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Four-Week Repeated Oral Toxicity Study of AIP1, a Water-soluble Carbohydrate Fraction from *Artemisia iwayomogi* in Mice

Sung Ha Ryu[1], Haeran Jo[2], Ji Won Kim[2], Hyun-Joo Youn[3] and Kyu-Bong Kim[4]

[1]Department of Pharmaceutical Engineering,
[2]Department of Smart Foods and Drugs,
[3]School of Biological Sciences,
and [4]College of Pharmacy, Inje University, Obang-dong, Gimhae, Gyeongnam 621-749, Korea

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Artemisia iwayomogi, a member of the Compositae, is a perennial herb easily found in Korea and used as a traditional medicine to treat liver disease. AIP1, a water-soluble carbohydrate fraction from *Artemisia iwayomogi*, showed anti-tumor and immuno-modulating activities in animal studies. A subacute toxicological evaluation of AIP1 was performed for 4 weeks in ICR mice. After administration of AIP1 (0, 20, 100, 500 mg/kg/day), the clinical signs, mortalities, body weight changes, hematology, blood clinical biochemistry, urinalysis, organ histopathology, organ weights and gross finding were examined. The results showed that there were no significant differences in body weight changes, food intakes, water consumptions, or organ weights among different dose groups. Also we observed no death and abnormal clinical signs during the experimental period. Between the groups orally treated with AIP1 and the control group, there was no statistical significance in hematological test or serum biochemical values. Histopathological examination showed no abnormal changes in AIP1 groups. These results suggest that no observed adverse effect level (NOAEL) of the oral administration of AIP1 for 4 weeks was considered to be more than 500 mg/kg/day in mice under the condition investigated in current study.

***Key words*:** AIP1, *Artemisia iwayomogi*, 4-week toxicity, Mice

INTRODUCTION

Artemisia iwayomogi, a member of the Compositae, is a perennial herb easily found in Korea and used as a traditional anti-inflammatory medicine to treat liver diseases. *A. iwayomogi* has been used to treat various liver diseases including hepatitis (Kang *et al*., 1993; Park *et al.*, 2000). *A. iwayomogi* has been shown to have various biological functions. Methanol extracts of *A. iwayomogi* inhibited nitric oxide production of lipopolysaccharide-activated macrophages (Ryu *et al.*, 2003) and two sesquiterpenes from *A. iwayomogi*, 3-O-methyl-isosecotanapartholide and isosecotanapartholide, were shown to inhibit the expression of inducible nitric oxide synthetase (iNOS) (Ahn *et al.*, 2003). In other study, methanol extracts of *A. iwayomogi* displayed scavenging activity of peroxy-nitrite ($ONOO^-$), a potent cytotoxic oxidant formed by the reaction between nitric oxide (NO) and superoxide radical (O_2^-) (Kim *et al.*, 2004). NO synthesized by action of iNOS and its derivatives, such as $ONOO^-$, play a detrimental role in inflammation and asthma (Ricciardolo, 2003). AIP1, a water-soluble carbohydrate fraction from *A. iwayomogi*, showed anti-tumor and immune-modulating activities (Koo *et al.*, 1994) and suppressed spontaneous or 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced apoptotic death of mouse thymocytes probably by down regulating Fas gene expression in previous studies (Ji *et al.*, 2005; Hwang *et al.*, 2005). A recent study showed that AIP1 could interfere with functional differentiation of pulmonary immature dendritic cells to mature dendritic cells (Lee *et al.*, 2008a) and expression of TNF-α was inhibited by AIP1 administration (Lee *et al.*, 2008b).

AIP1 has such an immune-modulating activity, but detailed studies on the toxicology of AIP1 have not been performed yet. Therefore, we tested the toxicity of a 4-week oral repeated trial of AIP1 in ICR mice in compliance with the toxicity test guideline from the Korea Food and Drug Admin-

Correspondence to: Kyu-Bong Kim, Department of Pharmacy, College of Pharmacy, Inje University, Obang-dong, Gimhae, Gyeongnam 621-749, Korea
E-mail: kyubong@inje.ac.kr
or Hyun-Joo Youn, School of Biological Sciences, Inje University, Obang-dong, Gimhae, Gyeongnam 621-749, Korea
E-mail: mbhjyoun@inje.ac.kr

istration (KFDA) for Nonclinical Laboratory Studies (2009).

