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

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].
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 (γGT), 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±SD.

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

| Dose of Angelica acutiloba (mg/kg) | 0     | 125   | 250   | 500   | 1,000 | 2,000 |
|-----------------------------------|-------|-------|-------|-------|-------|-------|
| Males                            |       |       |       |       |       |       |
| Liver (g)                        | 6.563±0.353 | 6.209±0.241 | 5.946±0.316 | 6.169±0.621 | 6.336±0.401 | 6.582±0.306 |
| (%BW)                            | 3.441±0.107 | 3.385±0.172 | 3.258±0.072 | 3.303±0.151 | 3.381±0.096 | 3.468±0.050 |
| Kidney (g)                       | 0.730±0.034 | 0.708±0.017 | 0.705±0.037 | 0.719±0.054 | 0.697±0.017 | 0.724±0.047 |
| (%BW)                            | 0.383±0.011 | 0.386±0.021 | 0.386±0.011 | 0.386±0.012 | 0.373±0.016 | 0.381±0.011 |
| Testis (g)                       | 1.089±0.101 | 1.122±0.061 | 1.104±0.080 | 1.086±0.150 | 1.081±0.135 | 1.088±0.118 |
| (%BW)                            | 0.570±0.036 | 0.611±0.017 | 0.605±0.030 | 0.580±0.056 | 0.576±0.057 | 0.573±0.048 |
| Thymus (g)                       | 0.362±0.039 | 0.460±0.282 | 0.359±0.025 | 0.361±0.033 | 0.361±0.026 | 0.352±0.014 |
| (%BW)                            | 0.190±0.025 | 0.248±0.143 | 0.197±0.014 | 0.194±0.013 | 0.193±0.020 | 0.186±0.013 |
| Heart (g)                        | 0.664±0.038 | 0.628±0.019 | 0.837±0.014 | 0.649±0.043 | 0.645±0.020 | 0.642±0.017 |
| (%BW)                            | 0.348±0.014 | 0.342±0.014 | 0.350±0.016 | 0.348±0.005 | 0.345±0.016 | 0.339±0.013 |
| Lung (g)                         | 0.812±0.015 | 0.816±0.032 | 0.814±0.043 | 0.817±0.047 | 0.814±0.010 | 0.832±0.020 |
| (%BW)                            | 0.426±0.020 | 0.444±0.015 | 0.447±0.021 | 0.438±0.017 | 0.435±0.019 | 0.439±0.016 |

| Females                          |       |       |       |       |       |       |
| Liver (g)                        | 4.203±0.185 | 4.013±0.116 | 4.005±0.159 | 4.118±0.137 | 4.075±0.123 | 4.166±0.131 |
| (%BW)                            | 3.145±0.064 | 3.101±0.080 | 3.139±0.073 | 3.183±0.087 | 3.110±0.106 | 3.204±0.092 |
| Kidney (g)                       | 0.553±0.015 | 0.528±0.032 | 0.521±0.023 | 0.529±0.028 | 0.531±0.045 | 0.535±0.024 |
| (%BW)                            | 0.414±0.008 | 0.408±0.020 | 0.409±0.032 | 0.409±0.021 | 0.405±0.029 | 0.412±0.025 |
| Thymus (g)                       | 0.321±0.019 | 0.313±0.035 | 0.297±0.020 | 0.312±0.025 | 0.330±0.019 | 0.309±0.030 |
| (%BW)                            | 0.241±0.019 | 0.241±0.022 | 0.233±0.016 | 0.241±0.015 | 0.252±0.017 | 0.237±0.019 |
| Heart (g)                        | 0.516±0.027 | 0.481±0.019 | 0.462±0.020 | 0.487±0.025 | 0.487±0.016 | 0.490±0.009 |
| (%BW)                            | 0.385±0.011 | 0.371±0.008 | 0.362±0.019 | 0.376±0.014 | 0.371±0.007 | 0.377±0.006 |
| Lung (g)                         | 0.698±0.058 | 0.678±0.057 | 0.655±0.034 | 0.695±0.037 | 0.702±0.019 | 0.679±0.023 |
| (%BW)                            | 0.522±0.029 | 0.523±0.029 | 0.514±0.024 | 0.537±0.021 | 0.536±0.007 | 0.522±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, clitorial 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 Angelica acutiloba (mg/kg) | 0   | 125 | 250 | 500 | 1000 | 2000 |
|-----------------------------------|-----|-----|-----|-----|------|------|
| Males                            |     |     |     |     |      |      |
| WBC (10³/mm³)                    | 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 (10³/mm³)                    | 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±6.4 | 37.8±6.3 | 38.8±1.8 |
| PLT (10³/mm³)                    | 604.3±48.3 | 586.4±51.6 | 586.5±50.2 | 569.2±71.2 | 585.5±103.5 | 589.4±72.8 |
| MV (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)                      | 34.0±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±3.3 |
| 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 |
| Lymphocytes (%)                  | 75.8±1.6 | 76.0±2.1 | 76.7±2.7 | 75.9±2.3 | 74.6±1.9 | 76.3±3.8 |
| 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 (10³/mm³)                    | 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 (10³/mm³)                    | 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 (10³/mm³)                    | 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 | 17.8±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 |
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...
groups suggest 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.

