



Preclinical safety assessment of *Angelica acutiloba* using a 13-week repeated dose oral toxicity study in rats

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Angelica acutiloba (AA), a Japanese species of Danggui, has been used worldwide as a traditional herbal medicine with several bioactivities including anti-diabetic, anti-allergic, anti-inflammatory, anti-tumor, and anti-obesity. However, there is lack of toxicological data available to evaluate potential long-term toxicity and the no-observed-adverse-effect level (NOAEL) of AA extract in accordance with the test guidelines published by the Organization for Economic Cooperation and Development. In the 14-day repeat-dose toxicity study, no adverse effects on mortality, body weight change, clinical signs, and organ weights was found following repeat oral administration to rats for 14 days (125, 250, 500, 1000, and 2000 mg/kg body weight), leading that 2000 mg/kg is the highest recommended dose of AA extract for the 13-week repeat-dose oral toxicity study. In the 13-week repeat-dose oral toxicity study, the AA extract was orally administered to groups of rats for 13 weeks (125, 250, 500, 1000, and 2000 mg/kg body weight) to compare between control and AA extract groups. The administration of AA extract did not produce mortality or remarkable clinical signs during this 13-week study. And, the data revealed that there were no significant differences in food/water consumption, body weight, hematological parameters, clinical chemistry parameters, gross macroscopic findings, organ weight and histopathology in comparison to the control group. On the basis of these results, the subchronic NOAEL of the AA extract was more than 2000 mg/kg/day when tested in rats. And, the AA extract is considered safe to use orally as a traditional herbal medicine.

Keywords: *Angelica acutiloba*, traditional medicine, toxicity, subchronic

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Danggui, the root of the species *Angelica* belonging to the family Umbelliferae, has been named 'female ginseng' and has been most commonly used traditional herbal medicine for the treatment of gynecological disorders, such as menoxenia and anemia, due to its hemogenic, analgesic, and sedative activities [1-3]. According to

geographical locations, three common species of Danggui are found in Asia: Korean Danggui (*Angelica gigas* in Korea), Chinese Danggui (*A. sinensis* in China), and Japanese Danggui (*A. acutiloba* in Japan). Various chemical constituents and pharmacological effects of these three species have been reported [4-6].

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Various bioactivities of the *A. acutiloba* (AA) extract were previously reported including antitumor [7], anti-complementary [8], antioxidant [9], and anti-inflammatory activity [10-13]. And, the AA treatment attenuated fat accumulation in high fat diet-induced obesity through the up-regulation of lipid metabolism [14]. Liu *et al.* [15] have demonstrated that the AA exerted to attenuate insulin resistance and to promote glucose homeostasis as an effective ethnomedicine for treating diabetic complications. Further, that the root of *Angelica acutiloba* was reported to exert a protective effect against allergic diseases and inflammatory diseases through the inhibition of the release of histamine from mast cells and the production of pro-inflammatory cytokines [13,16-18]. Also, the AA could be a potent anti-wrinkle agent in the field of traditional medicine through the enhancement of collagen synthesis and suppression of matrix metalloproteinases [1].

The use of natural herbal medicines is increasing for self-medication without supervision because it is believed that these do not have adverse effects compared with synthetic chemical drugs [19,20]. However, it is noteworthy that we have recently found the subchronic hepatotoxic or nephrotoxic potentials of various well-known traditional medicines such as *Paecilomyces tenuipes*, *Sophorae radix*, and vinegar-processed *Genkwa flos* although they are currently available on the market [21-23]. More importantly, data on the systemic oral toxicity of the AA extract is also lacking in spite of its use as an herbal medicine in East Asia. Therefore, we investigated the subchronic repeated dose oral toxic effects of the AA extract in rats in the present study.

Materials and Methods

Test substance and animals

A hot water AA extract was provided by the National Institute of Food and Drug Safety Evaluation (Osong, Korea). AA roots were purchased from an Oriental medicine market in Korea, and an extract of AA was obtained according to a method described previously [24]. In brief, dried AA roots were ground by a mixer, and incubated with distilled water (DW) at 100°C. After filtration through filter paper, the filtrate was freeze-dried and dissolved in DW for oral administration. The extraction yield of the hot water AA extract was 0.153 g of freeze-dried AA extract/g of dried AA root.

