Natural Product Sciences
21(1) : 34-41 (2015)

A 14-Day Repeated Dose Toxicity of Epimedii Herba Aqueous Extract Administered by Oral Gavage in F344 Rats

Hyoung-Yun Han[1], Young-Su Yang[1], Soo Nam Kim[1], Su-Cheol Han[1], Kang-Hyun Han[1], Jong-Hwa Lee[1], Ja Young Jeong[2], Hang-sik Roh[2], Ji Hyeon Seok[2], Jeong-Ah Kim[3], and Byung-Sun Min[4,*]

[1]Korea Institute Toxicology, 141 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Korea
[2]Toxicological Research Division, Toxicological Evaluation and Research Department, National Institute of Food and Drug Safety Evaluation, Chungcheongbuk-do 363-951, Korea
[3]College of pharmacy, Research Institute of pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Korea
[4]College of Pharmacy, Catholic University of Daegu, Gyeongsangbuk-do 712-702, Korea

Abstract – The objective of this study is to characterize a toxicity of Epimedii Herba (EH) in F344 rats and to find ~~a~~ dose levels for the 13 weeks toxicity study. EH is well known as medicinal herb in many Asian countries for traditional medicines of antibacterial and antiviral effects, estrogenic and antiestrogenic effects, and for treatment of osteoporosis, hypotensives, fatigue, kidney disorders, and related complications. However, the indispensable and basic information of toxicological evaluation of EH extract is insufficient to support its safe use. Therefore, we conducted toxicological evaluation of this drug in compliance with OECD and MFDS guideline in this study. The extract of EH was administered orally to F344 rats at dose levels of 0, 500, 1000, 2000, 3500, and 5000 mg/kg/day for 2 weeks. Each group was composed of 5 male and female rats. In this study, there were no treatment of EH-related adverse changes in clinical observations, mortality, body weights, food consumption, urinalysis, gross finding at necropsy, and organ weight examination. Total red blood cell count, hematocrit, mean corpuscular hemoglobin concentration, total cholesterol, and phospholipid were decreased in males and females at 5000 mg/kg/day compared to the control animals. Mean corpuscular volume and reticulocyte counts were increased in males and females at 5000 mg/kg/day compared to control animals. Therefore, we recommend that dose level of 5000 mg/kg/day is a highest treatment group in 13-week EH extract exposure study for further toxicity assessment.
Keywords – Epimedii Herba, Berberidaceae, Toxicity test, F344 rats, Water extract.

Introduction

Herbal medicine has been traditionally used in Asian countries for a long time. Recently, they have been widely used as an alternative medicine even in western countries. Herbs used traditionally for long-time are considered to be safety in human (Jordan *et al*., 2010).[1] Although herbal medicinal products are also considered to be lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effect (Smet, 2004).[2] Recently, the safety issue for herbal products has been increased.

Epimedii Herba (EH; *Epimedium koreanum*, *E. breviconum, E. sagittatum, E. pubescen or E. wushanense*) is well known as medicinal herb in many Asian countries for treatment of osteoporosis, hypotensives, fatigue, and kidney disorders and has been traditionally used for antibacterial and antiviral effects, estrogenic and antiestrogenic effects (Kang *et al*., 2012; Fan *et al*., 2011).[3,4] In addition, this herbal medicine was considered as hepatoprotective agent (Shindel *et al*., 2010).[5] EH contains a lot of flavonoids such as icariin, icariside II, epimedin, epimedosides, hyperoside, quercetin, and chlorogenic acid (Cho *et al*., 2012).[6] However, the indispensable and basic information of toxicological evaluation of EH extract is insufficient to support its safe use. Therefore, the objective of this study was to evaluate a toxicity of EH aqueous extract (EHAE) orally administered in male and female F344 rats and to find a dose level 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

*Author for correspondence
Byung-Sun Min, College of Pharmacy, Catholic University of Daegu, Gyeongsangbuk-do 712-702, Korea
Tel: +82-53-850-3613; E-mail: bsmin@cu.ac.kr

(OECD, 1997)[7] and the Ministry of Food and Drug Safety (MFDS, Korea, 2014).

