



Two-Week Repeated Dose Toxicity of *Atractylodis Rhizoma Alba* in F344 Rats

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Abstract – This research is to estimate the toxicity of *Atractylodis Rhizoma Alba* (ARA) in F344 rats and to find a dose level for the 13 weeks toxicity study. A hot water extract of ARA (ARWE) was administered orally to F344 rats at dose levels of 0 (vehicle control), 500, 1000, 2000, 3500, and 5000 mg/kg/day for 2 weeks. Each group was composed to five male and five female F344 rats. According to the result, there were no ARWE-related adverse changes in mortality, body weights, food consumption, urinalysis, hematology, clinical chemistry, gross finding at necropsy, and organ weight examination. Salivation was observed in 3500 and 5000 mg/kg/day in male and female rats but it could not have found any relationship with ARWE administration. Based on our findings, ARWE may not cause toxicity in rats under the experimental conditions. Therefore, dose level of 5000 mg/kg/day as a highest treatment group in 13-week exposure study is recommended for further toxicity assessment.

Keywords – *Atractylodis Rhizoma Alba*, Toxicity test, F344 rats, Water extract

Introduction

Medicinal herbs have been traditionally used in Korea, China, and Japan for a long time. Recently, medicinal herbs as complementary and alternative medicine have been widely used even in western countries. Traditional Oriental herbal prescriptions have become popular over the past decades; they are widely used for the treatment and prevention of various diseases because of their effectiveness (Jiang, 2005; Liu *et al.*, 2008).^{1,2} However, the safety of their use has recently been questioned due to several reports on side effects and fatalities (Ernst, 2002; Stewart *et al.*, 1999).^{3,4} Recently, researchers using protocols for evidence-based medicine have conducted extensive studies to establish scientific evidence of the efficacy of medicinal herbs. However, few scientific studies have examined on safety and toxicity of medicinal herbs.

Atractylodis Rhizoma Alba (ARA; the rhizome of *Atractylodes japonica* Koid. and *A. macrocephala* Koid.) is well known as medicinal herb in Korea, China, and

Japan for the treatment of dizziness, anticancer activity (Cheong *et al.*, 1999),⁵ arthritis (Kim *et al.*, 2012),⁶ gastrointestinal disease, and anti-obesity activity (Han *et al.*, 2012).⁷ ARA contains several eudesmane-type sesquiterpenoids including atractylon, atractylodes, atractylodiol (Nishikawa *et al.*, 1977),⁸ and sesquiterpene glycosides including atractyloside A and 10-*epi*-attractyloside A (Kitazima *et al.*, 2003).⁹ However, there is insufficient background information on toxicological evaluation of ARA extract to support the safety use. Therefore, the purpose of the present study was to evaluate the toxicity of ARA hot water extract (ARWE) orally administered in male and female F344 rats and to find a dose levels for the 13 weeks toxicity study. The present study was performed in compliance with the Good Laboratory Practice (GLP) of the Organization for Economic Cooperation and Development (OECD, 1997) and the Ministry of Food and Drug Safety (MFDS, Republic of Korea, 2014).

Experimental

Preparation of ARWE and HPLC analysis – Freeze-dried powdered ARWE was prepared from *Atractylodis Rhizoma Alba* purchased from oriental market in Ulsan-si