F344 rats (SLC, Hamamatsu, Japan) were used after a

week of quarantine and acclimatization. During the studies, the animal facility was maintained under standard conditions (22±2°C, 40-60% humidity, and 12 h light/dark cycle). The animals were fed a rodent diet (LabDiet 5002 Certified Rodent Diet, PMI Nutrition International, St. Louis, MO, USA) and tap water *ad libitum*. All of the animal experiments were approved by the Institutional Animal Care and Use Committee of the Biomedical Research Institute at the Seoul National University Hospital, and this study was performed in compliance with the guidelines published by the Organization for Economic Cooperation and Development (OECD) as well as the guidance for Good Laboratory Practices for toxicity tests issued by the Ministry of Food and Drug Safety [25].

Experimental design for the oral toxicity study

For the 14-day repeat-dose toxicity study, the hot water AA extract was administered to F344 rats (5/sex/group) by oral gavage at doses of 125, 250, 500, 1000, and 2000 mg/kg of body weight/10 mL DW once daily for 14 days. For the 13-week repeat-dose toxicity study, the hot water AA extract was administered to F344 rats (10/sex/group) by oral gavage at doses of 125, 250, 500, 1000, and 2000 mg/kg of body weight/10 mL DW once daily for 13 weeks in accordance with OECD guideline 408 [26] and the US National Toxicology Program (NTP) protocol (<https://ntp.niehs.nih.gov/testing/types/cartox/protocols/13week/index.html>). During the administration period, the rats were observed for general appearance daily, and body weights, food intake, and water consumption were recorded weekly. The rats were anesthetized with isoflurane one day after the final gavage.

Hematology and serum biochemistry

Blood samples were collected *via* the posterior vena cava. The hematology parameters were measured using an automatic hematology analyzer MS9-5 Hematology Counter (Melet Schloesing Laboratories, Osny, France) for the following parameters: total white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential WBC. And, the standard serum biochemistry parameters were analyzed with an automatic chemistry analyzer 7070 (Hitachi, Tokyo, Japan) to

evaluate the following serum biochemistry parameters: blood urea nitrogen (BUN), total cholesterol (TC), total protein (TP), albumin, total bilirubin (TB), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), γ -glutamyl transferase (γ GT), creatinine kinase (CK), creatinine, triglyceride (TG), and glucose.

Gross findings, organ weights, and histopathological assessments

At the end of the treatment period, animals were exsanguinated, and organs and tissues were observed macroscopically. Organ weights were obtained for the liver, kidney, testis, thymus, heart, and lung. The eyes with the Harderian glands were fixed in Davidson

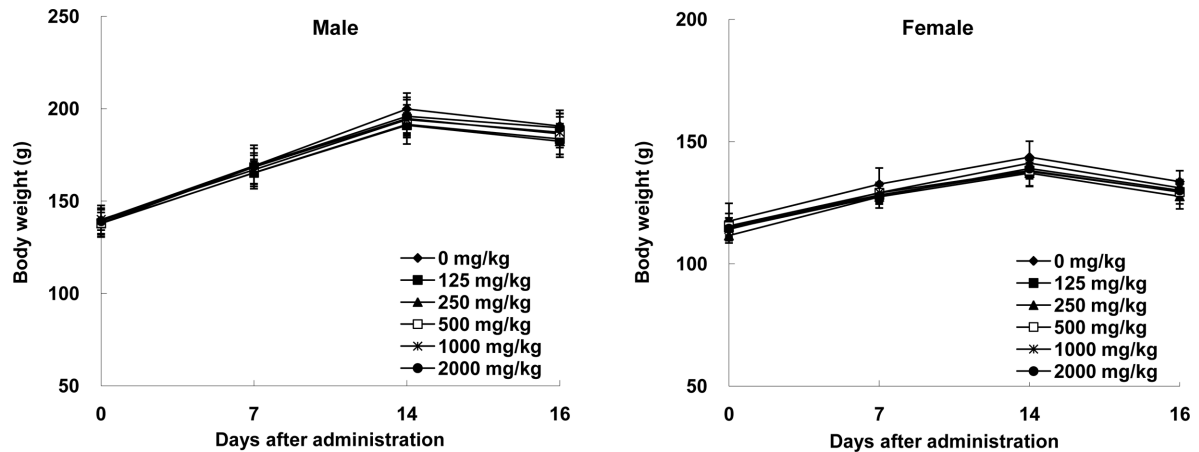


Figure 1. Effects of *Angelica acutiloba* extract on the body weight changes after oral administration in male and female rats for 14 days. Data expressed as means \pm SD.

