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Pharmacological evaluation of sedative–hypnotic activity and gastro-intestinal toxicity of *Rhizoma Paridis* saponins

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ABSTRACT

Ethnopharmacological relevance: *Rhizoma Paridis* saponins (RPS) have been well studied for antimicrobial, anti-hemorrhagic, and anticancer effects. However, scientific information on RPS regarding the toxic and neuropharmacological effects is limited. In this study, the acute oral toxicity, sedative–hypnotic activity and gastro-intestinal toxicity of RPS were investigated.

Materials and methods: The acute toxicity was carried out by administering single doses (800–5000 mg/kg) of RPS to adult mice. Rotarod test and sodium pentobarbital-induced hypnosis activity were used to evaluate the neuropharmacological effects on mice. Gastric emptying and intestinal transit were used to investigate the gastric–intestinal system effects.

Results: A single oral administration of RPS dose-dependently caused adverse effects on the general behavior and mortality rate of mice. LD_{50} value of oral acute toxicity was 2182.4 mg/kg, with 95% confidence limit of 1718.4–2807.8 mg/kg. In the test of sleeping mice, RPS acted in synergy with sodium pentobarbital at doses 250 and 500 mg/kg while motor coordination was not influenced within 120 min after treatment with RPS. Regarding the gastric–intestinal toxicity, RPS (100, 250, and 500 mg/kg) significantly inhibited gastric emptying but did not affect the intestinal transit.

Conclusions: RPS, which is a hypotoxic anticancer drug, possesses the sedative–hypnotic activity and gastric stimulus side effect.

1. Introduction

Rhizoma Paridis, the dried root of *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz (PPY) of Liliaceae family, is a traditional Chinese herb. It was recorded in "Shengnong Herb" and Li Shizhen's "Compendium of Materia". *Rhizoma Paridis* has properties of heat-clearing and detoxicating, detumescence, sedation, acesodyne and hemostasis and has been used in folk medicine for a long time. Extensive phytochemistry and pharmacological studies further identified steroidal saponins as the main active components of PPY (Cheng et al., 2008; Man et al., 2009a). Steroidal saponins played an important role in the medicine development for anti-tumor, immunity adjustment, analgesia, and anti-inflammation activities (Deng et al., 2008; Zhao et al., 2009), and show protective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats (Matsuda et al., 2003).

According to historical and Chinese Pharmacopoeia records, *Rhizoma Paridis* has mild toxicity (Committee of National Pharmacopoeia, 2010). In traditional clinical usage, excessive ingestion of *Rhizoma Paridis* could cause a toxic condition marked by nausea, vomiting, diarrhea and even heart palpitations and convulsions. No death or significant symptoms were observed in mice after being given the extraction of *Rhizoma Paridis* with gastric infusion (1:1, 0.4 mL, three times a day) for three consecutive days or by intravenous injection (1:500, 0.4 mL) (Wu et al., 2004). However, few studies on the pharmacological evaluation of the toxicity of *Rhizoma Paridis* have been investigated especially the toxicity of steroidal saponins. In our previous work, *Rhizoma Paridis* saponins (RPS), whose total steroidal saponins accounted for over 50%, were prepared and they showed potent anti-lung cancer activity while inhibiting metastases in pulmonary adenocarcinoma (Yan et al., 2009; Man et al., 2009b). Also, the mechanism of the antitumor effects *in vivo* and *in vitro*, and pharmaceutical and pharmacodynamic effects of RPS were researched (Man et al., 2009a, 2010, 2011a, 2011b, 2011c).

Abbreviations: RPS, *Rhizoma Paridis* saponins; PPY, *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz; i.p., intraperitoneal; i.g., intragastrically; CNS, central nervous system

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The aim of this study was to investigate the toxicity of RPS. According to the adverse symptoms in the acute toxicity, we further research the neuropharmacological and gastric–intestinal effects of RPS in mice.

2. Materials and methods

2.1. Plant material and preparation of RPS

The dried rhizomes of *P. polyphylla* Smith var. *yunnanensis* were collected in September 2010 from Lijiang, Yunan Province, China, and identified by Prof. Gao (Tianjin University, China). A voucher specimen (GWCL 201009) was deposited at the School of Pharmaceutical Science and Technology at Tianjin University, Tianjin, China. RPS were prepared in Zhongxin Pharmaceuticals (Tianjin, China) and the method was the same as that previously reported (Man et al., 2009b). In brief, the crushed root (30 kg × 3) of *P. polyphylla* var. *yunnanensis* was extracted with 70% ethanol (120 L × 3, 3 times) for 2 h under reflux. The combined 70% ethanol extracts were concentrated and then filtered and centrifuged. The supernatant dissolved in water was then eluted by 65% ethanol on macroporous adsorptive resin D101. The eluent was finally condensed with a vacuum rotary evaporator to give a gray, viscous extract (1.50 kg), which was RPS. The agent was lyophilized and stored at −20 °C until further studies.

2.2. Animals

Kunming mice, weighing about 18–20 g, of SPF degree, were purchased from Tianjin Experimental Animal Center (License no. SCXK (Jin) 2009-0002) and involved in this trial. The animals were housed in polycarbonate cages (10 animals in each cage) with white wood chips for bedding, and given free access to food and drinking water, under controlled temperature (23 ± 2 °C), humidity (50–60%) and photoperiod (12 h light and 12 h dark). Mice were allowed to be acclimated for one week and were fasted for 16 h prior to dosing. This animal study was approved by the Institutional Animal Care and Use Committee of China, and institutional guidelines for animal welfare and experimental conduct were followed.

