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Safety of 8-weeks oral administration of *Arctium lappa* L.

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Recently, worldwide dietary reference intakes have been considered an important guideline for public health. Some governments and the World Health Organization (WHO) provide guidelines concerning dietary intake. Although an ingredient may have a history of use as a culinary material, changes in the environment over time suggest that the acceptable maximum intake each of food/culinary material should be regularly evaluated. *Arctium lappa* L. has been used as a culinary material for many centuries in Korea and Japan and some recent studies have reported related therapeutic effects. However, there are no reports on the safety of repeated oral administration. In this study, we evaluated the safety of a 8-weeks repeated oral intake of *A. lappa*. We concluded that treatment with <250 mg/kg *A. lappa*, which was within the safety range, resulted in body weight decrease and blood glucose suppression.

Keywords: *Arctium lappa* L., safety of repeated oral intake (administration), body weight decrement, blood glucose suppression

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Currently, the nutritional aspects of excessive eating in developed countries are contrasted by severe problems, such as the scarcity of food, in developing countries. Many nations and the World Health Organization (WHO) provide guidelines for reference dietary intakes [1-4] to assist in public health. In 2013, the Ministry of Health and Welfare in Korea [1] provided dietary references for food intake that included a majority of the foods in Korean society. The guidelines contain not only a "Recommended Nutrient Intake" but also a "Tolerable Upper Intake Level" (UL). The UL is important, as it indicates that overconsumption of a nutritional food may lead to health problems. Although an ingredient may have been previously used as a culinary material, environmental changes that occur over time require that further safety studies for these ingredients should be conducted.

Arctium lappa L. has been used as a culinary material for centuries, but its recently reported bioactivities have included the suppression of renal interstitial fibrosis in obstructive nephropathy [5], a protective effect against ethanol-induced neurotoxicity [6], and gastroprotective [7] and anti-metastatic [8] effects.

However, there are no data for the safety of repeated oral intake and therefore we evaluated the safety of a 8-weeks repeated oral administration of *A. lappa* and the potential reduction of blood glucose levels.

Materials and Methods

Preparation of *Arctium lappa* extract

Arctium lappa was purchased in October 2016 from a traditional market in Naju-si Jeonnam province, Korea. A voucher specimen (DSUOB-AL-01) was deposited at

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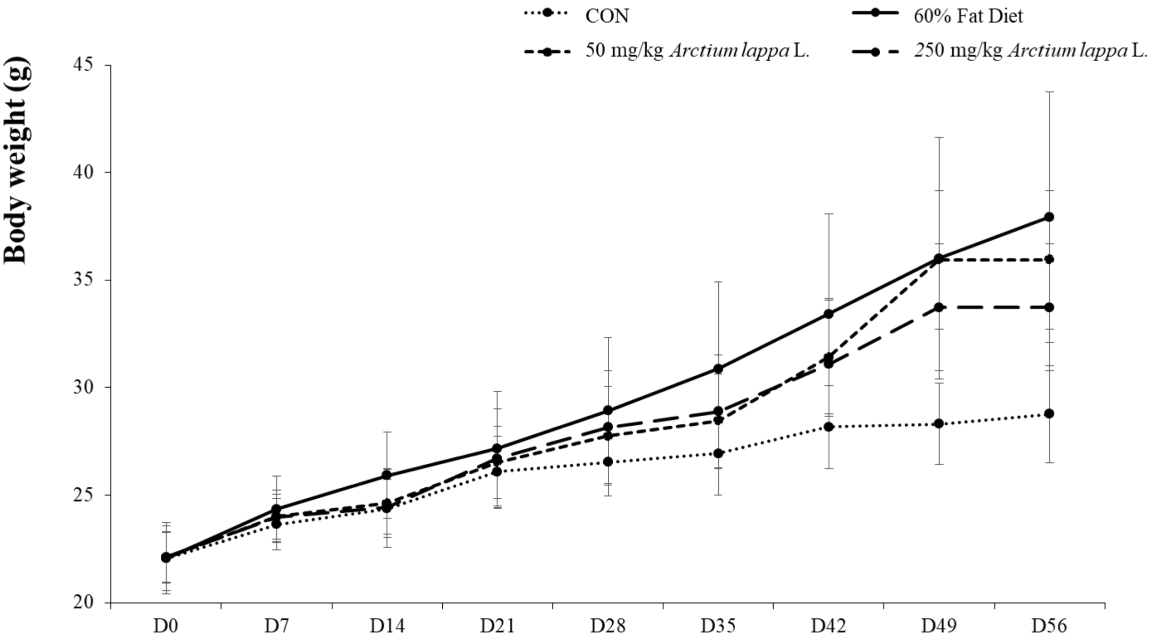
the College of Oriental Medicine, Dongshin University. The root was separated for the study and air-dried. The powdered *A. lappa* roots (250 g) were extracted twice with hot water (1 L) at room temperature for 3 days. After filtration, the water was evaporated, freeze dried, and stored at −50ºC. The crude extract was resuspended in distilled water and filtered through a 0.4-μm membrane. The yield of *A. lappa* roots extract with hot water was 5%.