MATERIALS AND METHODS

Animal treatments. Young ICR mice (6-week-old) of both sexes were purchased from Hyochang Science (Daegu, Korea) and used after a week of quarantine and acclimatization. They were in light-controlled room (light 8:00~20:00) maintained at 23 ± 2°C with humidity of 50 ± 10% and light intensity of 100~200 Lux (Inje University Animal Resource Center). Under the controlled conditions, animals were housed into cages accordingly and were divided into four groups: one control group and three treatment groups, and each group was consisted of fifteen animals. They were free access to foods and tap water. This experiment was approved by Institutional Animal Care and Use Committee of Inje University (IACUC, approval number, 2011-40). AIP1 (0, 20, 100, or 500 mg/kg/day) was administered to ICR mice for 4 weeks (7 days/week)

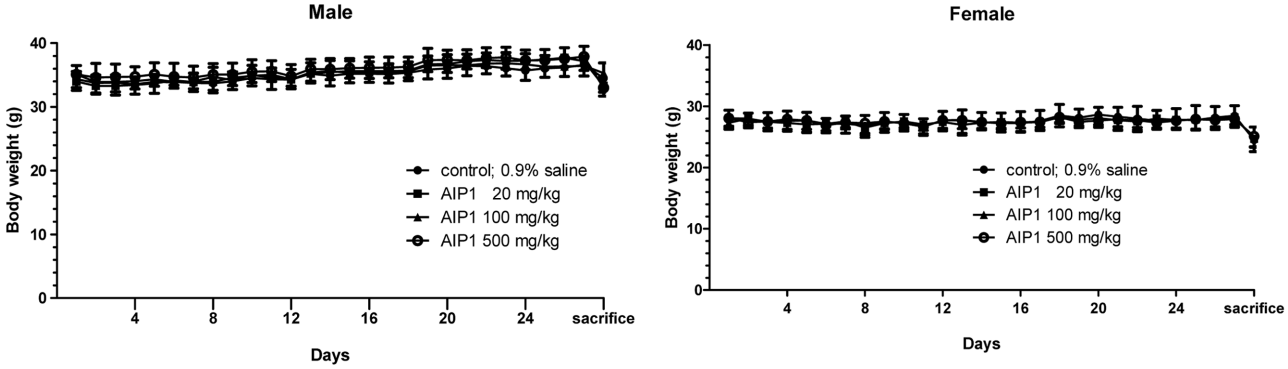

Fig. 1. Change of body weights in ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks. No significant changes were observed in all AIP1 administration groups as compared with control. All ICR mice were overnight fasted at sacrifice. Each value represents mean ± SD (n = 15).

Table 1. Absolute organ weights in ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks

Dose (mg/kg/day)	0	20	100	500
Male				
Spleen	0.15 ± 0.09	0.13 ± 0.04	0.16 ± 0.07	0.15 ± 0.05
Liver	1.45 ± 0.16	1.27 ± 0.14	1.44 ± 0.38	1.31 ± 0.12
Lung	0.22 ± 0.04	0.20 ± 0.04	0.18 ± 0.05	0.21 ± 0.03
Kidneys	0.71 ± 0.08	0.61 ± 0.05	0.61 ± 0.16	0.64 ± 0.05
Adrenals	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Heart	0.18 ± 0.01	0.16 ± 0.02	0.16 ± 0.04	0.17 ± 0.01
Thymus	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
Brain	0.46 ± 0.03	0.44 ± 0.04	0.41 ± 0.11	0.43 ± 0.04
Stomach	0.15 ± 0.02	0.14 ± 0.02	0.16 ± 0.04	0.19 ± 0.02
Prostate	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.01
Testes	0.22 ± 0.04	0.18 ± 0.05	0.20 ± 0.06	0.23 ± 0.01
Seminal vesicle	0.27 ± 0.08	0.24 ± 0.04	0.21 ± 0.07	0.19 ± 0.05
Female				
Spleen	0.09 ± 0.02	0.09 ± 0.03	0.10 ± 0.02	0.10 ± 0.02
Liver	1.01 ± 0.13	0.99 ± 0.10	1.05 ± 0.12	1.10 ± 0.14
Lung	0.24 ± 0.07	0.22 ± 0.06	0.23 ± 0.06	0.24 ± 0.05
Kidneys	0.36 ± 0.03	0.36 ± 0.03	0.39 ± 0.08	0.39 ± 0.04
Adrenals	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Heart	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
Thymus	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
Brain	0.46 ± 0.03	0.46 ± 0.03	0.50 ± 0.02	0.49 ± 0.03
Stomach	0.14 ± 0.01	0.15 ± 0.02	0.17 ± 0.03	0.16 ± 0.04
Ovaries	0.02 ± 0.00	0.02 ± 0.01	0.04 ± 0.04	0.02 ± 0.01
Uterus	0.09 ± 0.04	0.09 ± 0.02	0.12 ± 0.05	0.12 ± 0.06

Each value is expressed as mg.
Each value represents mean ± SD (n = 15).

consecutively. The high dose of 500 mg/kg/day was determined based on the result of single oral toxicity study which showed no observation of severe toxicity in SD rats treated with 10 g/kg bw. Administration of AIP1 was based on the ani-

Table 2. Relative organ weights in ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks

Dose (mg/kg/day)	0	20	100	500
Male				
Spleen	0.43 ± 0.24	0.38 ± 0.13	0.48 ± 0.18	0.46 ± 0.14
Liver	4.16 ± 0.36	3.82 ± 0.32	4.29 ± 0.41	3.96 ± 0.29
Lung	0.63 ± 0.14	0.61 ± 0.12	0.54 ± 0.04	0.65 ± 0.09
Kidneys	2.02 ± 0.19	1.82 ± 0.17	1.84 ± 0.17	1.93 ± 0.15
Adrenals	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Heart	0.51 ± 0.04	0.47 ± 0.05	0.47 ± 0.04	0.51 ± 0.05
Thymus	0.06 ± 0.03	0.06 ± 0.02	0.06 ± 0.03	0.11 ± 0.04
Brain	1.31 ± 0.10	1.32 ± 0.11	1.24 ± 0.13	1.31 ± 0.15
Stomach	0.44 ± 0.05	0.43 ± 0.04	0.46 ± 0.08	0.58 ± 0.06
Prostate	0.07 ± 0.03	0.09 ± 0.04	0.09 ± 0.04	0.12 ± 0.03
Testes	0.62 ± 0.09	0.56 ± 0.14	0.58 ± 0.11	0.69 ± 0.05
Seminal vesicle	0.76 ± 0.20	0.71 ± 0.12	0.62 ± 0.17	0.58 ± 0.13
Female				
Spleen	0.35 ± 0.06	0.36 ± 0.11	0.41 ± 0.06	0.39 ± 0.06
Liver	4.13 ± 0.31	4.15 ± 0.31	4.26 ± 0.33	4.39 ± 0.40
Lung	0.98 ± 0.26	0.91 ± 0.22	0.89 ± 0.26	0.94 ± 0.21
Kidneys	1.45 ± 0.09	1.53 ± 0.14	1.57 ± 0.27	1.56 ± 0.13
Adrenals	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Heart	0.51 ± 0.03	0.51 ± 0.05	0.54 ± 0.05	0.54 ± 0.04
Thymus	0.13 ± 0.05	0.15 ± 0.06	0.14 ± 0.05	0.15 ± 0.05
Brain	1.89 ± 0.19	1.93 ± 0.18	2.04 ± 0.14	1.95 ± 0.17
Stomach	0.57 ± 0.06	0.61 ± 0.07	0.67 ± 0.09	0.63 ± 0.17
Ovaries	0.08 ± 0.02	0.10 ± 0.05	0.15 ± 0.16	0.10 ± 0.03
Uterus	0.35 ± 0.14	0.37 ± 0.09	0.48 ± 0.17	0.47 ± 0.24

Each value is expressed as organ-to-body weight % ratio.
Each value represents mean ± SD (n = 15).