Experimental

Preparation of EHAE and HPLC analysis – Freeze-dried and powdered EHAE was extracted from leaves of *Epimedium koreanum* which were purchased from oriental market in Ulsan-si (Kwangmyungdang Medicinal Herbs Co.) and authenticated by Prof. Byung-Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. Botanical identification and HPLC analysis were performed by Prof. Byung-Sun Min, and the voucher specimen CUD-1484-1 was deposited at the Herbarium of the College of Pharmacy, Catholic University of Daegu, Korea. The authentic EH was proved to be safe from typical contaminations such as pesticides (total dichlorodiphenyltrichloroethane, total hexachlorocyclohexane, aldrin, endrin and dieldrin), heavy metals (As, Cd, Hg and Pb), and aflatoxin B1 (JH Keum *et al*., 2014).[8] EH was extracted according to a standard hot water extraction method of the Korea Pharmacopoeia and then freeze-dried. Components of EHAE were measured using a high performance liquid chromatography (HPLC) method. The content of icariin, the marker compound of EH, was 5.00 mg/g. EHAE was stable at 5 °C for 6 months (JH Keum *et al*., 2014).[8] 5 g of freeze-dried EHAE powder was suspended in 10 mL distilled water and the suspension of EHAE (highest dose group) was gradationally diluted to prepare lower dose groups.

Animals and maintenance – 60 male and female specific pathogen-free F344 rats were obtained from Orient Bio Co. (Seongnam-si, Republic of Korea) at 6 weeks of age. The animals were acclimated for 8 days and healthy animals were used on the study. 60 male and female rats were randomly assigned to 6 groups (one control group and 5 treatment groups) using Path/Tox system (Version 4.2.2, Xybion Medical Systems Corporation, USA). Each group consisted of 5 rats of each sex. The body weight range prior to the start of dosing ranged from 120.5 to 182.3 g for males and 96.4 to 115.7 g for females. The animals were housed in polycarbonate cage with bedding (Laboratory animal Aspen bedding, ABEDD BALTIC LTD., Latvia) throughout the study period. Sterilized tap water and pelleted food for experimental animals (PMI nutrition International, USA) were given to animals *ad libitum*. The animal room was maintained at a temperature of 23 ± 3 °C, relative humidity of 50 ± 10%, air ventilation of 10 to 20 times/hour and light intensity of 150 to 300 Lux with 12 hour light/dark cycle. This study was approved by Institutional Animal Care and Use Committee in Korea Institute of Toxicology and performed in compliance with Testing Guidelines for Safety Evaluation of Drugs (Notification No. 2014-6 issued by the Ministry of Food and Drug Safety on 29 January, 2013).

Treatment and toxicity assessment – Oral administration was chosen for this study because it is the intended clinical route of EHAE administration in humans and has been used in previous non-clinical study. The dosing volume of 10 mL/kg was calculated using Path/Tox system based on the most recent body weight. In a previous study of similar crude drug in Korea Institute of Toxicology (not published), doses of 0 (vehicle), 500, 1000, 2000, 3500 and 5000 mg/kg/day were well tolerated. The same doses with previous study were selected for this 2-week repeated-dose study. All record for the measurement and examination were performed using Path/Tox system. The condition and behavior of all animals was made once daily throughout the acclimation period. All animals were examined and clinical signs 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 once weekly thereafter. A final weighing was performed on the day of necropsy. Cage food consumption was recorded once during the acclimation period and once weekly during the treatment period. Individual food consumption was calculated as g/rat/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) was 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, red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpus-

