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Toxicity Study of AS6, a Triterpenoid Derivative: 4-Week Repeated Oral Administration in Rats

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Abstract – The present study was conducted to investigate the potential subacute toxicity of AS6, [(3β,4α)-3,23-dihydroxyurs-12-en-28-oic acid], by a 4-week repeated oral administration in Sprague-Dawley rats. To test the subacute toxicity, AS6 was administered once daily by gavage to rats at dose levels of 0, 250, 500, and 1000 mg/kg/day for 4 weeks. There were no treatment-related effects on mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, necropsy findings, organ weights, and histopathology in any treatment group. In the condition of this study, target organ was not observed and the no-observed-adverse-effect level (NOAEL) was considered to be 1000 mg/kg/day for both male and female rats.

Keywords □ AS6, 4-Week subacute toxicity, rats

Centella asiatica (L.) Urban has been used as a traditional herbal medicine in Asiatic countries for hundreds of years (Singh and Rastogy, 1969). Especially, asiaticoside (AS), a biologically active triterpenoid present in this plant has been known to have a variety of biological effects such as anti-inflammatory, wound-healing, antiulcer, hepatoprotective, skin-tumor prevention, and immunomodulatory effects (Price *et al.*, 1987; Nishino *et al.*, 1988; Maquart et al., 1999; Tan *et al.*, 1997). Among the biological effects of AS, hepatoprotection is one of the more notable (Liu *et al.*, 1994). Recently, Dong-Kook Pharmaceutical Co. synthesized 150 asiaticoside derivatives and compared the hepatoprotective effect in primary cultured liver cells of rats having acute liver injury induced by CCl_4 and galactosamine. Among them, AS6 was found to be the most effective in decreasing chemical-induced liver injury and also had the inhibitory effect in chronic liver cirrhosis (unpublished data). AS6 (mol. wt. 472.7 Da and melting point of 277-278°C) is white crystalline powder with the formula of [(3β,4α)-3,23-dihydroxyurs-12-en-28-oic acid]. In our previous acute toxicity study, a single oral administration of AS6 at 5000 mg/kg resulted in no toxicological effects in rats (unpublished data).

In this study we report the results of 4-week repeated oral dose toxicity study in Sprague-Dawley rats as a part of the preclinical safety evaluation program for AS6. This study was conducted according to the test guidelines from the KFDA and OECD guidelines for the testing of chemicals under modern Good Laboratory Practice Regulations.

MATERIALS AND METHODS

Materials

AS6 (purity ≥ 99.3%) was supplied from Dong Kook Pharmaceutical Co. (Jincheon, Korea) and was suspended in 0.5% methylcellulose solution. The oral administration was selected for animal treatment in the present study, because the oral route is a clinically intended route.

Dosing and dose selection

Healthy males and females were randomly assigned to four experimental groups: Based on the results of the preliminary study, 1000 mg/kg was selected for the high dose in this study.

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Doses of 500 and 250 mg/kg were selected as middle and low doses, respectively, using a common ratio of 2. The negative control rats received 0.5% methylcellulose solution and the daily volume of administration was calculated according to the most recent body weight. Each group consisted of 10 rats of each sex.

Animal treatment

For 4-week subacute toxicity study, forty-eight Sprague-Dawley rats of each sex were obtained from the Korea Institute of Toxicology Breeding Facility (KIT, Daejeon, Korea) at 4 weeks of age and used after one week of quarantine and acclimatization. The animals were kept in stainless wire cages. Only healthy animals were assigned to the study. An ambient temperature of 25±2°C, relative humidity of 50±2%, and photoperiod of 12 h was maintained throughout the study. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (Jeil Feed Co, Daejeon, Korea) *ad libitum*. All animal experiments were conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), and animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality. Detailed clinical observations were recorded and printed by Labcat Computer System (Innovative Programming Associates Inc., NJ, USA), respectively. At scheduled termination, all surviving animals were anesthetized by ether inhalation for blood sample collection, taken blood samples, and then sacrificed by exsanguination from the aorta. Complete gross postmortem examinations were performed on all terminated and dead animals. Body weight of each rat was measured at the initiation of treatment, twice a week thereafter, and on the day of scheduled autopsy.

Food and water consumption was measured per cage at the start of treatment and at weekly intervals thereafter. The amounts of food and water were calculated before they were supplied to each cage and their remnants were measured, which was regarded as a daily food and water consumption (g/rat/day). The absolute and relative (organ-to-body weight ratios) weights of following organs were measured in all survivors when they were sacrificed: brain, pituitary gland, adrenal glands, liver, spleen, kidneys, heart, thymus, lung, salivary glands, thyroid glands, testes, epididymis, seminal vesicles, prostates, ovaries, and uterus.

Ophthalmoscopy

External eye examination of all males and females was carried out shortly before the start of treatment. And the examination of all males and females was also conducted shortly before the termination of treatment. The ocular fundus was examined shortly before the termination of treatment using a indirect binocular ophthalmoscope (IO-H, Neitz Instruments Co., Japan) in the negative control and highest dose groups. Conjunctiva, sclera, cornea, lens and iris of each eye were also examined.