Table 3. Hematological findings of ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks

Dose (mg/kg/day)	0	20	100	500
Male				
WBC (× 10^3/μ*l*)	8.01 ± 1.77	7.74 ± 1.70	4.55 ± 0.37	10.13 ± 2.12
RBC (× 10^6/μ*l*)	10.50 ± 0.90	9.66 ± 1.26	7.54 ± 1.16	9.05 ± 0.82
HGB (g/d*l*)	14.44 ± 0.94	13.29 ± 1.85	10.66 ± 1.62	12.16 ± 1.44
HCT (%)	60.87 ± 2.13	56.71 ± 2.54	50.17 ± 3.19	52.53 ± 1.62
MCV (f*l*)	57.88 ± 1.62	56.96 ± 0.91	57.53 ± 2.22	55.37 ± 1.56
MCH (pg)	13.78 ± 0.59	13.72 ± 0.35	13.97 ± 0.62	13.26 ± 0.50
MCHC (g/d*l*)	23.85 ± 0.55	23.93 ± 0.80	24.29 ± 0.43	24.10 ± 0.75
PLT (× 10^3/ μ*l*)	1562.22 ± 143.70	1125.25 ± 112.68	1161.20 ± 119.64	1484.75 ± 78.24
Female				
WBC (× 10^3/ μ*l*)	4.05 ± 1.03	6.13 ± 0.59	5.65 ± 0.35	3.17 ± 0.23
RBC (× 10^6/ μ*l*)	9.90 ± 0.75	9.56 ± 0.69	11.75 ± 0.18	8.27 ± 0.09
HGB (g/d*l*)	13.55 ± 0.68	13.00 ± 1.37	16.55 ± 0.21	12.20 ± 1.73
HCT (%)	56.05 ± 1.06	54.30 ± 1.88	67.50 ± 1.13	49.30 ± 2.40
MCV (f*l*)	56.05 ± 1.20	58.94 ± 0.43	57.45 ± 0.07	58.80 ± 0.69
MCH (pg)	13.70 ± 0.41	13.44 ± 0.61	13.90 ± 0.35	13.80 ± 0.36
MCHC (g/d*l*)	23.18 ± 0.95	22.83 ± 1.09	24.70 ± 0.28	23.23 ± 0.78
PLT (× 10^3/ μ*l*)	1072.00 ± 66.69	1232.00 ± 81.55	1093.50 ± 19.09	914.67 ± 41.86

Each value represents mean ± SD (n = 15).
RBC, red blood cell; WBC, white blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet.

mal bodyweight measured every morning before dosing and performed between 09:00 and 10:00.

Preparation of A. iwayomogi extract and purification of AIP1 fraction. *A. Iwayomogi* was purchased from Daewon herbal medicine dealer (Changwon, Korea). A voucher specimen (CNUBio00101) was deposited at the Herbarium of the Department of Biology, Changwon National University. A crude extract was prepared by percolating dried leaves of *A. iwayomogi* with distilled water at 100°C for 2 h. The extract was filtered with Whatman No.1 paper and then lyophilized. The yield of dried extract from starting crude materials was about 10%. The dried extract was dissolved in water and autoclaved and then stored in room temperature. AIP1 fraction was isolated from the crude extract as described by Koo *et al.* (1994) and Ji *et al.* (2005). Briefly, a water soluble crude extract was fractionated using Sephadex G-50 size exclusion chromatography with distilled water. The fractions containing carbohydrates or similar compounds were determined by the modified phenol/sulfuric acid method of total sugar determination (Taylor, 1995), producing a minor peak around 50 kDa and a major peak smaller than 2 kDa. The fractions smaller than 2 kDa were pooled and used as AIP1 fraction (Koo *et al.*, 1994). The potential presense of endotoxin in AIP1 was tested by the Limulus amebocyte lysate assay (Sigma, USA). No detectable entotoxin was present in AIP1.