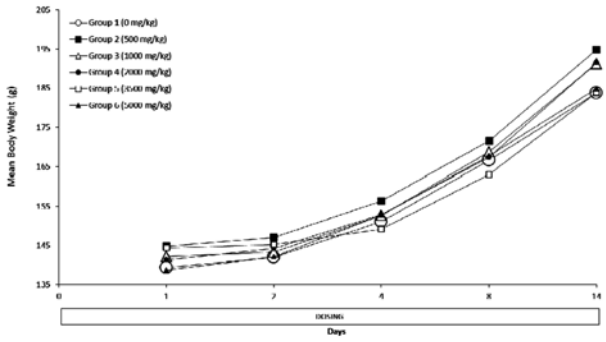

Fig. 1. Changes in body weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day in male of EHAE.

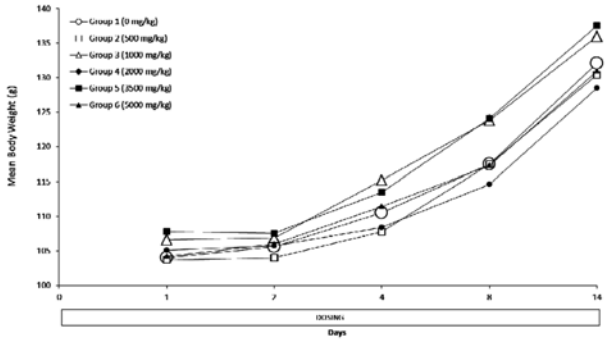

Fig. 2. Changes in body weights after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day in female of EHAE.

Table 1. Food consumption in grams after treatment with 500, 1000, 2000, 3500 and 5000 mg/kg/day of EHAE (Unit: g)

Group		G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)		0	500	1000	2000	3500	5000
Males							
Day 2	Mean	14.4	14.7	13.8	14.4	13.6	13.5
	S.D.	1.52	1.44	0.55	0.62	3.51	1.28
Day 9	Mean	13.2	15.2	15.4	14.6	13.7	12.4
	S.D.	2.93	1.45	1.47	1.11	1.54	0.99
Females							
Day 2	Mean	10.6	10.2	10.9	10.5	10.0	11.1
	S.D.	0.68	0.59	2.25	0.14	1.78	0.65
Day 9	Mean	10.9	11.0	11.5	10.2	11.1	10.7
	S.D.	0.12	0.25	0.81	0.59	1.47	0.40

* Significant differences from control group ($p < 0.05$).
+ Significant differences from control group ($p < 0.01$).

cular hemoglobin concentration, platelet, differential leucocyte absolute counts (neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cell), and reticulocyte absolute and relative (%) count were analyzed using ADVIA 2120i Hematology analyzer (Siemens, USA). In addition, blood samples treated with 3.2% sodium citrate were analyzed for prothrombin time and activated partial thromboplastin time using ACL Elite Pro coagulation analyzer (Instrumental Laboratory, Italy).

Blood samples for clinical chemistry analysis were collected into tubes without anticoagulant at the same time as for hematology, placed at the 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).

After blood sampling, the animals were killed by exsanguination from the vena cava and aorta under isoflurane anesthesia. Complete necropsy examination were performed in all animals. 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 – The collected data were statistically analyzed by multiple comparison methods. When the 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