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(Kwangmyungdang Medicinal Herbs Co.) and authenticated by Prof. Byung-Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. HPLC analysis was performed by Prof. Byung-Sun Min and the voucher specimen CUD-3064-1 was deposited at the Herbarium of the College of Pharmacy, Catholic University of Daegu, Korea. ARA samples were proved to be safe in the aspect of the contaminations of pesticides (total dichlorodiphenyltrichloroethane, total hexachlorocyclohexane, aldrin, endrin and dieldrin), heavy metals (As, Cd, Hg and Pb), and aflatoxin B1 (data not shown). Total dichlorodiphenyltrichloroethane and total hexachlorocyclohexane contents were less than 0.1 and 0.2 mg/kg, respectively. Aldrin, endrin, dieldrin, and aflatoxin B1 contents were less than 0.01 mg/kg. In addition, Cd content was less than 0.3 mg/kg. After the hazardous substances testing, the *Atractylodis Rhizoma Alba* extract was prepared according to the standard decoction method of the Korean Pharmacopoeia. Active components were quantitatively determined using a high performance liquid chromatography (HPLC) method. As a result, content of atractylenolide III, a marker compound of *Atractylodis Rhizoma Alba*, was 0.085 µg/g. The *Atractylodis Rhizoma Alba* hot water extract (ARWE) was chemically stable at 5 °C during 6 months (Keum *et al.*, 2014).¹⁰ Lyophilized ARWE powder was suspended in 10 mL distilled water (Dai Han Pharm. Co., Ltd, Korea) with concentrations of 0, 50, 100, 200, 350, and 500 mg/mL by serial dilution. The appropriate quantity of the test sample for the highest dose was measured and mixed with the vehicle. The mixture of the test sample with vehicle (distilled water) was prepared once a week and stored in a refrigerator at 5 °C.

Animals and maintenance – Specific pathogen free F344 rats were obtained from Orient Bio Co., Ltd (Seongnam-si, Korea). Thirty males and females were assigned to each group administered at 0, 500, 1000, 2000, 3500, and 5000 mg/kg/day for 2 weeks by oral gavage. Five animals per cage for the quarantine and acclimatization periods, and 2 animals per cage for the treatment period were maintained in a polycarbonate cage (255 W × 473 L × 200H mm). Each group consisted of 5 animals of each sex. The body weight range prior to the start of dosing ranged from 126.3 to 190.8 g for males and from 96.9 to 124.8 g for females. Sterilized tap water and pelleted food for rats (PMI nutrition International, USA) were given to animals *ad libitum*. Animal room environment was as following; temperature: 23 ± 3 °C, relative humidity: 50 ± 10%, 12-hour light/12-hour dark photoperiod with 150~300 lux (turn on at 8 a.m./ turn off at 8 p.m.), ventilation: 10 - 20 times/hr. All personnel in

the animal facility wore clothes, soft caps, masks, and gloves which had been autoclaved high pressure and steam (121 °C, 20 min). This study was reviewed and assessed by the Institutional Animal Care and Use Committee (IACUC) of KIT accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International in 1998.

Treatment and toxicity assessment – Oral administration was adopted for this toxicity assessment study because it is an intended route of ARWE to humans. The dosing volume (10 mL per kg of body weight) of test sample for each animal was calculated with the Path/Tox software based on the most recently recorded body weights before and after dosing. In a previous study of similar crude drug in Korea Institute of Toxicology (not published), doses of 0, 500, 1000, 2000, 3500, and 5000 mg/kg/day were well tolerated. Therefore, the highest dose level of 5000 mg/kg and the same doses with previous study were selected for this 2-week repeated-dose study.

Behavior changes and body conditions of all animals were checked once daily throughout the acclimation period. Clinical signs of the all animals were examined and recorded twice daily (before and after dosing) during the treatment period and once before on the day of necropsy. Animals were weighed prior to randomization on the day of arrival, before dosing on the first day of treatment and every other day thereafter. A final weighing was performed on the day of necropsy. Food consumption was measured and recorded once weekly during the pre-treatment and treatment periods. A fixed quantity of food was provided and food consumption for individual animal was measured during a week. The amount of food intake was calculated as g/animal/day.

Urine samples were collected overnight (for approximately 16 hours) from animals housed in metabolism cages in the last week of treatment. Each animal was housed in an individual metabolism cage, food was withdrawn overnight during urine collection but water was available. Urinalysis was performed using urine automatic analyzer (Cobas U411, Roche, Germany) and urine stick (Multistix, 10 TM, Roche, Germany) to evaluate the following parameters; urine volume, color, specific gravity, pH, protein, ketone body, occult blood, glucose (GLU), bilirubin (BIL), nitrite, and urobilinogen. Also microscopic examination for urine cast, epithelial cell, red blood cell (RBC), and white blood cell (WBC) were performed.