Bodyweight changes. Bodyweight of each mouse was measured at the initiation of administration, once a day thereafter, and on the day of scheduled autopsy.

Food and water consumption. Food and water consumption were measured per cage at the start of treatment and at daily intervals thereafter.

Gross finding. At the scheduled termination, all male and female ICR mice were anesthetized by CO_2 and sacrificed by exsanguination from the abdominal aorta. Complete gross postmortem examinations were performed on all animals.

Organ weights. At necropsy, all animals were carefully examined macroscopically and spleen, liver, lung, kidney, adrenals, heart, thymus, brain, stomach, prostate (male), testes (male), seminal vesicle (male), ovaries (female), and uterus (female) were isolated and weighed.

Urinalysis. During the last week of administration, urinalysis of four animals per group from each sex was con-

Table 4. Serum biochemical findings of ICR mice orally treated with AIP1 *Artemisia iwayomogi* for 4 weeks

Dose (mg/kg)	0	20	100	500
Male				
ALB (mg/d*l*)	3.13 ± 0.25	2.97 ± 0.15	2.67 ± 0.42	3.13 ± 0.15
ALP (U/*l*)	6.00 ± 1.73	15.00 ± 14.14	34.67 ± 12.10	46.67 ± 3.79
ALT (U/*l*)	61.00 ± 5.66	90.67 ± 24.58	82.33 ± 13.05	91.67 ± 62.18
T-BIL (mg/d*l*)	0.27 ± 0.06	0.23 ± 0.06	0.33 ± 0.12	0.20 ± 0.00
Ca (mg/d*l*)	10.90 ± 0.36	9.53 ± 0.95	9.57 ± 0.23	10.70 ± 0.30
CHOL (mg/d*l*)	125.67 ± 17.47	133.67 ± 16.20	113.00 ± 18.52	127.67 ± 8.14
CREA (mg/d*l*)	0.10 ± 0.00	0.13 ± 0.06	0.13 ± 0.06	0.10 ± 0.00
GLU (mg/d*l*)	85.50 ± 3.54	95.00 ± 14.73	115.00 ± 26.21	121.00 ± 19.00
PHOS (mg/d*l*)	11.60 ± 0.96	10.07 ± 1.07	9.30 ± 0.61	11.70 ± 0.44
TP (g/d*l*)	5.13 ± 0.67	5.47 ± 0.59	4.87 ± 0.32	5.27 ± 0.21
BUN (mg/d*l*)	25.00 ± 2.00	26.67 ± 1.53	26.33 ± 1.15	26.67 ± 2.08
Female				
ALB (mg/d*l*)	3.63 ± 0.21	3.87 ± 0.12	3.70 ± 0.10	3.80 ± 0.20
ALP (U/*l*)	63.33 ± 2.08	45.00 ± 26.85	38.00 ± 9.90	69.00 ± 11.31
ALT (U/*l*)	84.33 ± 31.79	68.67 ± 4.04	56.67 ± 5.51	69.00 ± 5.66
T-BIL (mg/d*l*)	0.23 ± 0.06	0.20 ± 0.00	0.17 ± 0.06	0.20 ± 0.00
Ca (mg/d*l*)	13.13 ± 5.31	12.60 ± 3.20	10.07 ± 0.47	10.63 ± 0.50
CHOL (mg/d*l*)	93.50 ± 13.44	94.00 ± 3.00	98.33 ± 0.58	96.00 ± 7.55
CREA (mg/d*l*)	0.10 ± 0.00	0.09 ± 0.07	0.10 ± 0.00	0.10 ± 0.00
GLU (mg/d*l*)	91.33 ± 7.64	105.33 ± 14.47	100.33 ± 13.32	93.00 ± 11.27
PHOS (mg/d*l*)	13.63 ± 2.74	11.77 ± 0.29	13.07 ± 0.51	12.67 ± 1.16
TP (g/d*l*)	4.87 ± 1.06	5.53 ± 0.32	5.73 ± 0.58	5.87 ± 0.06
BUN (mg/d*l*)	19.33 ± 0.58	19.67 ± 1.15	20.67 ± 1.53	19.33 ± 2.52

