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Evaluation of toxicity studies of flavonoid fraction of *Lithocarpus polystachyus* Rehd in rodents

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ABSTRACT

The aim of the study was to evaluate the safety of flavonoid fraction of *Lithocarpus polystachyus* Rehd (Sweet Tea-F, ST-F) in mice and rats through acute and sub-chronic toxicity studies respectively. For acute toxicity study, a single dose of 5000 mg/kg of ST-F was given orally to healthy KM mice. The mice were observed mortality and toxic symptoms for 24 h, then once a day up to 14 days. In the sub-chronic toxicity study, ST-F was administered orally at doses of 0, 70, 140, 560 mg/kg/day to rats for 26 weeks. Body weight and food intake were recorded weekly. Hematological, biochemical, coagulation and organ parameters were analyzed at the end of 26 weeks administration. Vital organs were evaluated by histopathology. In the acute toxicity study, ST-F caused neither significant toxic symptoms, nor mortality in mice. In sub-chronic toxicity study, daily oral administration of ST-F at the dose of 70 mg/kg resulted in a significant increase ($P < 0.05$) in the relative body weight at the 10-week, and the same situation brought at the dose of 140 mg/kg/day at the 22-week. Hematological and biochemical showed significant changes ($P < 0.01$ or $P < 0.05$) in WBC, GLU, ALP, AST and serum electrolytes levels at the dose of 560 mg/kg/day. The amount of RBC decreased significantly ($P < 0.05$) while the content of PLT slightly increased ($P < 0.05$) at the dose of 140 mg/kg/day. In additional, no obvious histological changes were observed in vital organs of ST-F treated animals compared to control group. The ST-F may be exit slight side effects at the dose of 560 mg/kg/day in rats. Thus, the overall results show that the no-observed adverse effect level (NOAEL) of ST-F was considered to be 140 mg/kg for male SD rats.

1. Introduction

In the last few decades, many Chinese herbal medicines in the form of compounds, extracts, single herbs, or formulae have been reported to be effective for the prevention and treatment of various kinds of diseases (Sham et al., 2014). However, some medicinal plants are bound to use them discreetly, because some studies have indicated that many medicinal plants applied in clinical treatments showed adverse reactions (Lao et al., 2009). Therefore, it emphasizes that traditional plant's safety should be guaranteed for the medicinal purposes. So, the acute and sub-chronic toxicity studies on medicinal plants or extracts, single herbs, or formulae are necessary.

Lithocarpus polystachyus Rehd is an autochthonous plant in Southern China with affluent nature resources, which belongs to the family Fagaceae and is commonly known as “Sweet Tea” (ST) (Wan yi et al., 2001; Tang et al., 1998). Its leaves have been used to prevent or treat hypertension, obesity and hypolipidemic (Geerts et al., 2015) for hundreds of years in southern China folk medicine (Maruthappu et al., 2015). Previous experiments have indicated that ST is rich in flavonoids and polyphenolic compounds (Li et al., 2014; Yang et al., 2007). These components have extensive

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pharmacologic action, including hypoglycemic (Desprez et al., 2015), cardiovascular (Majewska-Wierzbicka and Czeczot, 2012), anti-diabetic (Honti and Fenner, 2015), anti-hypertension (Hou et al., 2012) and anti-oxidative (Lohani et al., 2013). In recent years, studies show that flavonoid fraction of ST (ST-F) plays an important role in the treatment of diabetes and hypertension, and has certain therapeutic effects for chronic illness (Hou et al., 2011). However, up to now, the safety of ST-F has not been reported.

Therefore, the aim of the present paper was to study the acute and sub-chronic toxicities of KM mice and rats by oral administration of ST-F to obtain information on the safety of the extract to provide guidance for clinical applications.

2. Material and methods

2.1. Plant material

The fresh leaves of *Lithocarpus polystachyus* Rehd were purchased in Jiangxi Province in October 2012, and identified by Prof. Lai Xiao-Ping in the School of Chinese Pharmaceutical Science, Guangzhou University of Chinese Medicine. The leaves were dried in the shade. A voucher specimen is deposited in the herbarium of Guangzhou University of Chinese Medicine.

