Single Oral Dose Toxicity Test of Water Extracts of *Radix Araliae Cordatae* in ICR Mice

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Abstract – The object of this study was to evaluate the acute toxicity of lyophilized water extract of *Radix Araliae Cordatae* (RA) in male and female mice. The extract was administered to female and male ICR mice as an oral dose of 2000 mg/kg (body wt.) according to the recommendation of KFDA Guidelines. 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. As results, we could not find any mortality, clinical signs, changes in the body weight and gross findings except for increases of hypertrophy of lymph nodes in male RA extracts-dosing group. In addition, no RA extracts-treatment related abnormal changes in the organ weight and histopathology of principle organs except for some sporadic accidental findings. The results obtained in this study suggest that the RA extracts does not cause any toxicological signs. The LD₅₀ and approximate LD of RA extracts in both female and male mice were considered as over 2000 mg/kg.

Key words □ *Radix Araliae Cordatae*, *Aralia continentalis* Kitagawa, Water extracts, Single oral dose toxicity, Histopathology

INTRODUCTION

Radix Araliae Cordatae (RA) is a herbal drug consisted of the dried root-barks of *Aralia continentalis* Kitagawa, and has been used as a Korean traditional folk medicine for treating or preventing of various diseases, especially for inflammatory diseases (Park *et al.*, 2005), and the analgesic and anti-inflammatory effects of RA are relatively well documented (Lee *et al.*, 1995; Park *et al.*, 2005) with the chemical components (Wang *et al.*, 1989)

As increase of the concern in the functional food and well being in life, the demands and consumption of functional food originated from natural sources are increased (Lee *et al.*, 2003). However, the toxicological aspects about these natural origin-functional foods has been neglected because of the reasons that they has been used as various purpose for long times. Therefore, it is considered that more detailed and systemic toxicological studies should be performed to control the abuse and potential toxicities even if they have been used as traditional folk medicine. The toxicological studies about RA also have

been neglected and the reports dealing the toxicological aspects of RA extracts, even if the basic single dose toxicities in rodents are also seldom.

The objective of the present study, therefore, was to obtain the primary safety information about RA extracts, lyophilized water extract of dried root-barks of *Aralia continentalis*, and to further clarify their safety for clinical use.

MATERIALS AND METHODS

Animals and husbandry

Each of ten female and male ICR mice (6-wk old upon receipt, Charles River, Japan) was used after acclimatization for 8 days. Animals were allocated five per polycarbonate cage in a temperature (20-25°C) and humidity (45-50%) controlled room. Light : dark cycle was 12hrs : 12hrs, and feed (Samyang, Korea) and water were supplied free to access. All animals were overnight fasted before dosing and terminal necropsy. Animals were marked by picric acid. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the Korea Food and Drug Administration (KFDA) guidelines.

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Administration of RA extracts

Aqueous RA extracts (dried root-barks of *Aralia continentalis* Kitagawa: yield = 16~18%, extracted by Dong Wha Pharm. Ind. Co., Korea) were prepared by routine methods using rotary vacuum evaporator (Lab. Camp, Korea) and programmable freeze dryer (IlShin Lab., Korea). RA were purchased from Bu-young Pharmaceutical Ind. Co. (Seoul, Korea) collected in China after confirm the morphology under microscopy. Powders of RA extracts are light yellow powder, and they were stored in a refrigerator at -20°C to protect from light and degeneration. RA extracts were soluble up to 100 mg/ml concentration level and appeared to be a light yellowish solution. The animals were grouped into 4 with 5 mice per group upon receipt. RA extracts have been used as folk medicine and ingredients of medicinal food for long times, and no revealed toxicological data was available, the highest dosage level was selected as 2000 mg/kg according to the recommended by KFDA Guidelines (2005-60, 2005), the limited dosages. In addition, a vehicle control group was added as listed in Table I. Animals were given an oral dose of RA extract in water 100 mg/ml using a zonde attached to a syringe of 1ml after overnight fasting (about 18hr, water was not restricted). Food and water were restricted further for about 3 hrs after dosing.