Animal experiment

In order to evaluate the safety of *A. lappa*, 32 C57BL/6 mice were purchased from SamTako BioKorea (Osan, Korea) and acclimatized to experimental conditions for 7 days. The mice were divided into four treatment groups and treated for 8-weeks with *ad libitum* access to the relevant diet and water: (1) control; (2) 60% fat diet; (3) 60%-fat diet and 50 mg/kg/day *A. lappa* treatment; and (4) 60%-fat diet and 250 mg/kg/day *A lappa* treatment. The 60% fat-diet was provided by Research Diets, Inc. (Product #D12492, New Brunswick, NJ, USA; Table 2). All experiments were approved by the Institutional Animal Care and Use Committee at Chonnam National University (Approval No. CNU IACUC-YB-R-2016-50).

Macroscopic and histopathological evaluation

All animals were observed twice per day for physiological changes, such as movement, fur color/grooming, appetite, aggression, and body weight and dietary consumption was measured twice per week. At 3 h before sacrifice, feeding was restricted and the rats were anesthetized with intraperitoneal injections of 50 mg/kg Zoletil (Virbac, Fort Worth, TX, USA). After sacrifice, the appearance of all organs was observed with the naked eye to identify any toxicological changes. Whole blood



	D0	D56
CON	22.1 ± 1.66	28.8 ± 2.27
60% Fat Diet	22.1 ± 1.49	37.9 ± 5.83
50 mg/kg *Arctium lappa* L	22.1 ± 1.17	35.9 ± 3.23
250 mg/kg *Arctium lappa* L	22.1 ± 1.16	33.7 ± 2.96

Figure 1. The body weight changes over 8-weeks. Con, normal diet treatment group (control group); 60% fat diet, 60%-fat diet treatment group; 50 mg/kg *Arctium lappa* L., 50 mg/kg *Arctium lappa* L. treatment with 60%-fat diet group; 250 mg/kg *Arctium lappa* L., 250 mg/kg *Arctium lappa* L. treatment with 60%-fat diet group. The dots and the bars indicate the average and standard deviation.

Table 1. The analysis of blood and blood glucose levels

	CON	60% fat diet	60% fat diet + 50 mg/kg *A. lappa* L.	60% fat diet + 250 mg/kg *A. lappa* L.
WBC	16.6±8.32	52.4±18.36*	83.5±43.64*	64.9±33.53*
NE	2.5±1.66	13.6±10.81*	32.4±11.16**	25.4±9.53**
EO	0.3±0.23	3.8±3.96*	4.3±3.13*	3.7±3.22*
LY	9.6±5.93	32.4±10.64*	40.8±26.21	43.1±21.50*
MO	1.0±0.73	3.2±0.80*	4.3±3.30*	4.7±2.66*
Glucose[&]	241±32	474±41*	341±41*,$	230±41$$,#

The units of white blood cells and the differential cell count are ×10^2 cells/mL of whole blood and the unit of blood glucose is ×mg/dL of serum. Unit, ×10^2 cells/mL; [&]unit, mg/dL; *vs control group, P<0.01; **vs control group, P<0.005; [$]vs 60% fat diet treatment, P<0.01; [$$]vs 60% fat diet treatment, P<0.005; [#]vs 50 mg/kg *Arctium lappa* L. treatment with 60% fat diet, P<0.01

was collected from the heart and analyzed with a Hemavet Multispecies Hematology System (Drew Scientific Inc., Waterbury, CT, USA) (n=8 per group). Five organs (heart, lung, kidney, brain, and spleen) were collected for the evaluation of the morphological changes that result from the treatment with *A. lappa*. All organs were fixed in 10% (v/v) formaldehyde solution, dehydrated in a graded ethanol series (99.9, 90, 80, and 70%), and embedded in paraffin. The paraffin-embedded lung tissue was then sectioned (4 μm) longitudinally and stained with hematoxylin and eosin.