Urinalysis

During the last week of treatment, urinalysis of 5 animals per group from each sex was conducted with fresh urine to determine specific gravity, pH, protein, glucose, ketone body, occult blood, bilirubin, urobilinogen, and nitrite by using a CliniTek-100 urine chemistry analyzer (Ames Division, Miles Laboratory, USA). Urine sediment test was also carried out within three hours after taking samples during the last week of administration period. The urine collected for 17 hours was measured for the volume. During the collection, the rats were housed in metabolic cages, which allowed for separate collection of urine and feces.

Hematological and biochemical analysis

The blood samples were collected into CBC bottles containing EDTA-2K (Green Cross Medical Industry, Korea), and were analyzed within 20 minutes in our laboratory. Red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and white blood cell count (WBC) were determined using a Coulter counter T-540 (Coulter Counter Electronics, USA). Differential WBC counts were made with a glass-slide method using the remaining blood after automatic analysis. Smears were air-dried immediately and stained subsequently with Wrights stain. Then, 200 cells were randomly counted in each smear. Following evaluation of the differential cell counts, the resulting percentage data were converted into absolute numbers using the total WBC count. Reticulocyte count was carried out with blood smear samples that were stained with new methylene blue stain. Any red or white blood cell morphological changes were also noted from these blood films.

To get the sera for serum biochemistry, blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour after collection. The sera were stored in the −80°C freezer before they were analyzed. Serum biochemistry parameters including

aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), glucose, total protein (TP), albumin, albumin/globulin ratio (A/G ratio), blood urea nitrogen (BUN), creatinine, triglyceride, phospholipid, total cholesterol, total bilirubin, calcium, and inorganic phosphorus were evaluated by an autoanalyzer (Shimadzu CL-7200, Shimadzu Co., Japan). Serum electrolytes such as chloride, sodium, and potassium were measured by an ion autoanalyzer (644 Na/K/Cl Analyzer, Ciba-Corning Co., USA).

Histopathology

The following tissues were obtained from all animals: abnormal lesions, skin (including mammary gland), spleen, pancreas, jejunum, stomach, duodenum, ileum, cecum, colon, mesenteric lymph node, salivary gland, submandibular lymph node, ovaries, uterus, vagina, urinary bladder, epididymides, prostates, seminal vesicles, rectum, kidneys, adrenal glands, liver, sternum, thymus, heart, lung, trachea, esophagus, thyroids (including parathyroids), tongue, aorta, sciatic nerve, skeletal muscle, femur, thoracic spinal cord, Harderian glands, brain, pituitary gland, eyes, and testes. Eyes and testes were preserved in Davidson's fixative and Bouin's fixative, respectively. Other tissues were fixed with 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin, and sectioned at 3~5 μm. The sections were stained with Hematoxylin-Eosin stain for microscopic examination. All organs and tissues taken from all animals in the negative control and highest dose groups were examined microscopically. All gross lesions as defined by the study pathologist were also included in the examination.

Statistical analyses

Statistical analyses were performed by comparing the treatment groups with the negative control group using either Labcat Computer System or Statistical Analysis Systems (SAS/STAT User's Guide Version 6.12, NC, USA). Whenever, the data were presented as mean±SD. Variance of numerical data was checked by Bartlett's test. If the variance was homogeneous, the data was subjected to one-way analysis of variance (ANOVA) and, if not, they were analyzed by the Kruskal-Wallis nonparametric ANOVA. If either of these tests showed a difference between the groups, the data were analyzed by the multiple comparison procedure of the Dunnett's or Scheffe's post-hoc test. Results of urinalysis obtained with reagent strips were analyzed by the Kruskal-Wallis test followed by multiple comparisons using the Scheffe's test. Clinical observations, necropsy findings, and histopathological findings were represented in frequency and were subjected to the Fisher's exact probability test. The level of significance was taken as $p<0.05$ or $p<0.01$.

RESULTS

Clinical signs, mortality and body weight changes

No animals died during the study. There was one case of loss

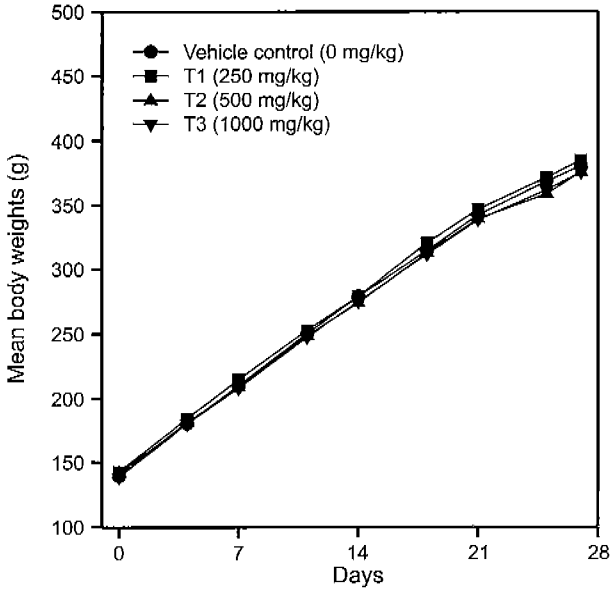

Fig. 1. Mean body weight changes of male rats treated with AS6.

