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RESEARCH ARTICLE

Acute and subchronic toxicity as well as evaluation of safety pharmacology of modified pulsatilla granules

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Abstract

The present study investigated acute and subchronic toxicity and safety pharmacology of modified pulsatilla granules (MPG) to provide a basis for a comprehensive understanding of MPG toxicity. The results of acute toxicity testing showed that the median lethal dose of MPG was more than 5000 mg kg^{-1}, suggesting that MPG was considered as practically non-toxic. The subchronic toxicity study for 30 days was conducted by daily oral administration at doses of 375, 750 and 1500 mg kg^{-1} in Sprague-Dawley rats. The results of subchronic toxicity study showed that the body weight and relative organ weight were not significantly changed by administration of MPG. The clinical chemistry study showed that MPG could induce kidney and liver damages. In histopathological, mild lesions in liver and kidney were also observed, suggesting that the liver and kidney might be potential target organs of MPG. In the safety pharmacology study, MPG did not exhibited any side effects to rats in cardiovascular system, respiratory system and central nervous system. These results suggested that MPG could be considered safe for veterinary use.

Keywords: modified pulsatilla granules, acute toxicity, subchronic toxicity, safety pharmacology

1. Introduction

Pulsatilla, a kind of traditional Chinese medicine, derives from the dried root of *Pulsatilla chinensis* (Bunge) Regel. It has been widely used for treatments of intestinal amebiasis, malaria, vaginal trichomoniasis, bacterial infections and malignant tumor (Cheng *et al.* 2008). Pulsatilla is also traditionally used to treat dysentery (Xu *et al.* 2006; Chen *et al.* 2014). Bai Tou Weng Tang, a Chinese herb decoction, is pulsatilla-based supplemented with Coptidis Rhizoma, Cortex Fraxini and Cortex Phellodendri Chinensis and has been clinically prescribed for hundreds of years to treat toxicosis (Xu *et al.* 2012).

The modified pulsatilla granules (MPG) was based on Bai Tou Weng Tang formula and supplemented with Radix Paeoniae Alba, Radix Angelicae Sinensis, Radix Glycyrrhizae, Caulis Akebiae, Semen Trichosanthis and Radix Vladimiriae (Liu *et al.* 2003). In veterinary clinic, MPG was applied in treatments of parvovirus enteritis infection and animal diarrhea (Yang *et al.* 2012). Although it is well accepted that

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traditional Chinese medicines had the property of relatively low toxicity, there are raising concerns about the scientific studies regarding the toxicities of herbal medicines. Moreover, the use of MPG is intensively increasing, it is essential to conduct a toxicological study of MPG. Therefore, this study aimed to assess the potential toxicology and safety pharmacology of MPG for the purpose of guiding clinical use of MPG.

2. Materials and methods

2.1. Modified pulsatilla granules

MPG (content 70%) was supplied by Departement of Pharmacy, Sichuan Agricultural University (Chengdu, China). MPG is composed of Pulsatilla (Batch number: 091003), Coptidis Rhizoma (Batch number: 091108), Cortex Fraxini (Batch number: 100303), Cortex Phellodendri Chinensis (Batch number: 100605), Radix Paeoniae Alba (Batch number: 100702), Radix Angelicae Sinensis (Batch number: 100705), Radix Glycyrrhizae (Batch number: 100601), Caulis Akebiae (Batch number: 100402), Semen Trichosanthis (Batch number: 100602) and Radix Vladimiriae (Batch number: 100506). They were bought from Huimingtang Pharmaceutical Chain Co., Ltd. (Ya'an, China).

2.2. Animals

Young adult male (average weight (125.52±6.46) g) and female (average weight (121.87±3.64) g) SPF Sprague-Dawley rats (28-day-old) were purchased from Chengdu Dossy Experimental Animals Co., Ltd. (License No. SCXK (Sichuan) 2008-24). They were kept in well ventilated sterile polypropylene cages in the animal houses of Sichuan Agricultural University (Ya'an, China). Each cage contained four rats of the same sex. Based on the Guidelines of the International Committee on Laboratory Animals, they were maintained at a controlled temperature of 20–25°C and relative humidity of (55±5)% and 12 h light/dark cycle with the lights off at 7 p.m. Experiments were started after the rats acclimating for 7 days. They were treated with a started diet from Nuvital Nutrients (Colombol, PR, Brazil) and allowed to access to distilled water *ad arbitrium*.

2.3. Oral acute toxicity

An oral study for calculating median lethal dose (LD_{50}) was performed according to the Organization for Economic Co-Operation and Development (OECD) Guideline 425 "Up-and-Down Procedure" (Bruce 1895; Jung and Choi 1994; OECD 2008). According to the results of preliminary experiment, this study failed to find out the dose which could cause 100% death of rats. Therefore, this study used the maximum dose (5 000 mg kg^{-1}) to evaluate acute peroral toxicity of MPG on rats. In each group, there were 20 rats, containing 10 females and 10 males. These rats in experiment group were treated with MPG at the dose of 5 000 mg kg^{-1} and the rats in control group were treated with normal saline. The animals were observed for gross behavioral neurologic, autonomic and toxic effects for 24 h and then daily for 7 days.

2.4. Thirty-day subchronic oral toxicity

Treatments The rats were divided into 4 groups (each containing 10 females and 10 males): high dose of MPG group (Group I, 1 500 mg kg^{-1}), medium dose of MPG group (Group II, 750 mg kg^{-1}), low dose of MPG group (Group III, 375 mg kg^{-1}) and control group (Group IV, physiological saline). The rats were treated once daily for 30 consecutive days. The animals were monitored for clinical and behavioral symptoms, such as diarrhea, immobility and mortality throughout the course of the study. Each rat was marked with a unique identification number and the body weight was measured once a week.

Clinical examination During the period of test, all animals were observed once daily for the clinical signs of toxicity. The changes of animals' behavior, hair, eyes, physical activity, mucous membrane, nervous system and respiratory system were recorded. The dead and endangered animals were timely dissected in order to reduce postmortem tissue autolysis.

Body weight and food consumption The food and water consumption were recorded for each group every week. The body weight of each rat was recorded prior to the beginning of experiment and once a week thereafter (with intervals of (7±1) days) throughout the course of experiment. The mean body weight of rats in each group was calculated.

Clinical pathology Blood chemistry and hematology analysis were performed on all animals for once at the end of the study. The blood samples which were about 0.5 mL each for hematology assessments were collected in a pre-calibrated tube containing heparin sodium. Approximately 1 mL blood samples were collected into a tube containing no preservative. After coagulation, these samples were centrifuged and the serum was separated for clinical chemistry assessments.

Hematology The hematological parameters included white blood count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count (PLT), hematocrit (PCT), red blood cell distribution width (RDW), mean platelet volume (MPV), mononuclear cells (MID), lymphocyte (LYM), and neutrophils (GRA).

Clinical chemistry The clinical chemistry parameters