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Research paper

Acute and subchronic toxicity as well as evaluation of safety pharmacology of *Galla chinensis* solution

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ABSTRACT

Galla chinensis has been popularly used in traditional Chinese medicine which is beneficial for the treatment of various diseases, such as inflammation, dysentery, toxicosis and sore. However, it has not previously been evaluated for safety through systematic toxicological studies. In the present study, acute and subchronic oral toxicity studies and safety pharmacology evaluation of *Galla chinensis* solution (GCS) were conducted in specific pathogen-free (SPF) Sprague–Dawley (SD) rats. Acute administration of GCS was done as single dose from 3333 mg to 6912 mg per kg/bodyweight (bw) and subchronic toxicity study for 30 days was done by daily oral administration of GCS at doses of 500, 1500 and 2500 mg/kg body weight in SPF SD rats. The acute toxicity study showed the LD_{50} of GCS was greater than 5000 mg/kg. The results of sunchronic toxicity study showed that the no-observed effect level of GCS was lesser than 1500 mg/kg bw day, which suggested three times higher than that of recommended dose for clinical applications (500 mg/kg bw day). The dose at 2500 mg/kg bw day of GCS may slow down the growth of rats and lead to degeneration and necrosis of tissue cells to some extent. In the safety pharmacology study, GCS did not produce any side effects to rats in central nervous system, cardiovascular system and respiratory system. Therefore, from the results of the study presented herein, it could be concluded that the use of appropriate levels (one to three times of recommended dose for clinical applications) of GCS as a topical preparations is considered safe.

1. Introduction

Traditional Chinese medicine (TCM) has been widely used throughout the world as a primary treatment strategy and alternative medicine (Kim et al., 2013). According to the reports of World Health Organization, about 80% of the world population, especially in developing countries, is dependent on natural medicines for their health care (World Health Organization, 2008). This increasing popularity is not only due to poverty and lack of access to the chemical drugs, but also the herbs are considered safe for humans by thousands of years' use (Jordan et al., 2010). However, the quality, safety and efficacy of many traditionally used herbal formulae are unknown (Firenzuoli and Gori, 2007; Wang et al., 2009). Moreover, there is a lack of data for many plants to guarantee their quality and safety.

Galla chinensis, found in many parts of China and mainly in Sichuan Province, is formed when the Chinese sumac aphid Baker (mainly *Melaphis chinensis bell*) parasitizes the plants of family *Anacardiaceae* (mainly *Rhus chinensis Mill*, *Rhus potaninii Maxim*, and *Rhus punjabensis var. sinica* (*Diels*) *Rehd. et Wils*) (Tian et al., 2009). *Galla chinensis* is composed of a large amount of gallotannin, a typical kind of hydrolysable tannin, whose content can exceed 70% of its weight (Sun, 1992). *Galla chinensis* is usually harvested in autumn and then dried after removal of the larvae to given the traditional Chinese medicine (Zhang et al., 2009).

Galla chinensis is traditionally used for the treatment of inflammation, dysentery, toxicosis, sore and so on (Tian et al., 2009). It was reported that *Galla chinensis* could promote the mineral ions deposit on the enamel surface layer of dental caries and then modify its remineralization (Cheng et al., 2010). The extracts of *Galla chinensis* including methyl gallate and penta-O-galloyl-b-D-glucoside could prevent platelet activation by suppressing ERK1/2 and PLCb3 phosphorylation (Lee et al., 2014). Pentagalloylglucose (the component of *Galla chinensis*) is a safe, effective potential parasiticide against *Ichthyophthirius multifiliis* (Zhang et al., 2013). It was reported that Chinese gallotannin could inhibit the formation of nitrosamine

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in vivo (Kong, 2005). The water extracts of *Galla chinensis* possessed antimutagenicity (Xie et al., 1997) and anti-aging activity (Li et al., 1999). It could also protect the alkaline phosphatase activity of periodontal ligament cell by inhibition of lipopolysaccharides (Wang et al., 2005). In addition, *Galla chinensis* also possessed other pharmacological activities such as antibacterial (Zhu et al., 2002; Ahn et al., 2005; Li et al., 2005; Tian et al., 2009), antifungal (Ahn et al., 1998) and antioxidant activity (Tian et al., 2009). In China, various skin tropic preparations of *Galla chinensis* are widely used in clinical practice (Jiang et al., 2013). Despite the wide use of *Galla chinensis* or its external preparations as herbal medicine for the treatment of a range of diseases, its toxicity is still unknown.

In general, the current medical therapeutics relies on scientific knowledge to improve health (Seeff, 2007; Tang et al., 2008). However, some aspects of non-scientific approach in medicinal practices are usually found. Among them herbal formulations are often not subjected to toxicity testing before application to animals or humans. Thus, there are raising concerns about the lack of modern scientific evidence regarding the efficacy and safety of herbal products (Seeff, 2007; Tang et al., 2008). TCM shows the property of "low-content, multi-component and multi-target" (Zhang et al., 2010; Wang et al., 2011), which leads to the conclusion that TCM has a relatively low toxicity and complicated toxicity mechanism; thus the toxicity evaluation of TCM must be comprehensive and sensitive. This study will provide information on the major toxic effects, provide an estimate of a no-observed-adverse-effect level (NOAEL) and define the safe range of GCS.

