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Acute and sub-chronic toxicity studies of the extract of *Thunberg Fritillary Bulb*

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ABSTRACT

The aim of this study was to investigate the acute and sub-chronic toxicity of extract of *Thunberg Fritillary Bulb*. For the acute toxicity tests, graded doses of the extract were administered orally to mice. The animals were observed for toxic symptoms and mortality daily for 14 days. In the sub-chronic toxicity study, rats were orally administered the extract at doses of 1 and 3 mg/kg body weight (BW) for 26 weeks. After 26 weeks, the rats were sacrificed for hematological, biochemical and histological examination. In the acute toxicity tests, the estimated median lethal dosage (LD_{50}) was 52.2 mg/kg body weight in the mice. In the sub-chronic toxicity tests, a dose of 1 mg/kg body weight presented no toxicity. Above the 1 mg/kg dose, the main adverse signs observed in male rats were body or head tremor and spontaneous motor activity reduction. There were no other significant changes observed in hematology, blood biochemistry, organ weight and organ histology. The overall findings of this study indicate that the extract of *Thunberg Fritillary Bulb* is non-toxic up to 1 mg/kg body weight, which can be considered a safe application dose.

1. Introduction

Thunberg Fritillary Bulb is the dry bulb of *Fritillaria thunbergii Miq.*, a perennial plant belonging to the Family Liliaceae and distributed in Hunan province, which is south of Jiangsu province and the north of Zhejiang province (Gao et al., 1996). *F. thunbergii Miq.* is the largest genus of *Fritillaria* in Family Liliaceae (Shang and Liu, 1995). In Chinese medical clinical practice, *Thunberg Fritillary Bulb* is often used for the treatment of a variety of conditions, such as cough, bronchitis, inflammation, hypertension, gastric ulcer, diarrhea, bacterial infection and tumor (Li et al., 2001; Zhang and Shen, 2007). In recent years, *Thunberg Fritillary Bulb* has been extensively used for the treatment of drug resistant leukemia and other cancer with good outcomes (Hu et al., 2004; Hyun-Ock et al., 2002; Li et al., 2013; Xiao et al., 1992; Zheng et al., 2010). However, the information regarding the toxicity of *Thunberg Fritillary Bulb* is very limited.

The present study was designed to evaluate the acute and sub-chronic toxicity of the extract of *Thunberg Fritillary Bulb*, with the aim of obtaining information on the safety of the extract to provide guidance for selecting a safe dose for clinical applications.

2. Materials and methods

2.1. Plant material and extraction

Thunberg Fritillary Bulb was obtained from Beijing Tongrentang Yinpian Co., Ltd. (Bozhou, China). The materials were extracted twice, 1 h each time, with 2% hydrochloric acid solution at six times the amount of the herbal material. The combined extract was concentrated and centrifuged. The pH of the collected supernatants was adjusted to 9.0 using 10% NaOH. After pH adjustment, the supernatants were extracted four times with an equal volume of methylene chloride. The collected methylene chloride portions

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; APTT, activated partial thromboplastin time; BW, body weight; BUN, urea nitrogen; CK, creatine kinase; CMC, carboxyl methyl cellulose; CNS, central nervous system; CRE, creatinine; FIB, fibrinogen; GAP, Good Agriculture Practice; GLU, glucose; HCT, hematocrit; HGB, hemoglobin; HPLC, high performance liquid chromatography; KM, Kun Ming; IAEC, Institutional Animal Ethics Committee; LD50, median lethal dosage; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PLT, platelets; PT, prothrombin time; RBC, red blood cells; SD, Sprague Dawley; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglyceride; TT, thrombin time; TP, total protein; UA, uric acid; WBC, white blood cells.

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were evaporated to dryness as the final extract. The dry extract obtained was purified and refined, and then the final extract was obtained and 0.05% of the herbal material. The total alkaloid content was 50% in the final extract and peimine and peiminine were detected by HPLC method.

2.2. Animals

Sixty 4–5 week-old Kun Ming (KM) mice of both sexes weighing 15–17 g were obtained from Beijing HFK Bioscience Co., Ltd., and one hundred and twenty 5–7 week-old Sprague Dawley (SD) rats of both sexes weighing 165–180 g were obtained from the Laboratory Animal Center of Academy of Military Medical Sciences of China. The animals were housed and maintained in standard conditions of 12 h light/darkness, humidity and temperature in the laboratory. They were fed a standard laboratory pellet diet and tap water ad libitum. The animals were acclimatized for one week before they were used in the experiments. The principles of laboratory animal care guidelines, approved by the Institutional Animal Ethics Committee (IAEC) of Drug Safety Evaluation Center in China Institute for Radiation Protection, were strictly followed.

