap water ad libitum. Environmental conditions were controlled to provide a temperature of 19–25 °C, relative humidity of 30–70%, a 12:12 h light:dark cycle, and fresh air ventilation (10–15 times per hour). The study was conducted at Biotoxtech Co., Ltd, Korea following Good Laboratory Practice (GLP) guideline inspected by MFDS. All procedures were performed in accordance with "Descriptions of NTP Study Types, Toxicology/Carcinogenicity, 13-Week Toxicity Study" (The National Toxicology Program (NTP), the U.S Department of Health and Human Services, Web page last updated on January 18, 2006), and also approved by the animal experiment committee of Biotoxtech Co., Ltd, Korea on the basis on the Animal Protection Act (Approval No. 09100).

The rats were assigned to 6 group (10 per sex per group), treated as follows: group 1 (vehicle, 5 mL distilled water/kg/day), group 2 (25 mg CR/5 mL/kg/day), group 3 (74 mg CR/5 mL/kg/day), group 4 (222 mg CR/5 mL/kg/day), group 5 (667 mg CR/5 mL/kg/day), and group 6 (2000 mg CR/5 mL/kg/day). The highest dose was selected as 2000 mg/kg/day on the basis of a 2-weeks repeat dose toxicity study performed previously (data not shown) where no adverse effects were observed. Animals were orally dosed 5 times per week for 13 consecutive weeks using a sonde attached to a disposable syringe. They were then sacrificed under isoflurane anesthesia after overnight fasting. Blood was collected for hematological and serum chemical analysis.

2.3. Animal observation

All animals were weighed individually just prior to dosing on day 1 and once a week thereafter. Body weight data from the day of necropsy was not included in the evaluation of body weights because these data reflected the fasted body weight of the animals. All animals were observed daily at 6-h intervals for mortality, general condition and clinical signs before the test and throughout dosing period. Clinical observation including motor activity, appearance and central and autonomic functions were performed once a week. Daily food consumption was measured before the initiation of dosing and once a week thereafter. Food consumption was calculated by subtracting leftover feed from the total feed provided.

2.4. Urinalysis

Urinalysis was performed for 5 rats in each group on week 13 of administration. Fresh urine (sampled within approximately 3 h of urine excretion) was collected to determine pH, protein, glucose, ketone body, bilirubin and occult blood using a urine analyzer (Miditron® Junior II; Roche Diagnostics, Germany) or test kits (Combur 10 Test®M stick; Roche Diagnostics, Germany). It was also visually observed for color and turbidity and the sediment was examined microscopically (Olympus CX31, Olympus Optical Co., Japan). In addition, 24-h urine (after excretion) samples were examined for urine volume, specific gravity, *N*-acetyl-β-glucosaminidase (NAG), creatinine (CRE), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) using a measuring cylinder, a specific gravimeter (Vet360;Reichert, USA.) or an automatic analyzer (7080; Hitachi, Japan).

2.5. Hematology and serum chemistry

All animals were fasted for at least 18 h before necropsy, when blood samples were collected from the abdominal aorta. Whole blood, collected in EDTA vacutainers, was analyzed for total erythrocyte counts (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count (PLT), total leukocyte counts (WBC), neutrophils (NEU, %), eosinophils (EOS, %), basophils (BASO, %), lymphocytes (LYM, %), monocytes (MONO, %) and reticulocytes (RET, %) using an automatic blood cell counter (Advia 120; Siemens, Germany). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using an automated coagulation analyzer (ACL 7000; Instrumentation Laboratory, USA) with plasma obtained after blood was mixed with 3.2% sodium citrate. Serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), blood urea nitrogen (BUN), creatinine (CRE), total bilirubin (T-BIL), total protein (TP), albumin (ALB), A/G (albumin/