Single Oral Dose Toxicity Test of Persicae Semen Aqueous Extracts in Mice

Hun-Bum Cho¹#, Ji-Ha Park¹#, Bu-Il Seo¹, Su-Yeon Cho²,³, Kyu-Ryul Park²,³, Seung-Hoon Choi², Chang-Kyun Han¹, Chang-Hyun Song², Soo-Jin Park²,³, Sae-Kwang Ku²,³*

¹ : Department of Herbology, College of Oriental Medicine, Daegu Haany University
² : Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University
³ : The Medical Research center for Globalization of Herbal Formulation, Daegu Haany University
4 : Department of Oriental Medicinal Materials & Processing, College of Life Science, Kyung Hee University

ABSTRACT

Objectives : This study was to evaluate the single dose toxicity of Persicae Semen (PS) in ICR mice.
Methods : Aqueous extracts of PS (Yield = 18.60%) were administered as an oral dose of 2,000, 1,000 and 500 mg/kg (body weight) according to the recommendation of Korea Food and Drug Administration (KFDA) guidelines (2009–116, 2009). Animals were monitored for the mortality and changes in body weight, clinical signs and gross observation during 14 days after dosing, upon necropsy: organ weight and histopathology of 12 principle organs were examined.
Results : Amygdalin contents in PS aqueous extracts were detected as 32.50±5.96 µg/ml. We could not find any PS extracts treatment related mortalities, clinical signs, changes on the body and organ weights, gross and histopathological observations up to 2,000 mg/kg in both female and male mice, except for transient and slight loss of locomotion detected in female and male mice treated with 2,000 mg/kg. In addition, pharmacological immunomodulatory effects related findings were also demonstrated in 2,000mg/kg treated female and male mice as hypertrophy and hyperplasia of lymphoid cells in the submandibular lymph nodes.
Conclusions : Based on the results of this experiment, the approximate lethal dose (ALD) of PS extracts after single oral treatment in female and male mice were considered above 2,000 mg/kg, respectively. It should be carefully used in clinics because the possibilities of respiratory or neurological disorders were observed when administered over 2,000 mg/kg of PS extract related to amygdalin.

Key words : Persicae Semen, Single oral dose toxicity, Mouse, Amygdalin, Histopathology
Maximowicz (Family: Rosaceae), and has been traditionally used as respiratory diseases including asthma, and hyperlipidemia related artherosclerosis in Korea as single crude extract or ingredient of Korean traditional herbal prescriptions\(^6\). Until now, various pharmacological effects of PS extracts have been experimentally studies focused on their traditional purpose like blood and cardiovascular\(^5\), anti-asthma\(^6\), anticancer\(^7\), acetylcholine esterase (ACE) inhibitory\(^8\), neuroprotective anti-inflammatory\(^9\) activities. Especially, PS extracts potentially inhibited the ACE in rat hippocampus, and possibly ameliorating cognitions on the Alzheimer disease\(^8\). Although favorable effects of ACE inhibitors on the Alzheimer have been suggested including seed extracts of Prunus species like PS\(^8\),\(^10\), ACE inhibitors also showed severe toxicities\(^11\),\(^12\); they have been used as insecticidal agent\(^13\). In addition, one of the main chemical components of PS is also known as toxic amygdalin, source of hydrogen cyanide, which can be induced life-threaten respiratory disorders like Armeniacae Semen, a dried seed part of Prunus armeniaca Linne var, ansu Maximowicz, a well-documented toxic herb\(^14\). In the previous study, 50% lethal dose (LD\(_{50}\)) of yield 19.0% Armeniacae Semen aqueous extracts after single oral administration in rats are known as 741.95 mg/kg\(^14\). However, there are no detailed toxicological assessment of PS extracts has been reported even if mouse single oral dose toxicity test, except for effects on pregnant rats, in which no abnormal changes on the reproductive indices were detected after 7 days repeated oral administration of concentrated PS extracts\(^4\).

In the present study, the amygdalin contents were observed in PS extracts by UPLC (Ultra Performance Liquid Chromatography) method, and single oral dose toxicity test of PS aqueous extracts were conducted in mice according to the Korea Food and Drug Administration (KFDA) Guidelines\(^15\) to obtain the primary safety information about PS and further clarifies their safety for clinical use.

**MATERIALS AND METHODS**

1. Preparation of PS aqueous extracts

Aqueous PS (yield = 18,60%) extracts were prepared by routine methods using rotary vacuum evaporator (Buchi Rotavapor R-144, Switzerland) and programmable freeze dryer (Freezone 1: Labconco Corp., USA) from dried seeds of Prunus persica Franchet var, davidiana Maximowicz, produced in China (Family: Rosaceae), which were purchased from Ominiherb (Korea) after confirm the morphology under microscopy. The voucher specimens documenting this purchase were deposited in the herbarium of the Medical Research center for Globalization of Herbal Formulation, Daegu Haany University. In the present study, prepared herbs were boiled at 80°C, 3 hrs and then, evaporated and lyophilized, Powders of PS extracts are light brown powder, and all extracts were stored in a refrigerator at −20°C to protect from light and degeneration until use. They were well soluble up to 100 mg/ml concentration levels in distilled water used as vehicle as clear light brown solution.

2. Measurement of amygdalin contents in PS extracts by UPLC

1) Chromatography conditions

The UPLC system (Waters, USA), equipped with a pump Waters ACQUITY™ ultra performance LC system (Waters, USA) and a Waters ACQUITY™ photodiode array detector (PDA), was used for analysis. The Empower Data System was used for recording of the output signal of the detector. A Waters ACQUITY™ BEH C18column(1,7µm, 2,1 × 100mm) was used for separation, The mobile phase was composed of 0,1% formic acid water and 0,1% formic acid acetonitrile (Sigma, USA) with the gradient elution system at a flow rate of 0,4 ml/min. The injection volume was 2 µL. The detection UV wavelength was set at 254 nm. The column temperature was set at room temperature.

2) Preparation of standard solutions and sample

Standard stock solutions of amygdalin (Wako, Japan) were prepared by dissolving at a concentration of 1000 µg/mL in 10mL of methanol. Working standard solutions were made by diluting the standard stock solution with methanol. Standard stock solutions and working solutions were stored at 4°C. For preparation of sample, the extract of PS was weighed and dissolved in methanol at a concentration of 10 mg/mL. Before UPLC analysis, the sample preparation was weighed through a 0,22 µm filter. Amygdalin contents were expressed as mean ± SD of three independent measurements as µg/ml, in this experiment.

3. Experimental Animals and administration of PS extracts

Each of twenty female and male ICR mice (6-wk old upon receipt, SLC, Japan) was used after