2.2. Preparation of ST-F extract

The extraction method of ST-F was strictly according to Hou (Hou et al., 2011). Briefly, the dry leaves (1 kg) were pulverized and extracted twice by reflux extraction with 4 L 70% ethanol for 2 h each time. Then extract solution was filtered and concentrated by using a rotary evaporator, and dried to powder in a vacuum dryer at 105 °C to get the flavonoid-rich extract. The flavonoid-rich fraction was prepared by low temperature equipment with different extracting pressure (ZLUPD-S-350A, Guangzhou Zeli Pharmtech Co., Ltd., Guangzhou, China). Then the extract solution was processed by ultrafilter membrane and purified by HPD-826 macroporous resin column chromatography. Finally the extract was dried to powder on an atomizing drier to yield a yellow powder extract (1 g of dried power is equivalent to 28.96 g dried drug herb). The concentration of total flavonoids were determined and calculated by spectrophotometer (UV-2550, SHIMADZU) at 284 nm, using phlorizin as a standard. The compounds identification of the flavonoid fraction of ST (ST-F) was analyzed by the RP-HPLC (Waters Milford, MA, USA) on a DIKMA Platisil ODS column (250 × 4.6 mm; particle size 5 μm; Dikma Technologies, CA, USA). HPLC purity of total flavonoids is over 90%. The extracts were freshly dissolved with distilled warm water just before administration to animals during experiment. According to the clinical use of the Chinese material medica (Medicine Shanghai, 1999), the estimates of doses as following: the doses for rats were designed according to people and animals doses equivalent ratio, the *Lithocarpus polystachyus* Rehd's dose of people is 10 g (dried herb)/day, so we designed the doses of sub-chronic toxicity study as follows: 70 mg (extract)/kg, 140 mg (extract)/kg, 560 mg (extract)/kg.

2.3. Animals and diet

Adult Kunming(KM)mice of both sexes weighing 18–22 g and adult male Sprague-Dawley (SD) rats, weighing between 200 and 240 g, were obtained from the Laboratory Animal Services Center, Guangzhou University of Chinese Medicine (Guangzhou, China). All animals were housed under standard environment condition of temperature at 20–25 °C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. The studies were approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine.

2.4. Acute oral toxicity study in mice

Acute oral toxicity test was conducted according to the guidelines of acute toxicity studies for the Organization for Economic Cooperation and Development (OECD 425) and traditional Chinese medicine. The acute toxicity test was decided to use a single dose of 5000 mg/kg body weight/oral ST-F. The mice were fasted for 12–16 h before the administration of the ST-F and assigned to groups according to the body weight. Mice were divided in 2 groups of 10 mice each (5 males and 5 females). A single dose of 5000 mg/kg of ST-F was administered orally to the treated group, Neither water nor diet was given up to 4 h after the treatment and after 4 h Animals were maintained in a cage with free access to a standard diet and water ad libitum, and observed for the initial 24 h after the administration. The mice were further observed once daily for 14 days for the number of deaths and parameters such as sedation, alertness, spontaneous motor activity, ptosis, dyspnea, convulsions, urination, diarrhea, postural reflex, piloerection, nociception and others (rearing, climbing, aggression, grooming and vocalization). The body weight and food consumption were monitored on Days 0, 3, 6, 9, 12 and 14.

2.5. Sub-chronic oral toxicity study in rats

The sub-chronic oral toxicity test was conducted according to the guidelines of toxicity studies for the traditional Chinese medicine. Four groups of 15 male rats received doses of 0, 70, 140 or 560 mg/kg/day of ST-F at daily gavage for 26 consecutive weeks. All animals were observed closely for any behavioral changes. The body weight of animals and food consumption were monitored weekly throughout the study period. The day before the end of the experiment, animals were fasted overnight and were then anesthetized with diethyl ether. Blood samples were collected from the posterior vena cava for hematological and biochemical value measurements. All organs were visually inspected, and parts of organs (liver, heart, spleen, lungs, kidney, adrenals, thymus, brain, testes, and epididymis) were weighed directly after dissection. Defined samples of the liver, brain, pancreas, stomach, lung, kidney, adrenals, epididymis, testes, thyroid, prostate, thymus, small intestine, bladder, trachea and parathyroid glands were placed in 10% neutral buffered formalin for pathological examination.

2.6. Hematological and biochemical analysis

The hematological parameters included: white blood cells (WBC), red blood cells (RBC), platelets (PLT), mean platelet volume (MPV), hemoglobin (HGB), lymphocyte, percent of lymphocytes (LYM%), monocyte, percent of monocytes (MONO%), neutrophil, percent of neutrophil, eosinophil (EOS), basophil (BASO), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), Platelet hematocrit (PCT), platelet distribution width (PDW) and red blood cell distribution width (RDW). Hematological analyses were performed at clinical lab of Guangdong province hospital of T.C.M.

For biochemical analysis, blood samples were centrifuged at 3000 rpm for 10 min. Serum was separated and stored at −80 °C. The serum was analyzed for the following biochemistry parameters: Na^+, K^+, Cl^{-1}, blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), total bilirubin (TB), albumin (ALB), globulin (GLO), total cholesterol (TC), triglyceride (TG). These parameters were performed at clinical