Statistical analysis

The data are shown as the mean±standard deviation (SD). Group differences were evaluated by one-way analysis of variance followed by Dunnett's multiple comparison test. Statistical significance was set at P<0.05 or P<0.01.

Results and Discussion

Arctium lappa decreases body weight gain from a high-fat diet

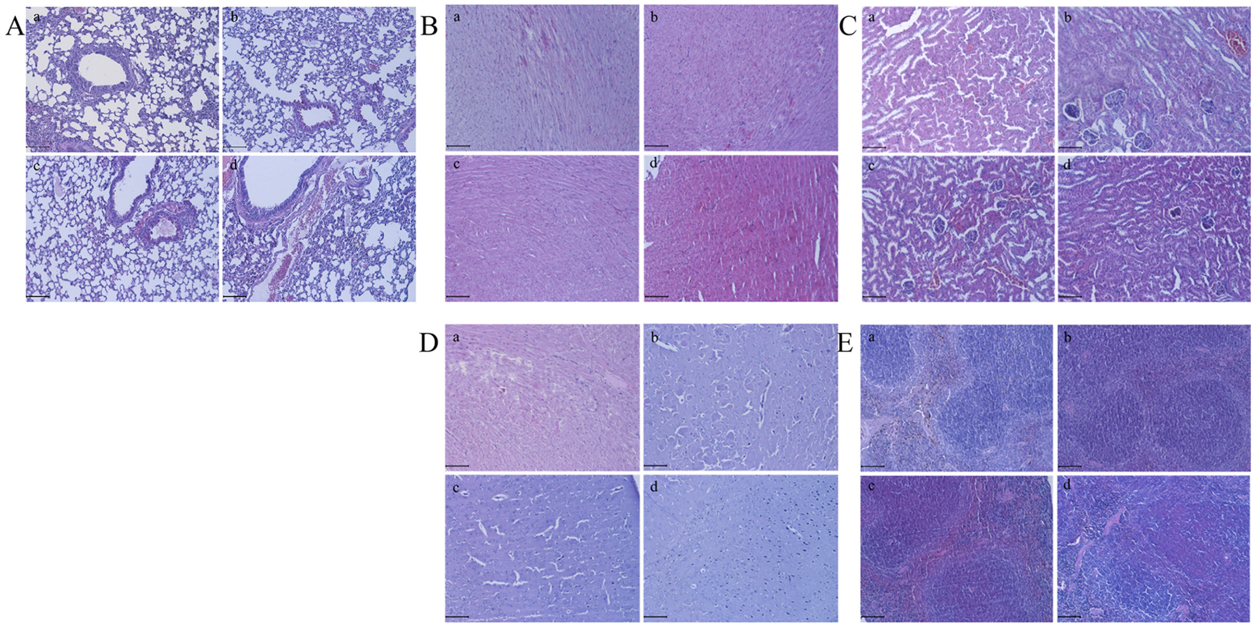
After acclimatization for 7 days, all mice were weighed and the mean body weight in each group was adjusted from 22.1±1.66 g in order to standardize the differences in body weight among each group (Figure 1). In the control group, the mice were fed a normal diet and the body weight increase was approximately 6.7 g; in comparison, the body weight of animals fed a 60%-fat diet had increased by approximately 15.8 g after 8-weeks. Although the body weight gain was the lowest in the control group, among the 60%-fat diet-fed groups, the mean body weight in the 250 mg/kg/day *A. lappa*-treated group was 33.7±2.96 g.

Arctium lappa treatment does not modulate the white blood cell level and differential count, but suppresses the blood glucose level

The comparison of the white blood cell (WBC)/differential cell counts in the control group in indicated a significant increase in the high-fat diet groups (Table 1). The number of WBC in the control group was 16.6±8.32×10^2 cells/mL and in the 60%-fat diet group it was 52.4±18.36×10^2 cells/mL. The number of WBC in the control group three times higher than that of the 60%-fat diet group. However, there was no difference between the animals fed the 60-fat diet and those fed the 60%-fat diet and treated with *A. lappa*. This result indicated that *A. lappa* treatment under 250 mg/kg/day for 8-weeks did not affect the changes in WBC. The results of the differential cell counts were similar to those of WBC. Although each cell involving WBC was increased by the 60% fat diet there, was no change between the 60%-fat diet and those fed the 60% fat-diet and treated with *A. lappa*. The results indicated that *A. lappa* L. treatment did not influence the changes of each cell in WBC.