2. Materials and methods

2.1. Galla chinensis extract

Galla chinensis was supplied by the Fangsheng biotechnology Co., Ltd. (Baoji, PR China). The water extracts of *Galla chinensis* and its solution (GCS) (the content of Gallic acid was more than 50%) was prepared in our laboratory. The extracts with a variety of concentrations including 5%, 15% and 25% were prepared by adding appropriate stabilizer and antioxidant.

2.2. Animals

Male and female Sprague–Dawley (SD) rats, 4- and 8-week old, were purchased from specific pathogen-free (SPF) facility at Chengdu Dossy Experimental Animals Co., Ltd. [License no. SCXK (Sichuan) 2008-24]. Based on the Guidelines of the International Committee on Laboratory Animals, they were maintained in environmentally controlled rooms at 20–25 °C with a relative humidity of 55 ± 5% and 12–15 air changes/h under a 12 h light–dark cycle (artificial lighting from 08:00 to 20:00). Animals were separated according to gender and were housed in well ventilated sterile polypropylene cages with bedding throughout the study period. They were treated with standard rat chow from Nuvital Nutrients (Colombol/PR, Brazil) and given free access to distilled water *ad libitum*. All rats were allowed to adjust to the new environment for 7 days before the study started.

2.3. Oral acute toxicity

The oral acute toxicity test for calculating LD_{50} was performed using the acute toxicity class method according to the Organization for Economic Cooperation and Development (OECD) guideline 425 Q4 "Up and Down procedure" (Jung and Choi, 1994; Rispin et al., 2002; OECD, 2008b). In this test, animals were dosed once at a time. If the animal survived, the dose of the next animal was increased; if the animal died, the dose for the next animal was decreased. Five experimental groups with 10 rats each, containing an equal number of both male and female, were formed. The five groups were treated with GCS at dose of 3333, 4000, 4800, 5760 and 6912 mg/kg. In each case, the product volume administered by gavage was 1 mL/100 g body weight. After administration, food was withheld for a further 3–4 h and animals were observed individually during the first 30 min, then 2, 4, 6 h after treatment, and thereafter daily for a total of 14 days. The observation focused on mortality, behavioral neurologic, autonomic and toxic effects. At the end of the 14 days, mortality was expressed as an LD_{50} value estimated according to the method described by improved Karber's method (Gu et al., 2009)

2.4. 30-day subchronic oral toxicity

2.4.1. Treatments

The determination of repeated dose 30-day oral toxicity was carried out according to OECD guideline 407, adopted on 3rd October 2008 (OECD, 2008a). Fifty SD rats were distributed in 5 groups of 10 animals each (5 female and 5 male). The five groups were as follows: saline control group (Group I), solvent control group (Group II), 500 mg/kg group (Group III), 1500 mg/kg group (Group IV), and 2500 mg/kg group (Group V). Animals were treated daily at 9 a.m. by gavage once a day for successive 30 days and observed once daily to detect signs of toxicity. The administered volume of GCS, saline or solvent was 1 mL/100 g body weight. Prior to administration, animals were marked, fasted overnight (animals had water but not food) and weighed. During the 30 days, the animals were monitored for clinical and behavioral symptoms such as diarrhea, immobility, and mortality. 24 h after the last administration, animals were euthanized under ether anesthesia after a 12 h overnight fasting.

2.4.2. Mortality and clinical signs

During the test, the condition and behavior of all animals were checked twice daily before and after dosing. The changes of animals' fur, eyes, mucous membrane, respiratory system, central nervous system, physical activity, behavior, and mortality (if any) were recorded. In order to reduce the residual interaction between the animals and postmortem tissue autolysis, the dead animals and endangered animals were dissected timely (Schneeman, 1987).

2.4.3. Bodyweight, food and water consumption

Animals were weighed on the first day of dosing, once a week thereafter (with intervals of 7 ± 1 days), and at termination. Food consumption was also recorded once before onset of dosing and approximately once a week thereafter. Water intake was monitored daily during the whole observational period.

2.4.4. Hematological assay

The blood samples, about 0.5 mL each for hematology assessments, were collected in a pre-calibrated tube containing sodium citrate. The hematological parameters included white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet count (PLT), and leukocyte differential count (lymphocytes, neutrophils, and monocytes).

2.4.5. Serum biochemistry

Blood samples were collected into non-heparinized tubes for separation of serum and biochemical analysis. The solidified blood samples in non-heparinized tubes were centrifuged at 3500 rpm (15 min at 4 °C) and the supernatant (serum) was collected. The clinical chemistry parameters included Albumin (ALB), total

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