All animals were fasted overnight before necropsy and blood sampling. Blood samples for hematology and clinical chemistry were collected from the vena cava of all animals at necropsy under isoflurane anesthesia. Blood

samples for hematology analysis were collected into tubes containing EDTA-2K and analyzed to evaluate white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), differential leucocyte absolute and relative (%) counts (neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cell), and reticulocyte absolute and relative (%) counts using a hematological analyzer (ADVIA 2120i, Siemens, USA). In addition, blood samples treated with 3.2% sodium citrate were analyzed for prothrombin time (PT) and activated partial thromboplastin time (APTT) using a coagulation analyzer (ACL Elite Pro, Instrumental Laboratory, Italy).

Blood samples for biochemical analysis were collected into tubes without anticoagulant at the same time as for hematology, placed at room temperature (for at least 90 minutes) and then centrifuged (approximately 3,000 rpm, 10 minutes, at room temperature) to obtain serum. The parameters including blood urea nitrogen (BUN), alanine

aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (CREA), GLU, total cholesterol (TCHO), albumin/globulin ratio (A/G), total protein (TP), albumin (ALB), creatine kinase (CK), triglycerides (TG), total TBIL, and phospholipids (PL) were measured using an automatic analyzer (TBA 120FR NEO, Toshiba Co., Japan).

On the scheduled termination sacrifice (Day 15 for males and females), all surviving animals were deprived of food overnight and anesthetized by isoflurane inhalation. After blood sampling, the animals were euthanized by exsanguination from the vena cava and aorta. A full macroscopic examination (abdominal, thoracic, and cranial cavities, etc) was performed. The absolute organ weight including brain, pituitary gland, adrenal gland, liver, spleen, kidneys, heart, thymus, lungs, salivary gland, thyroid gland, testes, epididymides, seminal vesicle, prostate, uterus, and ovaries were weighed and the relative organ weight (% of terminal body weight) were calculated.

Statistical analysis – Collected data were statistically analyzed by multiple comparison methods. When the

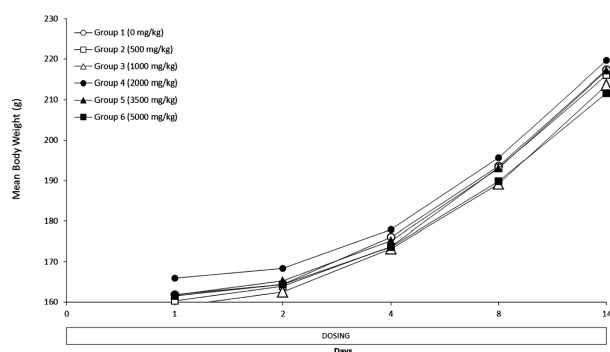


Fig. 1. Changes of body weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE in male rats.

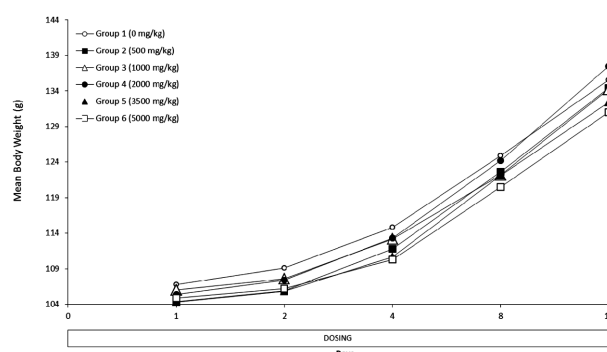


Fig. 2. Changes of body weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE in female rats.

Table 1. Comparison of food intake after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE between the treatment groups (Unit : g)

Group	G1	G2	G3	G4	G5	G6	
Dose (mg/kg/day)	0	500	1000	2000	3500	5000	
Males							
Day 2	Mean	16.3	15.4	16.1	15.9	15.8	16.3
	S.D.	1.01	1.69	1.45	0.94	0.31	1.37
Day 9	Mean	17.4	16.5	16.8	16.6	15.5	15.6
	S.D.	1.08	0.37	0.75	0.08	0.11	0.34
Females							
Day 2	Mean	11.3	10.2	10.6	10.8	10.4	9.7
	S.D.	0.58	1.33	0.32	1.00	1.32	1.77
Day 9	Mean	11.2	11.2	10.8	11.9	9.9	10.1
	S.D.	0.41	0.25	0.45	0.39	0.26	0.95