2.3. Drugs and drug administration

RPS were prepared in Zhongxin Pharmaceuticals (Tianjin, China) as mentioned above. Other drugs used in this study were sodium pentobarbital (Sigma-Aldrich, St. Louis, MO), estazolam (Tianjin Pacific Pharmaceutical Co., Ltd. China), domperidone (Xi'an-Janssen Pharmaceutical Co., Ltd. China), and atropine sulfate (Tianjin Jinhui Amino Acid Co., Ltd. China).

For oral administration, RPS, estazolam and domperidone were suspended in 0.9% saline solution. For intraperitoneal (i.p.) injection, sodium pentobarbital and atropine sulfate were dissolved in physiological saline.

2.4. Analysis of RPS by HPLC–ELSD

RPS were made up to a concentration of 2.5 mg/mL in 80% methanol and filtered through a 0.45 μm membrane before being used for HPLC analysis. Agilent 1100 liquid chromatography system (Agilent Technologies, USA), equipped with a quaternary pump, an online degasser, and a column temperature controller, coupled with an ELSD (Alltech Associates, USA) as the detector were used. The analytical column temperature was kept at 35 °C. The samples were separated with a Cosmosil 5C18-MS-II column (4.6 mm × 250 mm, 5 μm, NACALAI, Japan) using water (A) and acetonitrile (B) under gradient conditions similar to the previously reported (0–5 min, linear gradient 33–36% B; 5–10 min, linear gradient 36–39% B; 10–12 min, linear gradient 39–45% B; 12–13 min, linear gradient 45–47% B; 13–18 min, linear gradient 47–50% B; 18–20 min, isocratic 50% B; 20–23 min, linear gradient 50–43% B; 23–42 min, isocratic 43% B; 42–45 min, linear gradient 43–55% B; 45-50 min, isocratic 55% B; 50–53 min linear gradient 55–100% B) as the mobile phase (Man et al., 2010). The injection volume was 20 μL and flow rate was 1 mL/min. The drift tube temperature for ELSD was set at 110 °C, and the nebulizing gas flow rate was 2.9 L/min. Peaks were assigned by comparing their retention time with that of each reference compound eluted in this mobile phase and by spiking samples with reference compounds. Fig. 1 shows the HPLC fingerprint of RPS. The content of the total saponins of RPS was 55.61%, and the higher saponins like Paris-VII (Tg), Chonglouoside H, Formosanin C (Paris II) and Polyphyllin D (Paris I) were 3.46%, 7.05%, 3.19% and 9.13% respectively, as analyzed by HPLC. The water solution of RPS was a suspension and formed a few floccules at 4 °C.

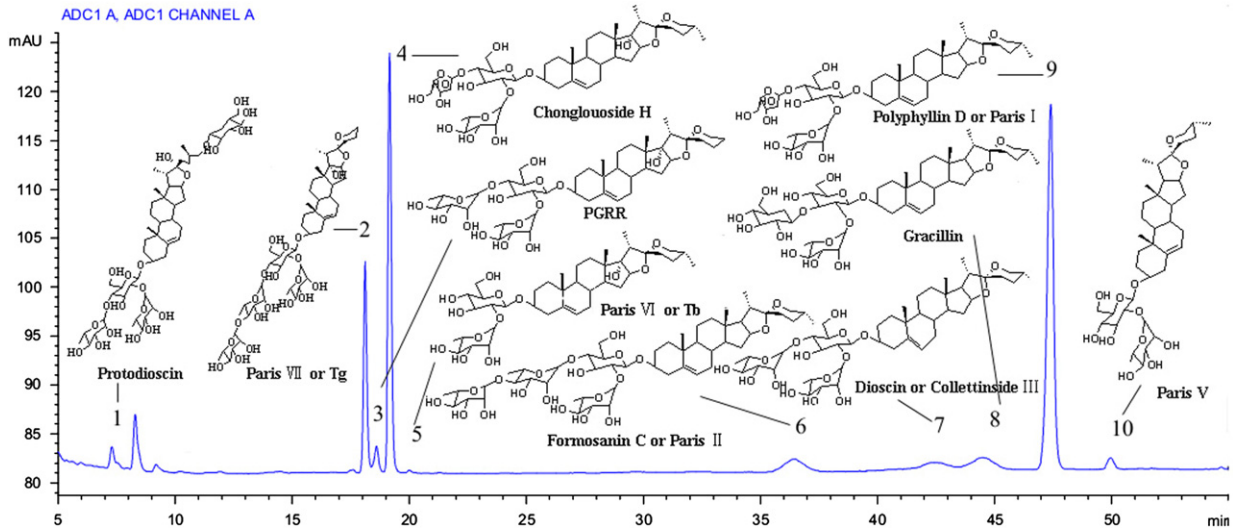

Fig.1. HPLC chromatograms of RPS. The peaks corresponding to Protodioscin, Paris VII (Tg), PGRR ((25R)-5-en-spirost-3,17-diol-3-O-L-rhamnopyranosyl-(1→4)-[-L-rhamnopyranosyl-(1→2)]-D-glucopyanoside), Chonglouoside H, Paris VI (Tb), Formosanin C (Paris II), Dioscin (Collettinside III), Gracillin, Polyphyllin D (Paris I) and Paris V were identified. ELSD was the detector, drift tube temperature was 110 °C. The injection volume was 20 μL and nebulizing gas flow rate was 2.9 L/min.