Each value represents mean ± SD (n = 15).
ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; T-BIL, total bilirubin; Ca, calcium; CHOL, cholesterol; CREA, creatinine; GLU, glucose; P, phosphorus; TP, total protein; BUN, blood urea nitrogen.

ducted with fresh urine to determine specific gravity (S.G), glucose, ketone, blood, leukocyte, pH, urobilinogen, bilirubin, nitrate, and protein contents using CYBOW™ Reader 300 and CYBOW™ 100 Strips (DEFI Co., Gimhae, Korea).

Hematology. Blood samples were drawn from the *abdominal aorta* by using a syringe with 23 gauge needle under anesthesia (CO_2). The blood samples were collected into CBC bottles containing EDTA-2K (Sewon Medical, Seoul, Korea). Hematological parameters were determined using POCH-100i (Green Medical, Gwangju, Korea), including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT).

Serum biochemistry. To get sera for serum biochemistry, blood samples were centrifuged at 4,000 rpm for 10 minutes after 30 minutes collection. The sera were stored in a -80°C freezer before they were analyzed. Serum biochemistry parameters including albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (GLU), phosphorus (P), total protein (TP), and blood urea nitrogen (BUN) were evaluated by Cobas C III (Hoffmann-La Roche Ltd., Bazel, Swiss).

Table 5. Urinalysis findings of ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks

Sex		Male				Female			
Dose (mg/kg/day)		0	20	100	500	0	20	100	500
No. of animal		4	4	4	4	4	4	4	4
S.G[a]		1.01 ± 0.000	1.01 ± 0.005	1.01 ± 0.000	1.01 ± 0.005	1.01 ± 0.003	1.01 ± 0.003	1.01 ± 0.004	1.02 ± 0.004
Uro[b]	norm	4	4	4	4	4	4	4	4
Glu[c]	-	4	4	4	4	4	4	4	4
Bili[d]	-	4	4	4	4	3	1	2	1
	1+	0	0	0	0	1	3	2	2
	2+	0	0	0	0	0	0	0	1
Ketone[e]	-	2	1	3	1	1	1	3	1
	T	2	3	1	3	3	3	1	3
Blood[f]	-	3	3	3	4	3	4	3	4
	1+	0	0	0	0	1	0	0	0
	2+	1	1	1	0	0	0	0	0
	3+	0	0	0	0	0	0	1	0
pH	6.5	0	0	0	1	2	2	2	2
	7	0	2	1	2	0	0	0	1
	7.5	0	0	1	0	1	0	1	0
	8	3	1	2	0	1	1	0	1
	8.5	1	1	0	1	0	1	1	0
Protein[g]	T	0	0	0	0	1	0	1	0
	1+	1	1	1	0	0	1	1	2
	2+	1	0	2	3	2	2	2	2
	3+	2	3	1	1	1	1	0	0
Nitrate[h]	-	1	2	1	3	3	3	3	3
	+	3	2	3	1	1	1	1	1
Leukocyte[i]	-	3	4	4	3	4	3	3	4
	1+	0	0	0	1	0	1	0	0
	2+	1	0	0	0	0	0	1	0