| Dose of Angelica acutiloba (mg/kg) | 0  | 125 | 250 | 500 | 1000 | 2000 |
|----------------------------------|----|-----|-----|-----|------|------|
| **Males**                        |    |     |     |     |      |      |
| Liver (g)                        | 10.71±0.598 | 9.97±0.587 | 10.55±0.942 | 10.42±0.854 | 10.53±0.771 | 10.37±0.549 |
| (%BW)                            | 3.11±0.173 | 3.00±0.136 | 3.08±0.240 | 3.08±0.177 | 3.07±0.223 | 3.04±0.172 |
| Kidney (g)                       | 1.03±0.051 | 0.98±0.083 | 1.06±0.106 | 1.04±0.054 | 1.08±0.085 | 1.03±0.059 |
| (%BW)                            | 0.30±0.011 | 0.29±0.015 | 0.31±0.029 | 0.30±0.011 | 0.31±0.021 | 0.30±0.017 |
| Testis (g)                       | 1.50±0.048 | 1.48±0.066 | 1.49±0.058 | 1.49±0.058 | 1.46±0.076 | 1.46±0.126 |
| (%BW)                            | 0.43±0.012 | 0.44±0.023 | 0.43±0.020 | 0.44±0.020 | 0.42±0.023 | 0.43±0.038 |
| Thymus (g)                       | 0.24±0.011 | 0.22±0.019 | 0.24±0.014 | 0.23±0.024 | 0.24±0.023 | 0.25±0.017 |
| (%BW)                            | 0.07±0.004 | 0.06±0.005 | 0.07±0.005 | 0.06±0.007 | 0.07±0.006 | 0.07±0.006 |
| Heart (g)                        | 0.94±0.052 | 0.90±0.070 | 0.93±0.040 | 0.90±0.028 | 0.93±0.054 | 0.91±0.047 |
| (%BW)                            | 0.27±0.011 | 0.27±0.011 | 0.27±0.006 | 0.27±0.010 | 0.27±0.012 | 0.26±0.005 |
| Lung (g)                         | 1.20±0.030 | 1.16±0.095 | 1.23±0.084 | 1.18±0.066 | 1.18±0.076 | 1.16±0.090 |
| (%BW)                            | 0.35±0.017 | 0.35±0.018 | 0.36±0.023 | 0.35±0.017 | 0.34±0.018 | 0.34±0.021 |
| **Females**                      |    |     |     |     |      |      |
| Liver (g)                        | 5.23±0.251 | 4.97±0.363 | 5.19±0.421 | 5.07±0.557 | 4.89±0.385 | 5.14±0.373 |
| (%BW)                            | 2.73±0.175 | 2.69±0.155 | 2.74±0.183 | 2.70±0.210 | 2.61±0.199 | 2.73±0.230 |
| Kidney (g)                       | 0.59±0.037 | 0.57±0.030 | 0.58±0.041 | 0.57±0.040 | 0.57±0.039 | 0.58±0.053 |
| (%BW)                            | 0.30±0.015 | 0.30±0.010 | 0.31±0.017 | 0.30±0.011 | 0.30±0.017 | 0.30±0.017 |
| Thymus (g)                       | 0.20±0.017 | 0.19±0.020 | 0.19±0.023 | 0.19±0.016 | 0.19±0.012 | 0.21±0.018 |
| (%BW)                            | 0.10±0.010 | 0.10±0.008 | 0.10±0.012 | 0.10±0.007 | 0.10±0.006 | 0.11±0.009 |
| Heart (g)                        | 0.60±0.039 | 0.58±0.030 | 0.60±0.037 | 0.60±0.028 | 0.59±0.026 | 0.58±0.027 |
| (%BW)                            | 0.31±0.016 | 0.31±0.014 | 0.31±0.013 | 0.32±0.009 | 0.31±0.007 | 0.31±0.009 |
| Lung (g)                         | 0.85±0.047 | 0.83±0.056 | 0.85±0.028 | 0.83±0.035 | 0.85±0.036 | 0.83±0.054 |
| (%BW)                            | 0.44±0.031 | 0.45±0.023 | 0.45±0.012 | 0.44±0.006 | 0.45±0.014 | 0.44±0.018 |
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Conflict of interests  The authors declare that there is no financial conflict of interests to publish these results.