Table 1. Organ weights for male and female F344 rats orally administered with *Angelica acutiloba* extract for 14 days

		Dose of <i>Angelica acutiloba</i> (mg/kg)					
		0	125	250	500	1,000	2,000
Males							
Liver	(g)	6.563 \pm 0.353	6.209 \pm 0.241	5.946 \pm 0.316	6.169 \pm 0.621	6.336 \pm 0.401	6.582 \pm 0.306
	(%BW)	3.441 \pm 0.107	3.385 \pm 0.172	3.258 \pm 0.072	3.303 \pm 0.151	3.381 \pm 0.096	3.468 \pm 0.050
Kidney	(g)	0.730 \pm 0.034	0.708 \pm 0.017	0.705 \pm 0.037	0.719 \pm 0.054	0.697 \pm 0.017	0.724 \pm 0.047
	(%BW)	0.383 \pm 0.011	0.386 \pm 0.021	0.386 \pm 0.011	0.386 \pm 0.012	0.373 \pm 0.016	0.381 \pm 0.011
Testis	(g)	1.089 \pm 0.101	1.122 \pm 0.061	1.104 \pm 0.080	1.086 \pm 0.150	1.081 \pm 0.135	1.088 \pm 0.118
	(%BW)	0.570 \pm 0.036	0.611 \pm 0.017	0.605 \pm 0.030	0.580 \pm 0.056	0.576 \pm 0.057	0.573 \pm 0.048
Thymus	(g)	0.362 \pm 0.039	0.460 \pm 0.282	0.359 \pm 0.025	0.361 \pm 0.033	0.361 \pm 0.026	0.352 \pm 0.014
	(%BW)	0.190 \pm 0.025	0.248 \pm 0.143	0.197 \pm 0.014	0.194 \pm 0.013	0.193 \pm 0.020	0.186 \pm 0.013
Heart	(g)	0.664 \pm 0.038	0.628 \pm 0.019	0.637 \pm 0.014	0.649 \pm 0.043	0.645 \pm 0.020	0.642 \pm 0.017
	(%BW)	0.348 \pm 0.014	0.342 \pm 0.014	0.350 \pm 0.016	0.348 \pm 0.005	0.345 \pm 0.016	0.339 \pm 0.013
Lung	(g)	0.812 \pm 0.015	0.816 \pm 0.032	0.814 \pm 0.043	0.817 \pm 0.047	0.814 \pm 0.010	0.832 \pm 0.020
	(%BW)	0.426 \pm 0.020	0.444 \pm 0.015	0.447 \pm 0.021	0.438 \pm 0.015	0.435 \pm 0.019	0.439 \pm 0.016
Females							
Liver	(g)	4.203 \pm 0.185	4.013 \pm 0.116	4.005 \pm 0.159	4.118 \pm 0.137	4.075 \pm 0.123	4.166 \pm 0.131
	(%BW)	3.145 \pm 0.064	3.101 \pm 0.080	3.139 \pm 0.073	3.183 \pm 0.087	3.110 \pm 0.106	3.204 \pm 0.092
Kidney	(g)	0.553 \pm 0.015	0.528 \pm 0.032	0.521 \pm 0.023	0.529 \pm 0.028	0.531 \pm 0.045	0.535 \pm 0.024
	(%BW)	0.414 \pm 0.008	0.408 \pm 0.020	0.409 \pm 0.032	0.409 \pm 0.021	0.405 \pm 0.029	0.412 \pm 0.025
Thymus	(g)	0.321 \pm 0.019	0.313 \pm 0.035	0.297 \pm 0.020	0.312 \pm 0.025	0.330 \pm 0.019	0.309 \pm 0.030
	(%BW)	0.241 \pm 0.019	0.241 \pm 0.022	0.233 \pm 0.016	0.241 \pm 0.015	0.252 \pm 0.017	0.237 \pm 0.019
Heart	(g)	0.515 \pm 0.027	0.481 \pm 0.019	0.462 \pm 0.020	0.487 \pm 0.025	0.487 \pm 0.016	0.490 \pm 0.009
	(%BW)	0.385 \pm 0.011	0.371 \pm 0.008	0.362 \pm 0.019	0.376 \pm 0.014	0.371 \pm 0.007	0.377 \pm 0.006
Lung	(g)	0.698 \pm 0.058	0.678 \pm 0.057	0.655 \pm 0.034	0.695 \pm 0.037	0.702 \pm 0.019	0.679 \pm 0.023
	(%BW)	0.522 \pm 0.029	0.523 \pm 0.029	0.514 \pm 0.024	0.537 \pm 0.021	0.536 \pm 0.007	0.522 \pm 0.006