2.3. Acute oral toxicity

Acute oral toxicity tests were conducted according to the guidelines of acute toxicity studies for natural and traditional Chinese medicine. The mice were fasted for 12–16 h before the administration of the extract and assigned to groups using a computerized random sort program so that body weight means for each group were comparable as follows: sixty KM mice were allocated into six groups of ten animals each (five males and five females). The extract was administered to mice in 0.5% carboxyl methyl cellulose (CMC) suspension at graded doses of 14.3, 22.0, 33.8, 52.0 and 80.0 mg/kg body weight, whereas the mice in the vehicle group received 0.5% CMC. The general behavior of mice and signs of toxicity were observed continuously for 3 h after extract administration. The mice were further observed twice a day up to 14 days for behavioral changes and signs of toxicity and/or death. The body weights were monitored on days 0, 3, 7 and 14, and their food consumption was monitored on days 0, 3 and 12.

2.4. Sub-chronic oral toxicity

The sub-chronic oral toxicity tests were conducted according to the guidelines for sub-chronic toxicity studies for natural and traditional Chinese medicine. Rats were assigned to groups using a computerized random sort program so that body weight means for each group were comparable as follows: SD rats of both sexes were assigned to three groups (two treatment groups and one vehicle group) of 40 animals each (20 males and 20 females). The rats in the vehicle group were orally fed with 0.5% CMC, and the rats in the treatment groups were orally administered 1 or 3 mg/kg body weight of the extract, respectively, six days a week for 26 weeks. The animals were observed closely for any behavioral changes. The body weights of animals, food consumption and water consumption were monitored weekly throughout the study period.

2.5. Coagulation parameters

At the end of 26 weeks, pentobarbital sodium was used to anaesthetize the animals before blood samples were collected from overnight fasted animals through abdominal aorta puncture into sodium citrate-coated vials. Plasma was separated for coagulation parameters, such as prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB) and thrombin time (TT), using a semi-automated coagulation analyzer (STA-4, Stago Co., Ltd.).

2.6. Measurement of biochemical and hematological parameters in blood

The blood samples collected in the sodium citrate-coated vials were analyzed for white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) content, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and platelets (PLT) using an auto-hematology analyzer (MEK-6318 K, Nihon Kohden).

K^+, Na^+, Cl^- and Ca^{2+} were determined using the ion-selective electrode method with an AC980 electrolyte analysis instrument (Audicom Medical Instruments Co., Ltd.).

The blood biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), urea nitrogen (BUN), creatinine (CRE), total cholesterol (TCHO), glucose (GLU), total bilirubin (TBIL), triglyceride (TG), creatine kinase (CK), lactate dehydrogenase (LDH) and uric acid (UA). These biochemical parameters were determined using an automatic biochemistry meter (SELECRTA-E, Vital Scientific).

2.7. Organ weight and histopathology

After blood collection, the animals were sacrificed and the organs, including brain, spinal cord, pituitary, thyroid, thymus, esophagus, salivary glands, stomach, small/large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, uterus, female mammary gland, prostate, urinary bladder, lymph nodes, bone marrow, skin, ovaries, testes and epididymis, were isolated for histological study. We also determined the absolute and relative organ weights (based on terminal body weights) for the brain, heart, kidneys, liver, lungs and spleen. The relative organ weights were calculated as follows:

$$\text{Relative organ weight} = \text{Absolute organ weight (g)}/\text{Body weight (g)} \times 100\%. \quad (1)$$

For the histological examination, all organs and tissues were fixed in 10% formalin, dehydrated with varying grades of alcohol, embedded in paraffin, cut into standard thick sections and stained with hematoxylin–eosin dye for microscopic observation.

2.8. Statistical analysis

All data are expressed as the mean ± standard error of the mean (S.E.M) and comparisons among different groups were performed by analysis of variance using an ANOVA test and DAS 1.0 statistical software. The LD_{50} value was determined according to the Bliss method (Bliss, 1938).

3. Results

3.1. Acute toxicity

The mortality as well as the acute toxicity increased progressively as the dose increased from 14.3 to 80.0 mg/kg (Table 1). The main behavioral signs of toxicity observed were locomotor activity reduction, asthenia and drowsiness. In the groups receiving the 33.8, 52.0 and 80.0 mg/kg extract doses, the dying mice presented asthma, abdominal breathing, restlessness, tics and urinary incontinence. Histological investigation suggested that pulmonary venous pleonaemia and respiratory failure may be the cause for death. The surviving mice recovered completely after 14 days. The LD_{50} of the extract in mice was calculated to be 52.2 mg/kg body weight. The extract treatments produced no