References

1. Park MA, Kim MJ, Kim YC. Anti-Photosaging Effects of Angelica acutiloba Root Ethanol Extract in Human Dermal Fibroblasts. Toxicol Res 2017; 33(2): 125-134.
2. Sheng YX, Li L, Wang Q, Guo HZ, Guo DA. Simultaneous determination of gallic acid, alibiforin, paconiforin, ferulic acid and benzoic acid in Si-Wu decoction by high-performance liquid chromatography DAD method. J Pharm Biomed Anal 2005; 37(4): 805-810.
3. Wen KC, Huang CY, Lu FL. Determination of baicalin and puerarin in traditional Chinese medicinal preparations by high-performance liquid chromatography. J Chromatogr A 1993; 631: 241-250.
4. Zhao KJ, Dong TT, Tu PF, Song ZH, Lo CK, Tsai KW. Molecular genetic and chemical assessment of radix Angelica (Danggui) in China. J Agric Food Chem 2003; 51(9): 2576-2583.
5. Lu GH, Chan K, Liang YZ, Leung K, Chan CL, Jiang ZH, Zhao ZZ. Development of high-performance liquid chromatographic fingerprints for distinguishing Chinese Angelica from related umbelliferae herbs. J Chromatogr A 2005; 1073(1-2): 383-392.
6. Piao XL, Park JH, Cui J, Kim DH, Yoo HH. Development of gas chromatographic/mass spectrometry-pattern recognition method for the quality control of Korean Angelica. J Pharm Biomed Anal 2004; 47(5): 1163-1170.
7. Yamada H, Konimaya K, Kiyohara H, Yamada H, Konimaya Y, Otsuka I. Structural characterization and antimutator activity of a pectic polysaccharide from the roots of Angelica acutiloba. Planta Med 1990; 56(3): 182-186.
8. Kiyohara H, Yamada H, Cyong JC, Otsuka Y. Studies on polysaccharides from Angelica acutiloba. V. Molecular aggregation and anti-complementary activity of arabinogalactan from Angelica acutiloba. J Pharmacobioepidemiol 1986; 9(4): 339-346.
9. Kim AR, Lee JJ, Lee MY. Antioxidative effect of Angelica acutiloba Kitagawa ethanol extract. J Life Sci 2009; 19: 117-122.
10. Yoon TS, Cheon MS, Lee DY, Moon BC, Lee HW, Choo BK, Kim HK. Effects of root extracts from Angelica gigas and Angelica acutiloba on inflammatory mediators in mouse macrophages. J Appl Biol Chem 2007; 50(4): 264-269.
11. Tanaka S, Kano Y, Tabata M, Konoshima M. Effects of "Tokii" (Angelica acutiloba Kitagawa) extracts on writhing and capillary permeability in mice (analgiesic and antiinflammatory effects]. Yakugaku Zasshi 1971; 91(10): 1098-1104.
12. Tanaka S, Ishihiro Y, Tabata M, Konoshima M. Anti-nociceptive substances from the roots of Angelica acutiloba. Arzneimittelforschung 1977; 27(11): 2039-2045.
13. Lee K, Soho Y, Lee MJ, Cho HS, Jang MH, Han NV, Shin K, Kim SH, Cho IH, Bu Y, Jung HS. Effects of Angelica acutiloba on mast cell-mediated allergic reactions in vitro and in vivo. Immunopharmacol Immunotoxicol 2012; 34(4): 571-577.
14. Liu IM, Tzeng TF, Liou SS, Chang CJ. Regulation of obesity and lipid disorders by extracts from Angelica acutiloba root in high-fat diet-induced obese rats. Phytother Res 2012; 26(2): 223-230.
15. Liu IM, Tzeng TF, Liou SS, Chang CJ. Angelica acutiloba root attenuates insulin resistance induced by high-fructose diet in rats. Phytother Res 2011; 25(9): 1283-1293.
16. Joo SS, Park D, Shin S, Jeon JH, Kim TK, Choi YJ, Lee SH, Kim JS, Park SK, Hwang BY, Lee DI, Kim YB. Anti-allergic effects and mechanisms of action of the ethanolic extract of Angelica gigas in diinflatoxygenase-induced inflammation models. Environ Toxicol Pharmacol 2010; 30(2): 127-133.