solution (30 mL 95% ethyl alcohol+20 mL formalin+10 mL glacial acetic acid+30 mL DW). The testis and epididymis were fixed in Bouin's solution. Other organs including the liver, kidney, adrenal gland, urinary bladder, spleen, pancreas, thymus, thyroid gland, parathyroid gland, trachea, esophagus, lung, heart, salivary gland, lymph node, stomach, duodenum, jejunum, ileum, colon, rectum,

preputial gland, clitoral gland, skin, brain, pituitary gland, prostate, seminal vesicle, ovary, uterus, and vagina were fixed in 10% neutral buffered formalin. The nasal cavity and femora were treated with a decalcification solution for up to 3 weeks. Tissue samples were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E). After staining, the histological

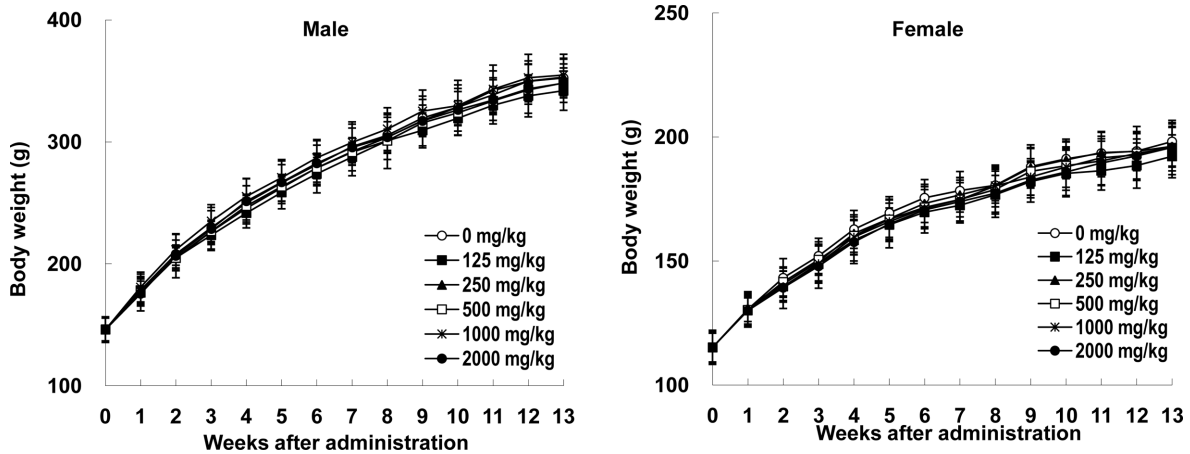


Figure 2. Effects of *Angelica acutiloba* extract on the body weight changes after oral administration in male and female rats for 13 weeks. Data expressed as means±SD.