[a]Specific gravity: Each value represents mean ± SD.
[b]Urobilinogen: norm = normal = 0.1 mg/d*l*.
[c]Glucose: - = negative.
[d]Bilirubin: - = negative; 1+ = small; 2+ = moderate.
[e]Ketone: - = negative: T 15 mg/d*l*.
[f]Blood: - = negative; 1+ = 10 RBC/μ*l*; 2+ = 50 RBC/μ*l*; 3+ = 250 RBC/μ*l*.
[g]Protein: T < 30 mg/d*l*; 1+ = 30 mg/d*l*; 2+ = 100 mg/d*l*; 3+ = 300 mg/d*l*.
[h]Nitrite: - = negative; + = positive.
[i]Leukocyte: - = negative; 1+ = 25 WBC/μ*l*; 2+ = 75 WBC/μ*l*.

Histopathology. All Organs were fixed and preserved in 10% formalin. Fixed tissues were routinely processed for embedding in paraffin, sectioned, and stained with Hematoxylin & Eosin (H&E). All processed tissues were grossly and microscopically (×400) examined.

Statistical analysis. Quantitative differences between the control and treatment groups in terms of body weight, urinalysis, hematology, serum biochemistry, and organ weights were statistically analyzed using the ANOVA followed by Bonferroni test (Prism 5.01, San Diego, CA, USA). $P < 0.05$ were considered statistically significant.

RESULTS

Body weight changes and food and water consumption. No significant changes on body weight and gains were observed in all AIP1 dosing groups (20, 100, or 500 mg/kg/day) tested as compared with the control (Fig. 1). Male mice gained body weights gradually, but female mice showed fluctuation of body weight changes. However, there were no significant difference of body weight changes between vehicle control and AIP1 treated groups. The last body weight observation showed decrease due to 12-h fasting for sacrifice. AIP1 treatment did not alter food and water consumption (data not shown).

Organ weights. No significant changes in absolute and relative organ weights were observed in all dosing groups (Table 1 and 2). All the values of absolute and relative organ weights are in the range of normal mice.

Hematology and serum biochemistry. AIP1 administration didn't consistently change hematology and serum biochemistry parameters (Table 3 and 4). AIP1 didn't increase the activity of serum toxicity makers (ALT, CREA, BUN), indicating normal liver and kidney function. All the values of hematology and serum biochemistry are in the range of normal mice.

Urinalysis. Urinalysis didn't show significant changes in urinary parameters from animals treated with AIP1 (Table 5).

Gross finding. Gross examination showed no abnormal changes in all animals. During administration of AIP1, excretion and hair quality and quantity didn't change.

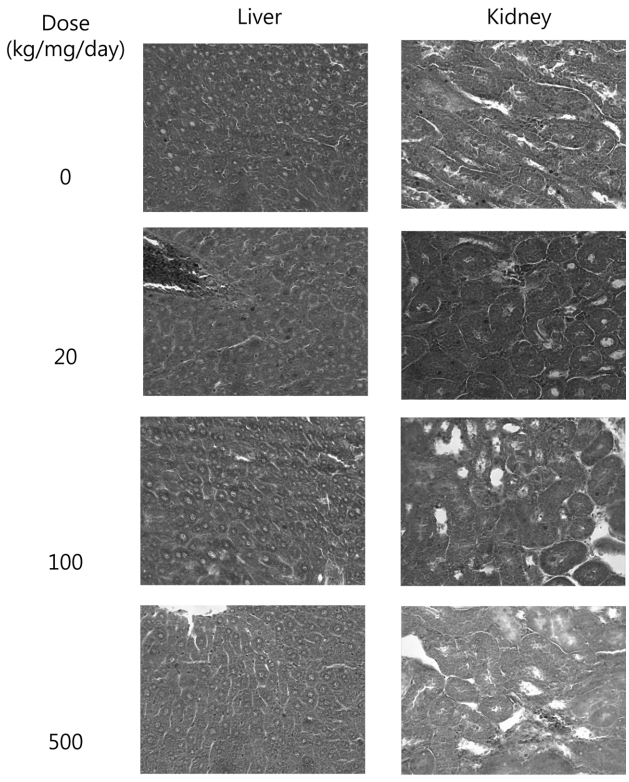

Fig. 2. Histopathological findings of male livers and kidneys from ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks. No significant changes were observed in all AIP1 administration groups as compared with control. All processed tissues were grossly and microscopically (×400) examined.