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

Group		G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)		0	500	1000	2000	3500	5000
Males							
WBC	x10^3/µL	5.47	5.31	5.44	5.49	5.7	5.41
RBC	x10^6/µL	9.14	9.28	9.24	8.97	9.16	8.24+
HGB	g/dL	16.2	16.3	15.7	15.9	16.3	14.8
HCT	%	51.1	52.1	51.8	50.5	51.8	47.9+
MCV	fL	56.0	56.1	56.1	56.3	56.5	58.1+
MCH	pg	17.7	17.6	16.9	17.7	17.8	17.9
MCHC	g/dL	31.6	31.4	30.2	31.4	31.4	30.8+
PLT	x10^3/µL	814.2	863.2	865.8	855.6	786.8	888
RET%	%	3.45	3.49	3.48	3.8	3.99	6.52+
RETA	x10^9/µL	314.8	323.1	321.9	340.5	365.3	534.7*
NEU%	%	20.0	22.4	23.2	22.4	23.7	27.9
LYM%	%	75.3	72.9	71.8	73.0	71.3	67.9
EOS%	%	0.9	0.7	0.9	0.9	1.0	0.6
MON%	%	2.2	2.2	2.3	2.1	2.2	2.1
BAS%	%	0.7	0.7	0.8	0.7	0.8	0.7
LUC%	%	0.9	1.2	1.0	0.9	1.0	0.9
NEUA	x10^3/µL	1.08	1.19	1.27	1.24	1.37	1.54
LYMA	x10^3/µL	4.17	3.88	3.90	4.00	4.04	3.65
EOSA	x10^3/µL	0.05	0.04	0.05	0.05	0.05	0.03
MONA	x10^3/µL	0.11	0.12	0.12	0.11	0.12	0.11
BASA	x10^3/µL	0.04	0.04	0.04	0.04	0.05	0.03
LUCA	x10^3/µL	0.05	0.06	0.06	0.05	0.06	0.05
Females							
WBC	x10^3/µL	7.43	6.9	6.12	7.16	6.5	6.05
RBC	x10^6/µL	9.41	9.49	9.42	9.31	9.35	8.73+
HGB	g/dL	17	16.9	16.9	16.8	16.9	16.0+
HCT	%	52.1	52.8	52.3	52.1	52.4	50.2
MCV	fL	55.4	55.6	55.5	55.9	56.1	57.5+
MCH	pg	18	17.9	17.9	18	18.1	18.2
MCHC	g/dL	32.5	32.1	32.3	32.2	32.2	31.7+
PLT	x10^3/µL	860.6	785	789	798.8	826	840.2
RET%	%	2.92	2.99	2.87	3.3	3.6	5.94+
RETA	x10^9/µL	274.96	284.26	269.83	306.28	336.02	516.96+
NEU%	%	12.7	13.7	14.2	14.7	15.6	13.8
LYM%	%	82.5	81.5	80.7	80.3	80.1	81.7
EOS%	%	0.9	1	0.9	0.8	1	0.8
MON%	%	2.2	2.1	2.6	2.2	1.9	2
BAS%	%	0.7	0.5	0.5	0.6	0.6	0.7
LUC%	%	1.1	1.1	1.2	1.5	0.9	0.9
NEUA	x10^3/µL	1.01	0.96	0.86	1.03	0.99	0.82
LYMA	x10^3/µL	6.04	5.61	4.94	5.76	5.23	4.96
EOSA	x10^3/µL	0.07	0.07	0.05	0.06	0.06	0.05
MONA	x10^3/µL	0.18	0.15	0.16	0.16	0.12	0.13
BASA	x10^3/µL	0.05	0.03	0.03	0.04	0.03	0.04
LUCA	x10^3/µL	0.09	0.08	0.07	0.11	0.07	0.06

* Significant differences from control group ($p < 0.05$).
+ Significant differences from control group ($p < 0.01$).

$P < 0.05$ level. When found significant in ANOVA, Dunnett's test was used to determine the difference between the control and treatment groups. In the case of significant deviations from variance homogeneity in the Bartlett's test, a non-parametric comparison test, Kruskal-Wallace (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-Wallace (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 prevalence or percentage). The level of probability was taken as 1 or 5%. Statistical analyses were performed by comparing the different dose groups with a vehicle control group using Path/Tox (version 4.2.2, Xybion Medical Systems Corporation).