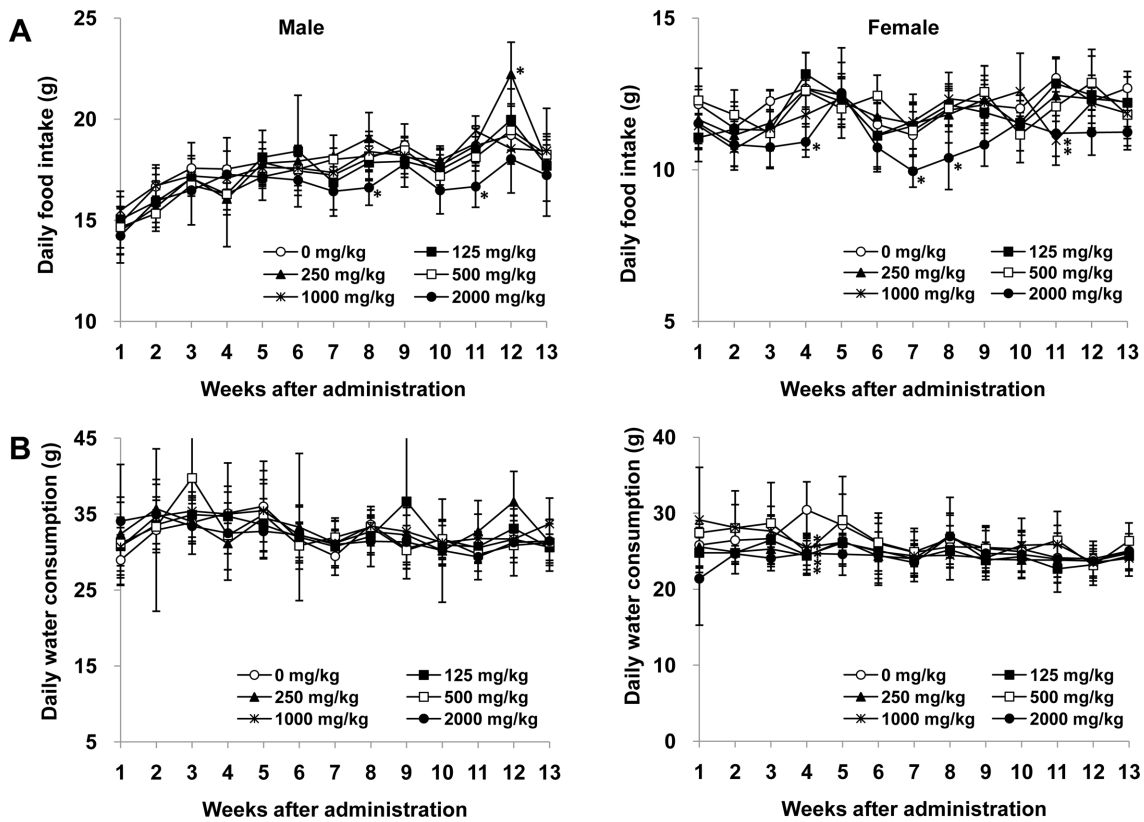


Figure 3. Effects of *Angelica acutiloba* extract on the daily food intake and water consumption after oral administration in male and female rats for 13 weeks. (A) Daily food intake. (B) Daily water consumption. Data expressed as means±SD. (*) indicates a significant difference relative to the control group (0 mg/kg) ($P<0.05$).

preparations from animals in the control, 1000, and 2000 mg/kg groups were initially examined *via* light microscopy. With respect to the organs and tissues showing significant histological changes, preparations of all rats in the other groups were examined microscopically.

Statistical analysis

All of the values are expressed as mean±SD. The statistical analysis was performed using a one-way ANOVA, followed by a multiple comparison procedure with a Tukey/Duncan test using SPSS software version 19 (SPSS Inc., Chicago, IL, USA). *P* values of less than 0.05 were considered to be statistically significant.

Results and Discussion

14-day repeat-dose oral toxicity study

In the 14-day repeat-dose oral toxicity study, the treated animals did not show any abnormal changes in the general appearance and clinical signs, and body

weights (Figure 1) throughout the test period at all selected dose levels following the oral administration of the AA extract. Likewise, there was no significant difference in gross macroscopic necropsy findings at all doses at the end of the 14 days of the experimental period. The parameters of absolute and relative organ weights showed no significant differences between the AA extract-treated groups and the control group (Table 1). On a basis of these results, 2000 mg/kg is the highest recommended dose of the AA extract for the 13-week repeat-dose oral toxicity study.

General observation, body weight, and feed/water consumption in 13-week repeat-dose oral toxicity study

In all groups, the administration of the AA extract for 13 weeks did not show increases in mortality and abnormal toxic symptoms in males and females. Similarly, the body weight gradually increased for 13 weeks both in the control group and the AA extract-

Table 2. Hematological data for male and female F344 rats orally administered with *Angelica acutiloba* extract for 13 weeks