Abnormal behavior, clinical sign and body weight

All abnormal clinical signs and behaviors were recorded before and after dosing at least twice a day based on the functional observational battery test (Irwin, 1968; Dourish, 1987). Body weights were measured on the day of dosing (Day 0) prior to treatment, 1, 2, 7, 13 and 14 days after dosing. In addition, to reduce the erratum originated from individual body weight differences of animals at initial dosing, body weight gains during Day 0~Day 7, Day 7~Day 13 and Day 0~Day 13 was also calculated based on measured body weight at each points.

Necropsy

Necropsy was done on the animals found dead during the

experiment and all animals were subjected to terminal necropsy at the end of experiment at Day 14 after overnight fasting (about 18hr, water was not restricted). Animals were euthanized by carbon dioxide and gross necropsy was performed.

Organ weight measurements and sampling

The absolute organ weight was measured and then relative organ weight (% for body weight) was calculated. The following organs were collected for histopathological observation.

Measured and sampled organs: lung, heart, thymus, left kidney, left adrenal gland, spleen, left testis or ovary, liver, splenic lobe of pancreas, brain, left epididymis or total uterus and left popliteal lymph node.

Histopathology

Samples were fixed in 10 % neutral buffered formalin. After 18 hrs of fixation, paraffin embedding was conducted and 4 µm sections were prepared by routine histological methods. Representative sections of each specified organs were stained with hematoxylin-eosin for light microscopical examination.

Statistical analyses

Changes of body weights and organ weights were analyzed by Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) compared to those of vehicle control. LD₅₀ was calculated by Probit method. Statistical analyses were conducted using SPSS for Windows (Release 6.1.3., SPSS Inc., USA) and a *p*-value of less than 0.05 was considered to be a significant difference.

RESULTS

Mortality rate

No sudden mortality was detected in all dose levels tested. All animals (5/5; 100%) survived in all dose levels tested including vehicle control.

Abnormal clinical signs detected

In this study, no RA extracts-treatment related abnormal

Table I. Experimental design used in this study

Group	Sex	No. of animals	Total Dose (mg/kg) of <i>Radix Araliae Cordatae</i> extracts
G0M*	Male	5	0
GRAM	Male	5	2000
G0F*	Female	5	0
GRAF	Female	5	2000

*Vehicle control; distilled water 20 ml/kg as vehicle in this study; All test articles in vehicle were once orally dosed.

clinical signs were observed during observation periods regardless of male and female mice except for hair loss that was randomly detected from 5 days after dosing including vehicle control groups (Table II).

Changes of body weights

No significant changes in body weight were detected compared to that of vehicle control in all dose levels tested (Table III).

Changes of organ weights

No significant changes in the absolute and relative weight of 12 principle organs were observed in all dose groups compared to that of vehicle control (Table IV, V).

Necropsy findings

No significant changes in the gross findings of 12 principle organs were observed in all dosing groups compared to that of vehicle control except for increased incidences of the hypertrophy encountered in RA extracts female group. Some animals showed sporadic accidental findings such as congestion spots of lung, atrophy of thymus, cyst and discolorization of kidney and spleen atrophy (Table VI).

Histopathological findings

No significant histopathological findings were observed in 12 principle organs of all dose groups tested compared to that

of vehicle control except for hypertrophy of lung alveolus wall with hemorrhage (Fig. 1), focal necrotic foci and fatty changes in liver (Fig. 2) (Table VII).

DISCUSSION

In the present study, we investigated the acute toxicity of single oral dose with RA extracts, lyophilized water extract of dried root-barks of *Aralia continentalis* to mice as a part of the safety test. In order to observe 50% lethal dose (LD₅₀) and

Fig. 1. Histopathological changes detected in the lung. Note that hypertrophy of alveolus as congestion with hemorrhage were randomly detected and dispersed throughout the all tested groups including vehicle control. All Hematoxylin & Eosin stain; Scale bars = 100 μ m.