Table 2. Hematological values observed after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE

Group		G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)		0	500	1000	2000	3500	5000
Male							
WBC	$\times 10^3/\mu\text{L}$	6.35	5.65	5.97	5.5	5.94	6.22
RBC	$\times 10^6/\mu\text{L}$	8.97	9.26	9.14	9.04	9	8.94
HGB	g/dL	15.8	16.2	16.1	15.8	15.9	15.7
HCT	%	49.8	51.8	51.4	50.3	50	49.3
MCV	fL	55.5	56	56.2	55.7	55.6	55.2
MCH	pg	17.5	17.5	17.6	17.5	17.7	17.6
MCHC	g/dL	31.6	31.3	31.3	31.4	31.8	31.8
PLT	$\times 10^3/\mu\text{L}$	958.6	959.4	951.8	986.2	891.8	923.6
RET%	%	3.56	3.85	3.85	3.76	3.74	3.67
RETA	$\times 10^9/\mu\text{L}$	316.9	354.9	351.2	337.3	336.3	325.7
NEU%	%	21.6	19.3	20.4	19.2	19.1	21.4
LYM%	%	73.7	75.7	74.6	75.9	75.8	73.1
EOS%	%	0.8	0.8	0.9	0.8	0.8	0.9
MON%	%	2.3	2.4	2.3	2.0	2.3	2.7
BAS%	%	0.7	0.7	0.7	0.9	0.8	0.7
LUC%	%	0.8	1.1	1.0	1.1	1.2	1.1
NEUA	$\times 10^3/\mu\text{L}$	1.36	1.10	1.22	1.07	1.16	1.33
LYMA	$\times 10^3/\mu\text{L}$	4.69	4.27	4.45	4.17	4.47	4.55
EOSA	$\times 10^3/\mu\text{L}$	0.05	0.04	0.06	0.05	0.05	0.06
MONA	$\times 10^3/\mu\text{L}$	0.15	0.14	0.14	0.11	0.14	0.17
BASA	$\times 10^3/\mu\text{L}$	0.05	0.04	0.05	0.05	0.04	0.05
LUCA	$\times 10^3/\mu\text{L}$	0.05	0.06	0.05	0.06	0.08	0.07
Female							
WBC	$\times 10^3/\mu\text{L}$	6.76	6.38	5.6	6.21	6.25	5.86
RBC	$\times 10^6/\mu\text{L}$	9.22	9.06	9.23	8.96	9.3	9.21
HGB	g/dL	16.6	16.3	16.8	16.2	16.8	16.7
HCT	%	52.2	50.7	52	50.8	52.4	51.9
MCV	fL	56.6	56	56.3	56.7	56.3	56.4
MCH	pg	18	18	18.2	18.1	18.1	18.2
MCHC	g/dL	31.9	32.2	32.3	31.9	32.1	32.2
PLT	$\times 10^3/\mu\text{L}$	891.8	933.8	881	981.6	855.6	881.2
RET%	%	3.43	3.36	3.28	3.74	3.36	3.04
RETA	$\times 10^9/\mu\text{L}$	316.7	304.8	302.0	332.4	312.6	280.1
NEU%	%	10.8	13.3	11.9	12.1	13.6	13.2
LYM%	%	79.4	81.3	83.5	83.2	81.8	81.8
EOS%	%	0.8	0.9	0.9	0.9	1.1	1.3
MON%	%	5.3	2.5	2.3	2.2	2.2	2.2
BAS%	%	0.6	0.6	0.6	0.6	0.5	0.7
LUC%	%	3.1	1.4	0.8	1.1	0.7	0.9
NEUA	$\times 10^3/\mu\text{L}$	0.78	0.88	0.67	0.75	0.85	0.77
LYMA	$\times 10^3/\mu\text{L}$	5.47	5.14	4.68	5.16	5.12	4.80
EOSA	$\times 10^3/\mu\text{L}$	0.05	0.06	0.05	0.06	0.07	0.07
MONA	$\times 10^3/\mu\text{L}$	0.27	0.16	0.12	0.14	0.14	0.13
BASA	$\times 10^3/\mu\text{L}$	0.04	0.03	0.03	0.04	0.03	0.04
LUCA	$\times 10^3/\mu\text{L}$	0.15	0.10	0.05	0.07	0.05	0.05