	Dose of <i>Angelica acutiloba</i> (mg/kg)					
	0	125	250	500	1000	2000
Males						
WBC ($10^3/\text{mm}^3$)	7.5±0.9	7.0±1.1	7.4±1.2	7.7±1.3	7.5±1.3	7.0±0.8
RBC ($10^6/\text{mm}^3$)	8.4±0.4	8.1±0.4	8.2±0.3	7.8±0.9	7.8±1.2	8.0±0.4
HGB (g/dL)	14.0±0.6	13.8±0.4	13.9±0.4	13.7±0.3	13.8±0.5	13.6±0.5
HCT (%)	40.5±1.9	39.2±1.6	39.9±1.8	37.4±4.6	37.9±6.3	38.8±1.8
PLT ($10^3/\text{mm}^3$)	604.3±46.3	586.4±51.6	586.8±50.2	569.2±71.2	585.5±103.5	589.4±72.8
MCV (fl)	48.3±0.7	48.6±1.0	48.5±1.0	48.3±0.6	48.5±1.0	48.2±0.8
MCH (pg)	16.7±0.3	17.1±0.5	16.9±0.5	18.0±3.2	18.4±5.2	17.0±0.5
MCHC (g/dL)	34.5±0.9	35.1±1.0	34.8±1.2	37.3±6.6	38.2±11.8	35.2±1.0
Neutrophils (%)	17.4±1.4	17.3±1.6	16.9±2.5	17.8±2.0	18.9±1.8	17.2±2.3
Eosinophils (%)	0.3±0.2	0.3±0.2	0.3±0.1	0.2±0.2	0.3±0.2	0.3±0.1
Basophils (%)	0.7±0.1	0.6±0.1	0.6±0.2	0.6±0.1	0.6±0.2	0.6±0.2
Lymphocytes (%)	75.8±1.6	76.0±2.1	76.7±2.7	75.9±2.3	74.6±1.9	76.3±2.8
Monocytes (%)	4.4±0.3	4.5±0.6	4.2±0.5	4.1±0.3	4.4±0.4	4.3±0.5
Females						
WBC ($10^3/\text{mm}^3$)	5.9±0.5	5.1±1.2	5.7±1.2	5.7±0.5	5.6±0.7	5.5±0.6
RBC ($10^6/\text{mm}^3$)	7.4±0.5	7.1±0.6	7.7±0.7	7.5±0.6	7.6±0.5	7.4±0.7
HGB (g/dl)	13.1±0.5	12.8±0.5	13.5±0.5	13.3±0.5	13.3±0.4	13.2±0.5
HCT (%)	39.0±3.5	37.5±3.1	40.4±3.8	39.8±2.5	39.5±2.8	39.3±3.8
PLT ($10^3/\text{mm}^3$)	625.1±88.2	622.3±69.8	654.2±89.0	617.1±69.1	649.5±62.0	625.0±85.7
MCV (fl)	52.7±1.4	52.7±1.5	52.3±1.4	52.9±2.3	52.1±1.4	52.9±2.0
MCH (pg)	17.7±0.8	18.1±1.1	17.5±1.0	17.6±1.0	17.5±0.8	17.8±1.5
MCHC (g/dL)	33.7±2.2	34.3±1.9	33.5±2.0	43.4±31.7	33.6±1.7	33.7±2.4
Neutrophils (%)	12.1±2.6	14.4±5.8	12.5±1.7	12.7±1.6	14.6±3.9	12.7±2.1
Eosinophils (%)	0.2±0.2	0.2±0.2	0.2±0.2	0.3±0.1	0.3±0.2	0.2±0.1
Basophils (%)	0.5±0.2	0.4±0.1	0.4±0.1	0.5±0.1	0.4±0.1	0.4±0.1
Lymphocytes (%)	82.6±3.4	80.8±6.9	82.9±2.0	82.2±1.8	79.9±4.1	82.2±2.4
Monocytes (%)	3.6±0.6	3.2±0.7	3.1±0.5	3.2±0.5	3.7±0.6	3.4±0.5

Table 3. Serum biochemistry data for male and female F344 rats orally administered with *Angelica acutiloba* extract for 13 weeks