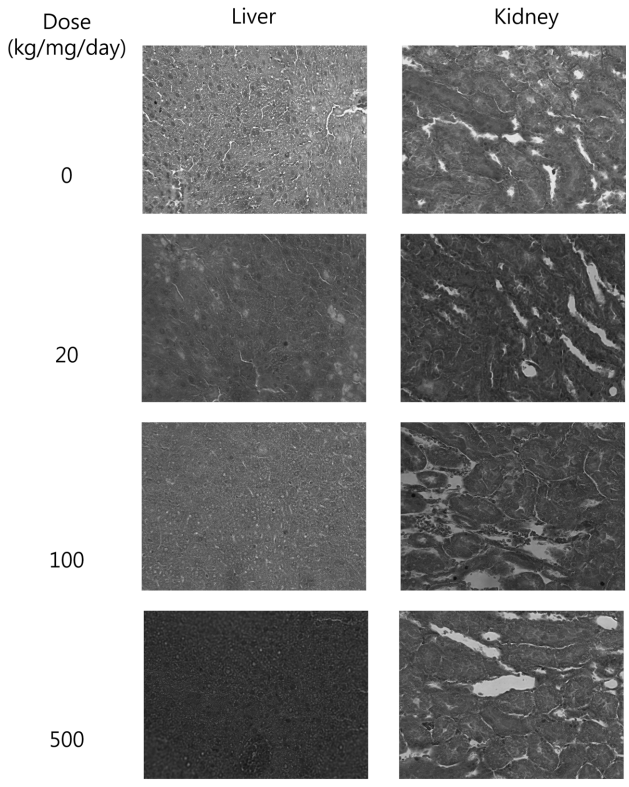

Fig. 3. Histopathological findings of female livers and kidneys from ICR mice orally treated with AIP1 from *Artemisia iwayomogi* for 4 weeks. No significant changes were observed in all AIP1 administration groups as compared with control. All processed tissues were grossly and microscopically (×400) examined.

Histopathology. No histopathological findings were observed all AIP1 administration groups as compared with control. Administration of AIP1 didn't affect the liver and kidney tissue or produce morphological variation in their linings (Fig. 2 and 3). Administration of AIP1 didn't induce pathological variation in spleen, lung, adrenals, heart, thymus, brain, stomach, prostate (male), testes (male), seminal vesicle (male), ovaries (female), and uterus (female) (data not shown).

DISCUSSION

AIP1 has anti-tumor and immuno-modulating activity (Koo *et al.*, 1994), but detailed studies on the toxicology of AIP1 have not been performed. Therefore we tested to investigate the potential subacute toxicity of AIP1 in mice. AIP1 was administered to ICR mice at dose levels of 0, 20, 100 and 500 mg/kg/day for 4 weeks, consecutively. After administration of AIP1 (0, 20, 100, 500 mg/kg/day), the clinical signs, mortalities, body weight changes, hematological parameters, serum biochemical parameters, urinalysis, histopathological changes, organ weights and gross finding were examined.

As the results, we could not find body weight changes, food intakes, water consumptions or organ weights among different dose groups. Also we observed no death and abnormal clinical signs during the experimental period. Between the groups orally administered AIP1 and the control group, there was no statistical significance in hematological test or serum biochemical values ($p > 0.05$). Single oral toxicity study was performed for AIP1 using rats previously (data not shown). Maximum dose of 10 g AIP1/kg was treated to Sprague-Dawley rats and did not show any adverse effect.

These results suggest that no observed adverse effect level (NOAEL) of the oral application of AIP1 was considered to be more than 500 mg/kg/day in mice under the condition investigated in this study. Current study is the toxicological data for AIP1 and will be useful to design clinical trial for development of AIP1.

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