Results and Discussion

Although the pharmacological effect or the analysis for extracts of Epimedii Herba (EH) have been revealed by a numerous reports, the information on its safety or toxicity could not be found anywhere. To obtain a toxicity data of EH aqueous extract (EHAE), F344 rats were orally treated with EHAE for 2 weeks. Rats consisted of six groups,

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

oup		G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)		0	500	1000	2000	3500	5000
Male							
GLU	mg/dL	124.3	124.1	136.7	132.1	120.2	131
BUN	mg/dL	16.09	16.02	15.03	15.76	16.83	14.71
CREA	mg/dL	0.53	0.53	0.52	0.53	0.53	0.52
TP	g/dL	6.56	6.75	6.76	6.63	6.59	6.35
ALB	g/dL	4.64	4.7	4.72	4.65	4.64	4.55
A/G	ratio	2.41	2.3	2.32	2.36	2.39	2.54
AST	IU/L	119.7	115.3	120	105.6	107.5	107
ALT	IU/L	52.6	49	51.5	42.3	50	44.5
TBIL	mg/dL	0.081	0.085	0.108	0.083	0.082	0.078
ALP	IU/L	560.9	580.8	565.1	564.4	573.8	577.2
TCHO	mg/dL	53.8	57.0	55.8	58.6	52.6	45.3*
TG	mg/dL	39.5	54.2	53.8	49.3	41.5	34.1
CK	IU/L	750.8	756.0	806.6	716.2	638.0	640.3
PL	mg/dL	96.0	101.4	102.0	101.4	96.0	83.8*
Female							
GLU	mg/dL	77.6	77.4	91.7	91.5	76.6	82.7
BUN	mg/dL	19.85	18.47	18.31	18.97	18.88	18.49
CREA	mg/dL	0.53	0.52	0.54	0.54	0.5	0.54
TP	g/dL	6.57	6.56	6.6	6.32	6.43	6.32
ALB	g/dL	4.58	4.62	4.63	4.47	4.52	4.56
A/G	ratio	2.31	2.39	2.35	2.41	2.37	2.61+
AST	IU/L	120.9	122.3	111.9	104.2	108.9	118.3
ALT	IU/L	39.4	39.9	39	38.2	42.3	47.8
TBIL	mg/dL	0.087	0.094	0.089	0.09	0.083	0.08
ALP	IU/L	504.7	516.5	484.9	482.9	477.9	493.8
TCHO	mg/dL	93.0	85.0	89.5	97.2	90.6	76.4*
TG	mg/dL	81.2	88.0	92.5	91.6	95.0	82.8
CK	IU/L	983.8	921.4	829.5	696.8	738.2	771.4
PL	mg/dL	158.4	151.8	158.0	164.2	159.8	136.4

* Significant differences from control group ($p < 0.05$).
+ Significant differences from control group ($p < 0.01$).

which were treated with a vehicle control or 500 mg/kg/day, 1000 mg/kg/day, 2000 mg/kg/day, 3500 mg/kg/day, and 5000 mg/kg/day EHAE. Mortality, clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, macroscopic findings, and organ weight were observed.

In 14-day repeated oral toxicity study, there were no treatment-related mortalities and clinical signs in any groups throughout the study period. In addition, none of groups treated with EHAE exhibited drug-related changes in body weight (Figs. 1 and 2) and food consumption (Table 1), compared with the vehicle control group. There were no significant changes found in urinalysis test for volume, specific gravity, pH, protein, ketone body, GLU, BIL, nitrite, urobilinogen, urine cast, epithelial cell, RBC, and WBC, which were compared with control group (data not shown).

In hematology shown as Table 2, RBC count (0.90X control), hemoglobin (HGB, 0.91X), hematocrit (HCT, 0.94X), and mean corpuscular hemoglobin concentration (MCHC, 0.97X) were decreased at 5000 mg/kg/day male group. Mean corpuscular volume (MCV, 1.04X), reticulocyte count (1.70X), and reticulocyte ratio (1.89X) were increased at 5000 mg/kg/day male group (Table 2). RBC count (0.93X control), HGB (0.94X), HCT (0.96X), and MCHC (0.98X) were decreased at 5000 mg/kg/day female group. MCV (1.04X), reticulocyte count (1.88X), and reticulocyte ratio (2.03X) were increased at 5000 mg/kg/day female