	Dose of <i>Angelica acutiloba</i> (mg/kg)					
	0	125	250	500	1000	2000
Males						
BUN (mg/dL)	18.5±2.7	18.6±3.7	18.1±1.9	19.8±2.8	19.0±3.4	17.8±2.5
TC (mg/dL)	65.1±2.9	67.2±6.6	66.7±7.4	68.6±8.1	67.2±4.8	71.6±4.5
TP (g/dL)	6.6±0.2	6.7±0.4	6.5±0.2	6.7±0.2	6.6±0.2	6.6±0.1
Albumin (g/dL)	3.0±0.1	3.0±0.2	2.9±0.1	3.0±0.1	3.0±0.1	3.0±0.1
TB (mg/dL)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
ALP (IU/L)	193.4±31.4	185.7±27.5	185.1±27.0	192.6±35.2	175.9±21.4	184.4±24.9
AST (IU/L)	101.0±13.3	93.5±18.3	94.5±11.0	99.8±14.4	101.6±15.9	93.4±13.3
ALT (IU/L)	66.3±5.9	62.0±13.6	61.5±6.9	60.2±5.3	71.7±15.1	61.9±12.5
γGT (IU/L)	0.2±0.4	0.2±0.4	0.0±0.0	0.2±0.4	0.3±0.5	0.4±0.7
CK (IU/L)	446.0±85.0	317.7±130.9	342.0±156.0	476.1±269.4	290.1±89.2*	346.7±68.3
Creatinine (mg/dL)	0.6±0.1	0.6±0.0	0.5±0.1	0.6±0.1	0.5±0.1	0.6±0.1
TG (mg/dL)	192.5±62.6	186.2±40.3	174.6±49.2	193.1±64.8	180.8±36.0	196.7±40.9
Glucose (mg/L)	163.5±11.0	176.5±15.1	170.5±12.2	167.0±17.4	180.0±10.7	171.4±11.2
Females						
BUN (mg/dL)	17.5±2.4	17.0±2.4	16.7±4.3	16.2±2.8	17.7±4.7	18.7±5.4
TC (mg/dL)	93.1±6.8	87.5±5.9	88.1±4.7	83.9±5.7*	81.8±7.3*	83.9±4.8*
TP (g/dL)	6.4±0.2	6.2±0.2	6.4±0.3	6.2±0.1	6.3±0.2	6.4±0.2
Albumin (g/dL)	3.0±0.1	2.9±0.1	3.0±0.1	2.8±0.1*	2.9±0.1	3.0±0.1
TB (mg/dL)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
ALP (IU/L)	167.3±39.8	168.5±28.1	164.9±24.1	162.0±20.7	148.2±28.3	177.9±15.6
AST (IU/L)	94.5±8.4	88.1±14.7	100.8±20.9	95.8±15.0	96.2±18.0	100.4±15.8
ALT (IU/L)	56.7±4.4	55.5±11.1	61.3±10.5	56.6±6.8	57.3±10.1	60.0±8.1
γGT (IU/L)	0.9±0.7	0.6±0.7	0.5±0.5	0.8±0.9	0.7±0.8	1.4±2.5
CK (IU/L)	604.7±229.7	502.3±205.1	639.1±287.0	598.9±255.7	532.4±230.6	493.2±190.2
Creatinine (mg/dL)	0.6±0.1	0.6±0.1	0.6±0.0	0.6±0.1	0.5±0.1	0.6±0.2
TG (mg/dL)	83.7±26.8	69.8±20.3	75.8±24.1	68.4±23.8	76.3±19.1	78.1±16.2
Glucose (mg/L)	154.2±18.2	164.5±14.6	156.1±24.9	150.8±21.8	151.0±15.8	160.0±15.2

*Significantly different from Control group ($p<0.05$).

treated groups throughout the study (Figure 2). And, a few incidental significant differences in the food (Figure 3A) and water intake (Figure 3B) of the animals between the AA extract-treated groups and the untreated control group were not dose-dependent. These indicate that the AA extract did not obstruct the normal growth of experimental animals.

Hematology and clinical chemistry in 13-week repeat-dose oral toxicity study

Table 2 summarizes the results of the effects of the AA extract on different hematological parameters in the 13-week repeat-dose toxicity study. All parameters were not significantly different in the treatment groups from the controls within physiologically normal ranges. It indicates that the AA extract did not affect hematopoiesis and leukopoiesis.

Table 3 shows the effects of the AA extract on different biochemical parameters. The TC levels in

females treated with 500 (83.9±5.7), 1000 (81.8±7.3), and 2000 mg/kg (83.9±4.8) of the AA extract were significantly lower than that in the control group (93.1±6.8). The albumin levels significantly decreased in females treated with 500 mg/kg (2.8±0.1) of the AA extract relative to the control females (3.0±0.1). The CK levels in males treated with the AA extract with a dose of 1000 mg/kg (290.1±89.2) were significantly lower than those in the control group (446.0±85.0). However, these statistically significant findings were not considered to be test material-related because they were within normal biological variability. In the toxicological research, liver and kidney are the two most important target organs since most of the drugs undergo many interactions such as hepatic metabolism and renal excretion following the oral administration [27,28]. Serum levels of creatinine and BUN were used as the primary indicators for kidney function [29,30]. The lack of significant changes in the levels of serum BUN and creatinine in the AA extract

Table 4. Organ weights for male and female F344 rats orally administered with *Angelica acutiloba* extract for 13 weeks