Table II. Clinical signs observed in female and male mice after a oral dose of RA extracts

Group ID		Clinical signs
		Hair loss
MALE	G0M	2/5 (40%)
	GRAM	1/5 (20%)
FEMALE	G0F	3/5 (60%)
	GRAF	1/5 (20%)

Values are expressed as observed animals/total observed animals (n = 5) (percentages)

Table III. Body weight gains in female and male mice after once orally dose of RA extracts

Group ID		Interval		
		Day 0 ^a - Day 7	Day 7 - Day 13	Day 0 - Day 13
MALE	G0M	10.30±3.68	3.68±1.21	9.12±1.63
	GRAM	10.60±0.84	4.08±0.54	9.76±0.67
FEMALE	G0F	5.42±0.98	1.94±1.13	4.10±0.78
	GRAF	4.78±1.01	3.56±1.64	5.94±1.86

Values are expressed as mean $\pm$ S. D., (n=5); ^a Day of dosing.

Fig. 2. Histopathological changes detected in the liver. Note that fatty changes and necrotic foci were randomly observed including vehicle controls as accidental findings. All Hematoxylin & Eosin stain; Scale bars = 100 μ m.

Table IV. Changes in the absolute organ weights observed in female and male mice after a oral dose of RA extracts

Group ID	Principal Organs											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis/Ovary L	Liver	Pancreas S	Brain	Epididymis L/Uterus	Lymph node L ^a
G0M	0.208±0.014	0.176±0.015	0.064±0.016	0.355±0.059	0.008±0.004	0.110±0.016	0.128±0.025	1.739±0.213	0.202±0.030	0.488±0.013	0.044±0.002	0.023±0.011
GRAM	0.210±0.021	0.191±0.024	0.068±0.009	0.341±0.069	0.009±0.005	0.120±0.050	0.119±0.008	1.772±0.121	0.210±0.030	0.510±0.018	0.044±0.004	0.028±0.010
G0F	0.180±0.011	0.145±0.015	0.072±0.016	0.203±0.020	0.009±0.003	0.124±0.021	0.040±0.013	1.268±0.112	0.178±0.016	0.468±0.035	0.136±0.032	0.022±0.003
GRAF	0.187±0.013	0.147±0.008	0.069±0.012	0.210±0.008	0.009±0.002	0.119±0.028	0.050±0.007	1.385±0.153	0.195±0.035	0.479±0.021	0.142±0.038	0.025±0.006

Values are expressed as mean±S. D., (n=5); L, left sides; S, splenic lobes; ^aPopliteal lymph node.

Table V. Changes in the relative organ weights observed in female and male mice after a oral dose of RA extracts

Group ID	Principal Organs											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis/Ovary L	Liver	Pancreas S	Brain	Epididymis L/Uterus	Lymph node L ^a
G0M	0.533±0.036	0.450±0.030	0.161±0.033	0.906±0.106	0.021±0.010	0.282±0.028	0.331±0.080	4.442±0.309	0.516±0.040	1.257±0.116	0.113±0.006	0.059±0.028
GRAM	0.530±0.053	0.482±0.053	0.172±0.022	0.858±0.151	0.024±0.013	0.304±0.125	0.301±0.028	4.465±0.192	0.530±0.083	1.287±0.061	0.112±0.014	0.071±0.026
G0F	0.611±0.034	0.491±0.035	0.243±0.065	0.688±0.051	0.030±0.009	0.421±0.062	0.135±0.048	4.302±0.327	0.605±0.043	1.588±0.065	0.460±0.101	0.075±0.009
GRAF	0.583±0.021	0.457±0.022	0.215±0.030	0.656±0.034	0.027±0.003	0.369±0.065	0.154±0.019	4.304±0.270	0.604±0.082	1.493±0.077	0.446±0.130	0.078±0.020