17. Sarker SD, Nahar L. Natural medicine: the genus Angelica. Curr Med Chem 2004; 11(11): 1479-1500.
18. Uto T, Tung NH, Taniyama R, Miyawakwi T, Morinaga O, Showama Y. Anti-inflammatory Activity of Constituents Isolated from Aerial Part of Angelica acutiloba Kitagawa. Phytother Res 2015; 29(12): 1956-1963.
19. Markman M. Safety issues in using complementary and alternative medicine. J Clin Oncol 2002; 20: 39-41.
20. Shin SH, Koo KH, Bae JS, Cha SB, Kang JS, Kang MS, Kim HS, Heo HS, Park MS, Gil GH, Lee YJ, Kim KH, Li Y, Lee HK, Song SW, Choi HS, Kang BH, Kim JC. Single and 90-day repeated oral dose toxicity studies of fermented Rhiz verniciflua stem bark extract in Sprague-Dawley rats. Food Chem Toxicol 2013; 55: 617-626.
21. Che JH, Yun JW, Cho EY, Kim SH, Kim YS, Kim WH, Park JH, Son WC, Kim MK, Kang BC. Toxicologic assessment of Paeclomycenes tenuepis in rats: renal toxicity and mutagenic potential. Regul Toxicol Pharmacol 2014; 70(2): 527-534.
22. Che JH, Yun JW, Kim YS, Kim SH, You JR, Jang JJ, Kim HC, Kim HH, Kang BC. Genotoxicity and subchronic toxicity of Sophorae radix in rats: hepatotoxic and genotoxic potential. Regul Toxicol Pharmacol 2015; 71(3): 379-387.
23. Yun JW, Kim SH, Kim YS, You JR, Kwon E, Jang JJ, Park IA, Kim HC, Kim HH, Che JH, Kang BC. Evaluation of subchronic (13-week) toxicity and genotoxicity potential of vinegray-processed Genkwa Flos. Regul Toxicol Pharmacol 2015; 72(2): 386-393.
24. Yun JW, Che JH, Kwon E, Kim YS, Kim SH, You JR, Kim WH, Kim HH, Kang BC. Safety evaluation of Angelica gigas: Genotoxicity and 13-weeks oral subchronic toxicity in rats. Regul Toxicol Pharmacol 2015; 72(3): 473-480.
25. Ministry of Food and Drug Safety (MFDS), 2005. Good laboratory practice regulation for non-clinical laboratory studies (Notification no. 2005-79).
26. OECD, 1998. OECD guideline for testing of chemicals, Test No. 408: repeated dose 90-day oral toxicity study in rodents.
27. Lim KT, Lim Y, Chin JH. Subacute oral toxicity study of ethanol leaves extracts of Stroblanthus crispus in rats. Asian Pac J Trop Biomed 2012; 2(12): 948-952.
28. Worasuttayangkurn L, Watcharasit P, Rangkadilok N, Suntararuks S, Khakmong P, Satayavivad J. Safety evaluation of longan seed extract: acute and repeated oral administration. Food Chem Toxicol 2012; 50(11): 3949-3955.
29. Vaidya VS, Ozer JS, Dieterle F, Collins FB, Ramirez V, Trosh T, Muniaplla N, Thudiam D, Gerhold D, Holder DJ, Bobadilla NA, Marrer E, Perentes E, Cordier A, Vonderscher J, Maurer G, Goering PL, Sistare FD, Bonventre J. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol 2010; 28(5): 478-485.
30. Chen Y, Brett D, Luo W, Gangl E, Barthlow H, Lengel D, Fikes J, Kinter L, Valentin JP, Bialecki R. Assessment of traditional biomarkers of kidney injury using an integrated rodent platform. Toxicol Appl Pharmacol 2013; 268(3): 352-361.
31. Han YD, Song SY, Lee JH, Lee DS, Yoon MH. Multizyme-modified biosensing surface for the electrochemical analysis of aspartate transaminase and alanine transaminase in human plasma. Anal Bioanal Chem 2011; 400(3): 797-805.
32. Ozdil B, Kece C, Cosar A, Akkiz H, Sandikci M. Potential benefits of combined N-acetylcysteine and ciprofloxacin therapy aspartate transaminase and alanine transaminase in partial biliary obstruction. J Clin Pharmacol 2010; 50(12): 1414-1419.
33. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008; 22(3): 659-661.