		Dose of <i>Angelica acutiloba</i> (mg/kg)					
		0	125	250	500	1000	2000
Males							
Liver	(g)	10.714±0.598	9.977±0.587	10.557±0.942	10.426±0.854	10.533±0.771	10.376±0.549
	(%BW)	3.112±0.173	3.004±0.136	3.085±0.240	3.084±0.177	3.077±0.223	3.047±0.172
Kidney	(g)	1.032±0.051	0.987±0.083	1.067±0.106	1.043±0.054	1.081±0.085	1.039±0.059
	(%BW)	0.300±0.011	0.297±0.015	0.312±0.029	0.309±0.011	0.316±0.021	0.305±0.017
Testis	(g)	1.502±0.048	1.480±0.066	1.492±0.058	1.492±0.058	1.467±0.076	1.465±0.126
	(%BW)	0.436±0.012	0.446±0.023	0.437±0.020	0.442±0.012	0.429±0.023	0.430±0.038
Thymus	(g)	0.244±0.011	0.227±0.019	0.249±0.014	0.232±0.024	0.248±0.023	0.254±0.017
	(%BW)	0.071±0.004	0.068±0.005	0.073±0.005	0.069±0.007	0.072±0.006	0.074±0.006
Heart	(g)	0.945±0.052	0.909±0.070	0.936±0.040	0.906±0.028	0.932±0.054	0.918±0.047
	(%BW)	0.274±0.011	0.273±0.011	0.274±0.006	0.268±0.010	0.272±0.012	0.269±0.005
Lung	(g)	1.206±0.030	1.166±0.095	1.236±0.084	1.185±0.066	1.180±0.076	1.169±0.090
	(%BW)	0.351±0.017	0.351±0.018	0.361±0.023	0.351±0.017	0.344±0.018	0.343±0.021
Females							
Liver	(g)	5.231±0.251	4.971±0.363	5.196±0.421	5.075±0.557	4.889±0.385	5.145±0.373
	(%BW)	2.739±0.175	2.694±0.155	2.746±0.183	2.703±0.210	2.616±0.199	2.732±0.230
Kidney	(g)	0.591±0.037	0.571±0.030	0.587±0.041	0.577±0.040	0.577±0.039	0.583±0.053
	(%BW)	0.309±0.015	0.309±0.010	0.310±0.017	0.307±0.011	0.309±0.017	0.309±0.017
Thymus	(g)	0.209±0.017	0.197±0.020	0.198±0.023	0.199±0.016	0.192±0.012	0.210±0.018
	(%BW)	0.109±0.010	0.107±0.008	0.105±0.012	0.106±0.007	0.102±0.006	0.111±0.009
Heart	(g)	0.604±0.039	0.587±0.030	0.602±0.037	0.607±0.028	0.596±0.026	0.588±0.027
	(%BW)	0.316±0.016	0.318±0.014	0.318±0.013	0.324±0.009	0.319±0.007	0.312±0.009
Lung	(g)	0.853±0.047	0.835±0.056	0.856±0.028	0.830±0.035	0.854±0.036	0.839±0.054
	(%BW)	0.447±0.031	0.452±0.023	0.453±0.012	0.443±0.006	0.457±0.014	0.445±0.018

groups suggests that the repeated administration of the AA extract did not affect kidney function in experimental animals. Liver damages such as hepatocellular damage [31] or biliary obstruction [32] can cause serum hepatic biochemical parameters (ALT, AST, and ALP) to leak into the blood circulation and rise in the serum levels. In the present study, there were no significant differences in these serum liver biomarker enzymes of the groups treated with the AA extract compared to the control, indicating that the repeated administration of the AA extract did not interfere with liver function in experimental animals.

Organ weights and histopathological changes in 13-week repeat-dose oral toxicity study

As shown in Table 4, the AA extract did not appear to affect the absolute and relative (organ-to-body weight ratios) organ weights of the male and female rats at all doses tested. The necropsy showed no significant changes in organ gross anatomy in the AA extract-treated rats when compared with the untreated rats. The histopathological studies of important major organs (i.e. liver,

kidney, lung, spleen, pancreas, and brain) indicated no toxic alteration in tissue structures following the long-term administration of the AA extract.

Based on the results of the present study, it was concluded that oral administration of the AA extract did not induce any significant adverse toxic reaction when tested for subchronic toxicity. The subchronic NOAEL for the AA extract in both sexes of rat is greater than 2000 mg/kg, which can be extrapolated to the human dose 324 mg/kg for further clinical study by the surface-area-guided dosing adjustment of the US Food and Drug Administration [33]. Among thousands of herbal products consumed in Asia, United States, and United Kingdom, AA is widely available as a functional food and traditional medicine [17]. To the best of our knowledge, there is no data available for potential long-term toxicological concerns of the AA extract, and this study is the first attempt to evaluate the subchronic toxicity in accordance with the OECD and the GLP regulations although further study is needed for major bioactive components of the AA extract.

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Conflict of interests The authors declare that there is no financial conflict of interests to publish these results.

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