Bartlett's test indicated no significant deviations from variance homogeneity, the ANOVA test was conducted to determine if any of the group means differed at the $p < 0.05$ level. When found significant in ANOVA, Dunnett's test was used to determine the difference between control group and treatment groups. In the case of significant deviations from variance homogeneity in the Bartlett's test, a non-parametric comparison test, Kruskal-Wallis (H) test, was conducted to determine if any of the group means differed at the $P < 0.05$ level. When a significant difference was observed in the Kruskal-Wallis (H) test, the Dunn's Rank Sum test was conducted to quantify the specific pairs of group comparison, which are significantly different. The Fisher's exact test was conducted to determine the pairs of group comparison (including the

prevalence or the percentage). The level of probability was taken as 1 or 5%. Statistical analyses were performed by comparing the different dose groups with the vehicle control group using Path/Tox (version 4.2.2, Xybion Medical Systems Corporation).

Result and Discussion

Many studies have reported the pharmacological effect or the analysis for extracts of *Atractylodes Rhizoma Alba*. However, there is no information on its safety or toxicity as a subchronic toxicity. Therefore, to obtain the toxicity data of ARWE and to find a dose levels for the 13 weeks toxicity study, F344 rats were orally treated with ARWE for 2 weeks. Rats consisted of six groups including 0

Table 3. Clinical chemical values observed after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE

Group		G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)		0	500	1000	2000	3500	5000
Male							
GLU	mg/dL	127.6	138.9	137.6	139.1	119.9	134.5
BUN	mg/dL	16.15	16.21	16.55	16.14	16.64	15.38
CREA	mg/dL	0.58	0.56	0.57	0.58	0.57	0.52
TP	g/dL	6.88	7.02	7	7.31	6.88	6.56
ALB	g/dL	4.73	4.81	4.8	4.93	4.74	4.62
A/G	ratio	2.21	2.17	2.18	2.13	2.22	2.4
AST	IU/L	108.1	119	115.5	113.6	139.2	100.9
ALT	IU/L	47.8	53.4	50.1	50.7	66.3	43.5
TBIL	mg/dL	0.086	0.088	0.088	0.091	0.091	0.084
ALP	IU/L	605.2	604.6	652.3	628.6	620.1	577.8
TCHO	mg/dL	56.8	59.8	58.0	60.8	58.2	47.2
TG	mg/dL	51.7	57.3	55.3	50.4	49.8	43.8
CK	IU/L	701.8	736.2	771.8	707.2	884.2	660.0
PL	mg/dL	102.2	107.0	107.4	107.6	102.8	89.0
Female							
GLU	mg/dL	86.5	77.3	77.2	71.9	87.8	84
BUN	mg/dL	19.08	18.55	18.31	18.24	18.07	17.95
CREA	mg/dL	0.53	0.53	0.52	0.55	0.54	0.53
TP	g/dL	6.54	6.54	6.58	6.43	6.65	6.56
ALB	g/dL	4.59	4.6	4.6	4.5	4.65	4.62
A/G	ratio	2.36	2.37	2.33	2.34	2.34	2.38
AST	IU/L	111.4	111.3	106	108.4	137	101.1
ALT	IU/L	37.2	35.7	35.5	37.1	51.9	35.9
TBIL	mg/dL	0.089	0.085	0.088	0.091	0.087	0.084
ALP	IU/L	508.4	498.3	470.9	509.1	493.0	492.1
TCHO	mg/dL	95.8	88.0	91.2	92.0	87.4	90.4
TG	mg/dL	76.9	76.1	70.2	78.8	69.6	69.0
CK	IU/L	947.6	869.0	813.2	891.0	952.8	744.0
PL	mg/dL	161.6	151.0	158.6	155.6	151.0	157.4

(vehicle control), 500, 1000, 2000, 3500, and 5000 mg/kg/day. Mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, macroscopic findings, and organ weight were observed. In 2-week repeated oral toxicity study, a certain treatment-related mortality was not found in any groups throughout the study period. In addition, there were no treatment-related body weights (Fig. 1 and 2), food consumption (Table 1), urinalysis, hematology (Table 2), clinical chemistry (Table 3), and organ weight (Table 4 and Table 5).