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

Group	G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)	0	500	1000	2000	3500	5000
Males						
Brain	1.712	1.719	1.711	1.71	1.709	1.678
Pituitary gland	0.004	0.005	0.004	0.005	0.004	0.005
Liver	5.91	5.89	5.863	5.934	5.831	5.847
Spleen	0.433	0.424	0.41	0.435	0.423	0.458
Heart	0.644	0.633	0.658	0.685	0.619	0.645
Thymus	0.306	0.327	0.298	0.324	0.285	0.291
Salivary glands	0.32	0.315	0.302	0.312	0.313	0.318
Seminal vesicle	0.399	0.363	0.261	0.339	0.354	0.384
Prostate	0.078	0.075	0.068	0.069	0.135	0.064
Kidneys	1.375	1.423	1.411	1.435	1.370	1.407
Adrenal glands	0.037	0.039	0.038	0.04	0.035	0.042
Testes	2.395	2.369	2.332	2.415	2.348	2.326
Epididymides	0.355	0.393	0.35	0.403	0.375	0.362
Lung	0.809	0.845	0.812	0.873	0.794	0.83
Thyroid/Parathy.	0.008	0.011	0.009	0.009	0.008	0.01
Females						
Brain	1.603	1.605	1.636	1.601	1.67	1.641
Pituitary gland	0.006	0.006	0.007	0.007	0.006	0.007
Liver	4.223	4.076	4.511	4.083	4.631	4.388
Speen	0.329	0.31	0.339	0.347	0.352	0.33
Heart	0.506	0.491	0.53	0.495	0.532	0.508
Thymus	0.301	0.298	0.295	0.281	0.287	0.293
Salivary glands	0.261	0.257	0.26	0.258	0.266	0.264
Kidneys	1.075	1.023	1.1	1.031	1.142	1.102
Adrenal glands	0.046	0.042	0.044	0.041	0.049	0.051
Ovaries	0.75	0.675	0.728	0.679	0.755	0.721
Lung	0.009	0.007	0.009	0.007	0.009	0.009
Thyroid/Parathyroid	0.25	0.252	0.233	0.338	0.28	0.268
Uterus/Cervix	0.068	0.06	0.065	0.058	0.069	0.063

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

Group	G1	G2	G3	G4	G5	G6
Dose (mg/kg/day)	0	500	1000	2000	3500	5000
Males						
Brain	1.02	0.996	1.003	1.007	1.038	1.012
Pituitary gland	0.003	0.003	0.003	0.003	0.003	0.003
Liver	3.507	3.38	3.408	3.451	3.516	3.523
Spleen	0.258	0.244	0.24	0.253	0.257	0.276
Heart	0.382	0.365	0.383	0.399	0.374	0.388
Thymus	0.184	0.191	0.176	0.191	0.174	0.176
Salivary glands	0.19	0.181	0.176	0.182	0.19	0.191
Seminal vesicle	0.232	0.199	0.149	0.186	0.206	0.228
Prostate	0.046	0.042	0.039	0.041	0.08	0.038
Kidneys	0.817	0.818	0.821	0.836	0.827	0.847
Adrenal glands	0.022	0.022	0.022	0.024	0.021	0.025
Testes	1.417	1.355	1.347	1.408	1.406	1.397
Epididymides	0.21	0.22	0.2	0.233	0.22	0.217
Lung	0.481	0.487	0.472	0.512	0.48	0.5
Thyroid/Parathy.	0.005	0.006	0.006	0.005	0.005	0.006
Females						
Brain	1.38	1.39	1.349	1.403	1.362	1.418
Pituitary gland	0.006	0.005	0.006	0.006	0.005	0.006
Liver	3.614	3.523	3.721	3.57	3.77	3.768
Speen	0.283	0.268	0.279	0.304	0.287	0.284
Heart	0.435	0.424	0.437	0.433	0.432	0.438
Thymus	0.258	0.258	0.243	0.247	0.234	0.251
Salivary glands	0.224	0.223	0.214	0.226	0.216	0.227
Kidneys	0.922	0.883	0.907	0.903	0.93	0.948
Adrenal glands	0.039	0.037	0.036	0.036	0.04	0.044
Ovaries	0.058	0.051	0.053	0.051	0.056	0.054
Lung	0.648	0.582	0.6	0.595	0.614	0.621
Thyroid/Parathyroid	0.008	0.006	0.007	0.006	0.008	0.008
Uterus/Cervix	0.214	0.22	0.192	0.288	0.227	0.231