Values are expressed as mean±S. D., (n=5); L, left sides; S, splenic lobes; ^aPopliteal lymph node.

approximate lethal dosage (LD), test substances were administered orally to female and male ICR mice at dose levels of 2000 mg/kg. However, we could not find any mortality, abnormal signs, significant changes in the body weight and gross observation except for increase of hypertrophy of lymph nodes in male RA extracts-dosing group. In addition, no RA extracts-treatment related abnormal changes were noted in the organ weight and histopathology of principle organs except for some sporadic accidental findings.

No RA extracts related histopathological changes were detected in lymph nodes, although the hypertrophy of lymph nodes was detected as one of accidental findings. The hyperplasia of lymphoid cells and follicles were frequently observed after treatment of immune enhancers (Lee *et al.*, 2005), with increase in the weights of spleen or lymphatic organs (Klein *et al.*, 1987; Iqbal *et al.*, 2001). However, RA extracts only increase the incidences of the hypertrophy.

In KFDA Guidelines (2005-60, 2005), the recommended highest dose of test materials is 2000 mg/kg or the maximum solubility in a volume below 20 ml/kg for studies of acute toxicity in mice. In the present study, the highest dose of RA extracts was selected as 2000 mg/kg because RA extracts have been used as folk medicine and ingredients of medicinal food for long times and no revealed toxicological data was available.

Hair loss detected in this study were not RA extracts-treatment related signs because they were also detected in the vehicle control with higher frequencies encountered. Congestion spots of lung, atrophy of thymus, cyst and discolorization of kidney and spleen atrophy detected as gross findings, and hypertrophy of lung alveolus wall with hemorrhage, focal necrotic foci and fatty changes in liver detected as histopathological findings were also considered as accidental findings, and they were not considered as RA extracts-treatment related abnormal gross or histopathological findings because they were restricted in some individual animals and most of the case, also observed in vehicle control. In addition, most of them were rarely observed in normal mice (Lee *et al.*, 2005; Lee *et al.*, 2006).

Hodge and Sterner (1949) classify as non toxic materials for those with LD₅₀ of 5000~15000 mg/kg and those with LD₅₀ of 500~5000 mg/kg as relatively low toxic (Class III) in US environmental Protection Agency (OPPTS 870.100; 1998). However, recent guideline by Korean Food and Drug Administration (2005-60, 2005) recommended that the highest oral dose of test materials should be 2000mg/kg. The LD₅₀ and approximate LD in mice after single oral dose of RA extracts

Table VI. Necropsy findings in female and male mice after once orally dose of RA extracts

Group ID	MALE		FEMALE	
	G0M	GRAM	G0F	GRAF
Lung				
Congestion	2/5	1/5	3/5	0/5
Thymus				
Atrophy	2/5	0/5	1/5	0/5
Kidney				
Cyst	0/5	1/5	1/5	1/5
Discolorization	1/5	1/5	1/5	0/5
Spleen				
Atrophy	2/5	2/5	1/5	1/5
Politeal lymph node				
Hypertrophy	2/5	4/5	1/5	1/5

Values are expressed as observed animals/total observed animals (n = 5)

Table VII. Histopathological findings in female and male mice after once orally dose of RA extracts

Group ID	MALE		FEMALE	
	G0M	GRAM	G0F	GRAF
Lung				
Congestion	3/5	3/5	2/5	1/5
Hemorrhage	0/5	1/5	0/5	0/5
Liver				
Focal necrosis	1/5	1/5	0/5	0/5
Fatty changes	2/5	0/5	1/5	0/5

Values are expressed as observed animals/total observed animals (n = 5)

were detected over 2000 mg/kg in both male and female in the present study. Therefore, oral administration of RA extracts caused no serious toxic effect to the male and female mice upto 2000 mg/kg - the highest dosage tested in this study and is likely to be safe in humans.

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