In clinical observations, salivation was observed in 3500 and 5000 mg/kg/day groups in both sex. However, these clinical observations were not considered to be related to the ARWE because these signs were temporarily observed, and it was not showed dose-response relationship. In macroscopic examination, diaphragmatic hernia of liver

was observed at vehicle control, 2000 mg/kg/day in males, and 500 mg/kg/day in females. However, this finding was not considered to be related to the ARWE because diaphragmatic hernia is species-specific and spontaneously occurred in F344 rats (Boorman, 1990).¹¹

In conclusion, ARWE was administered to male and female F344 rats at doses of 0 (vehicle control), 500, 1000, 2000, 3500, and 5000 mg/kg/day for 2 weeks by oral gavage. There were no ARWE-related adverse changes in mortality, body weights, food consumption, urinalysis, hematology, clinical chemistry, macroscopic finding at necropsy, and organ weight examination. Salivation was observed in 3500 and 5000 mg/kg/day groups in both sex. However, the incidence and severity were insignificant and salivary gland weight was not changed. Therefore, it is considered that ARWE may not cause toxicity in rats

Table 4. Absolute organ weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE (Unit : g)

Group Dose (mg/kg/day)	G1 0	G2 500	G3 1000	G4 2000	G5 3500	G6 5000
Males						
Brain	1.760	1.739	1.796	1.808	1.771	1.749
Pituitary gland	0.005	0.006	0.006	0.006	0.006	0.006
Liver	6.509	6.862	6.676	6.718	6.838	6.550
Spleen	0.492	0.475	0.502	0.506	0.515	0.479
Heart	0.721	0.708	0.712	0.729	0.738	0.716
Thymus	0.326	0.31	0.341	0.346	0.32	0.361
Salivary glands	0.351	0.353	0.35	0.355	0.361	0.348
Seminal vesicle	0.535	0.495	0.547	0.545	0.602	0.565
Prostate	0.123	0.106	0.129	0.103	0.100	0.106
Kidneys	1.530	1.597	1.587	1.667	1.622	1.573
Adrenal glands	0.037	0.038	0.039	0.043	0.043	0.040
Testes	2.653	2.654	2.723	2.602	2.666	2.657
Epididymides	0.483	0.465	0.511	0.488	0.486	0.517
Lung	0.942	0.869	0.934	0.967	0.99	0.906
Thyroid/Parathyroid	0.013	0.012	0.014	0.011	0.012	0.011
Females						
Brain	1.640	1.604	1.650	1.669	1.633	1.638
Pituitary gland	0.007	0.007	0.007	0.008	0.007	0.007
Liver	4.252	4.357	4.351	4.553	4.424	4.394
Speen	0.328	0.346	0.327	0.365	0.326	0.340
Heart	0.527	0.528	0.497	0.525	0.525	0.499
Thymus	0.312	0.306	0.301	0.317	0.301	0.292
Salivary glands	0.263	0.254	0.267	0.282	0.271	0.273
Kidneys	1.062	1.096	1.101	1.107	1.083	1.087
Adrenal glands	0.045	0.046	0.041	0.046	0.045	0.048
Ovaries	0.741	0.750	0.687	0.753	0.716	0.750
Lung	0.010	0.008	0.009	0.011	0.009	0.009
Thyroid/Parathyroid	0.283	0.325	0.247	0.214	0.301	0.265
Uterus/Cervix	0.069	0.071	0.064	0.071	0.074	0.081

Table 5. Relative organ weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day ARWE (Unit : %)