group. In clinical chemistry shown as Table 3, TCHO (0.82X, 0.84X control) and PL (0.86X, 0.87X control) were decreased at 5000 mg/kg/day male and female groups. These hematology or clinical chemistry values showed apparent difference from the control mean values, and/or were not normal limits (Charles River Laboratories Japan, Inc, Japan). Therefore, these changes of parameters in hematology or clinical chemistry are considered to be closely related to the treatment of EHAE, and require having further study for the long-term outcomes. Absolute organ weights are shown in Table 4. There were no treatment-related in either the absolute and relative organ weights changes in the treated groups compared to the vehicle control group (Table 5). In addition, no treatment-related macroscopic findings were observed in any of the treated animals.

In conclusion, EH 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 treatment-related adverse changes in clinical observations, mortality, body weights, food consumption, urinalysis, macroscopic finding at necropsy and organ weight examination. Total RBC count, HCT and MCHC, TCHO, PL were decreased in males and females at 5000 mg/kg/day compared to the control animals. MCV and reticulocyte counts were increased in males and females at 5000 mg/kg/day compared to the control animals. Therefore, we recommend that a dose level of 5000 mg/

kg/day is a highest treatment group in 13-week EH extract exposure study for further toxicity assessment.

Acknowledgments

This study was supported by a grant (13182KFDA602) from Korea Food and Drug Administration in 2013.

References

(1) Jordan, S. A.; Cunningham, D. G.; Marles, R. J. *Toxicol. Appl. Pharmacol.* **2010**, *243*, 198-216.

(2) De Smet, P. A. *Clin. Pharmacol. Ther.* **2004**, *76*, 1-17.

(3) Kang, H. K.; Choi, Y. H.; Kwon, H.; Lee, S. B.; Kim, D. H.; Sung, C. K.; Park, Y. I.; Dong, M. S. *Food Chem. Toxicol*. **2012**, *50*, 2751-2759.

(4) Fan, J. J.; Cao, L. G.; Wu, T.; Wang, D. X.; Jin, D.; Jiang, S.; Zhang, Z. Y.; Bi, L.; Pei, G. X. *Molecules* **2011**, *16*, 10123-10133.

(5) Shindel, A. W.; Xin, Z. C.; Lin, G.; Fandel, T. M.; Huang, Y. C.; Banie, L.; Breyer, B. N.; Garcia, M. M.; Lin, C. S.; Lue, T. F. *J. Sex Med.* **2010**, *7*, 1518-1528.

(6) Cho, W. K.; Kim, H.; Choi, Y. J.; Yim, N. H.; Yang, H. J.; Ma, J. Y. *Evid. Based Complement Alternat. Med*. **2012**, *2012*, 985151.

(7) Organization for Economic Cooperation and Development; Guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407. Repeated Dose 28-Day Oral Toxicity Study in Rodents. 2008.

(8) Keum, J. H.; Han, H. Y.; Roh, H. S.; Seok, J. H.; Lee, J. K.; Jeong, J.; Kim, J. A.; Woo, M. H.; Choi, J. S.; Min, B.S. *Korean Journal of Pharmacognosy* **2014**, *45*, 135-140.

Received October 31, 2014
Revised November 18, 2014
Accepted November 18, 2014