However, the increased blood glucose level that was observed in the 60%-fat diet group was dose-dependently suppressed by *A. lappa* treatment. The blood glucose level in the 60%-fat diet group was approximately two times higher (474±41 mg/dL) compared with the control group (241±32 mg/dL), but the levels were 341±41 mg/dL in with 60%-fat diet and 50 mg/kg *A. lappa* treatment and 230±41 mg/dL with 60% -fat diet and 250 mg/dL *A. lappa* treatment. Thus, *A. lappa* treatment significantly suppressed the increase in blood glucose.

Many blood glycemic controllers have been reported as culinary ingredients and/or traditional medicine materials, such as *Aloe vera* [9], bilberry (*Vaccinium myrtillus*)



A: Lung B: Heart C: Kidney D: Brain E: Spleen

a: CON b: HFD c: 50 mg/kg *Arctium lappa* L. d: 250 mg/kg *Arctium lappa* L.

Scale bar 100 μm

Figure 2. Histopathological images from rats in each group. A, photographs of the lung; B, photographs of the heart; C, photographs of the kidney; D, photographs of the brain; E, photographs of the spleen. A, normal diet treatment group (control group); B, 60%-fat diet treatment group; C, 50 mg/kg *Arctium lappa* L. treatment with 60%-fat diet; D, 250 mg/kg *Arctium lappa* L. treatment with 60%-fat diet. Scale bar: 100 μm

[10], Okra (*Abelmoschus esculentus*) [11], and *Cinnamon* sp. [12]. However, most of their blood glucose suppressive effects were induced by extracts, not by a single isolated component of the extracts. Recently, biological effects of *A. lappa* have been reported, including the suppression of renal interstitial fibrosis [5], neuroprotection [6], gastroprotection [7], and anti-metastasis [8]. However, no reports have covered the blood glucose suppression effect and although most of anti-diabetic agents have anti-glucose effect, it is necessary to investigate the mode of action and candidate molecules of glucose suppression.

Arctium lappa treatment under 250 mg/kg is safe for 8-weeks

In order to evaluate the histopathological changes in lung (Figure 2A), heart (Figure 2B), kidney (Figure 2C), brain (Figure 2D), and spleen (Figure 2E), the relevant organs were stained with H&E. No morphological changes related to the *A. lappa* treatment were found in the organs, except for the liver. All images in the "a" panels in each photograph were considered normal as they were from the animals fed the normal diet. The "b" panels are representative images of the 60%-fat diet group. There was no difference between the control group and the

Table 2. Formula of 60% fat diet

Product#	g%	Kcal%
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Total kcal/g	5.24	
Ingredient	g	kcal
Casein, 80 Mesh	200	800
L-Cysteine	3	12
Maltodextrin 10	125	500
Sucrose	68.8	275.2
Cellulose, BW200	50	0
Soybean Oil	25	225
Lard	245	2,205
Mineral Mix, S10026	10	0
Di-Calcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium Citrate, 1 H_2O	16.5	0
Vitamin Mix, V10001	10	40
Choline Bitartrate	2	0
FD&C Blue Dye #1	0.05	0
Total	773.85	4,057

60%-fat diet group. *A. lappa* treatment did not induce morphological changes in any of the organs observed.

Many natural products have been used worldwide as a culinary material or medicine for centuries. However, although ingredients may have been previously considered safe, recent toxicological analysis techniques has classified them as toxic. In 2015, *Cynanchum auriculatum* was distributed as *C. wilfordii* [13] in Korea, but the U.S. Food & Drug Administration classified *C. auriculatum* as a poisonous plant based on the research of Han et al. [14]. For hundred years, *A. lappa* has been used as a culinary ingredient that is boiled down in soy sauce in Korea and Japan and is a frequently ingested food. However, as the toxicity of *A. lappa* repeated administration had not been previously evaluated, we conducted an assessment of the toxicity of a 8-weeks oral administration of *A. lappa*. The results indicated that *A. lappa* was very safe and had therapeutic effects, including the suppression of body weight gain and blood glucose.

Authors' Contribution

S-H Bok and SS Cho contributed equally to this work; they collected test data and drafted the manuscript. C-S Bae conducted the experiments, including analyses of the raw data. D-H Park and K-M Park designed the study and interpreted the results.

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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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