Group Dose (mg/kg/day)	G1 0	G2 500	G3 1000	G4 2000	G5 3500	G6 5000
Male						
Brain	0.902	0.903	0.94	0.912	0.898	0.914
Pituitary gland	0.003	0.003	0.003	0.003	0.003	0.003
Liver	3.323	3.535	3.481	3.379	3.466	3.410
Spleen	0.252	0.246	0.262	0.255	0.260	0.250
Heart	0.369	0.366	0.371	0.367	0.373	0.373
Thymus	0.167	0.161	0.178	0.176	0.163	0.189
Salivary glands	0.180	0.182	0.182	0.178	0.183	0.182
Seminal vesicle	0.274	0.251	0.284	0.27	0.303	0.289
Prostate	0.063	0.054	0.066	0.051	0.050	0.054
Kidneys	0.782	0.824	0.828	0.839	0.820	0.819
Adrenal glands	0.019	0.020	0.020	0.021	0.022	0.021
Testes	1.359	1.367	1.419	1.304	1.350	1.383
Epididymides	0.248	0.237	0.264	0.242	0.245	0.266
Lung	0.483	0.450	0.487	0.487	0.502	0.472
Thyroid/Parathyroid	0.006	0.006	0.007	0.006	0.006	0.006
Female						
Brain	1.365	1.339	1.376	1.358	1.382	1.396
Pituitary gland	0.006	0.006	0.006	0.006	0.006	0.006
Liver	3.526	3.638	3.624	3.696	3.748	3.736
Spleen	0.272	0.288	0.273	0.295	0.276	0.289
Heart	0.437	0.441	0.414	0.426	0.445	0.424
Thymus	0.259	0.255	0.251	0.257	0.254	0.248
Salivary glands	0.219	0.212	0.222	0.229	0.229	0.232
Kidneys	0.882	0.914	0.917	0.900	0.916	0.925
Adrenal glands	0.037	0.038	0.035	0.037	0.038	0.041
Ovaries	0.057	0.059	0.053	0.057	0.063	0.069*
Lung	0.617	0.626	0.572	0.612	0.605	0.637
Thyroid/Parathyroid	0.008	0.007	0.008	0.009	0.007	0.008
Uterus/Cervix	0.233	0.267	0.206	0.174	0.255	0.226

* Significant differences from control group ($p < 0.05$).

under the given dose conditions. This study will be a valuable basic research for in-depth toxicological study such as 13-week exposure assessment.

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References

- (1) Jiang, W. Y. *Trends Pharmacol. Sci.* **2005**, *26*, 558-563.
- (2) Liu, S.; Yi, L. Z.; Liang, Y. Z. *J. Sep. Sci.* **2008**, *31*, 2113-2137.
- (3) Ernst, E. *Trends Pharmacol. Sci.* **2002**, *23*, 136-139.
- (4) Stewart, M. J.; Moar, J. J. *Forensic Sci. Int.* **1999**, *101*, 177-183.
- (5) Cheong, B. S.; Shin, M. G. *Iconographical Folk Medicine Encyclopedia*; Younglim Co., Ltd: Seoul, **1999**, p 1026.
- (6) Kim S. H.; Park, Y. K. *Kor. J. Herbology* **2012**, *27*, 1-6.
- (7) Han, Y. K.; Jung, H. W.; Park, Y. K. *BMC Complement. Alternat. Med.* **2012**, *12*, 154.
- (8) Nishikawa, Y.; Seto, T.; Watanabe, Y.; Yasuda, I. *Yakugaku Zasshi* **1977**, *97*, 515-518.
- (9) Kitajima, J.; Kamoshita, A.; Ishikawa, T.; Takano, A.; Fukuda, T.; Isoda, S.; Ida, Y. *Chem. Pharm. Bull.* **2003**, *51*, 152-157.
- (10) Keum, J. H.; Han, H. Y.; Roh, H. S.; Seok, J. H.; Roh, H. S.; Lee, J. K.; Jeong, J. Y.; Kim, J. A.; Woo, M. H.; Choi, J. S.; Min, B. S. *Korean Journal of Pharmacognosy* **2014**, *45*, 135-140.
- (11) Boorman G. A. *Pathology of the Fischer Rat - Reference and Atlas*; Academic Press, Inc. **1990**.

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