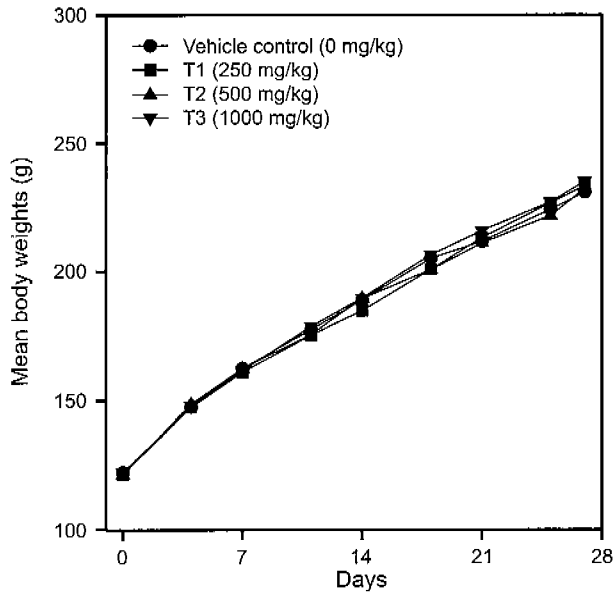

Fig. 2. Mean body weight changes of female rats treated with AS6.

of fur in male rats at 1000 mg/kg (data not shown) and no treatment-related body weight changes was found at any doses tested (Fig. 1.).

Food and water consumption

Food consumption of the male rats at 1000 mg/kg was significantly decreased on the 1st day of treatment. In female rats, however, there was no remarkable difference between the groups during the course of the study (Table 1). Water consumption of male rats at all doses was significantly increased on the day 21. The female rats at 500 mg/kg also showed significant increase in water consumption on the 1st day (Table 2).

Ophthalmoscopy and urinalysis

Ophthalmologic examinations did not reveal ocular lesions in all of the animals (data not shown). Also, no significant difference between treatment groups and controls was seen for any other urinary parameters (Data not shown).

Table I. Mean food consumption in male and` female rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	0	250	500	1000
Male				
Day 1	22.7±1.94[a]	23.3±0.52	21.9±0.81	21.2±0.90**
Day 8	29.4±1.37	30.4±1.06	29.4±1.24	28.7±0.90
Day 15	33.4±1.84	34.8±1.62	31.9±0.65	32.5±2.13
Day 21	34.1±1.77	35.3±2.28	34.6±2.26	33.6±1.21
Day 27	37.2±2.21	37.9±3.02	37.8±0.97	35.6±1.75
Female				
Day 1	17.9±0.77	18.9±0.90	18.4±1.83	18.0±1.00
Day 8	19.3±1.82	19.2±1.39	20.7±2.03	19.1±0.96
Day 15	21.7±2.05	21.7±2.13	21.5±1.15	23.6±1.11
Day 21	21.3±2.46	22.2±2.06	21.2±1.55	21.9±3.42
Day 27	24.0±1.74	25.7±5.01	24.8±2.33	25.4±2.93

[a]Values are presented as means±SD (g).
**Significant difference at the $p<0.01$ level when compared with the control group.

Table II. Mean water consumption in male and female rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	0	250	500	1000
Male				
Day 1	29.8±5.61[a]	28.0±1.84	28.0±1.46	28.5±1.43
Day 8	34.3±1.78	35.4±2.63	36.7±2.92	35.6±2.21
Day 15	40.6±1.98	45.4±4.11	39.5±6.60	42.2±4.17
Day 21	39.2±2.66	42.8±2.83**	46.2±3.61**	42.1±3.18*
Day 27	47.4±11.9	45.6±2.06	47.9±3.97	45.9±5.22
Female				
Day 1	24.7±1.08	27.9±2.83*	26.3±2.91	24.8±1.43
Day 8	27.5±4.06	27.3±3.08	29.9±4.74	27.3±1.84
Day 15	30.6±3.88	32.0±7.92	30.9±4.23	34.4±6.57
Day 21	29.1±5.27	24.9±5.06	27.7±3.92	31.2±5.15
Day 27	33.4±2.69	34.2±6.85	34.1±6.06	33.3±5.92

[a]Values are presented as means±SD (g).
*, ** Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

Table III. Hematological findings in male and female rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	0	250	500	1000
Male				
Erythrocytes ($\times10^{12}$/L)	7.42±0.366[a]	7.31±0.427	7.51±0.237	7.40±0.223
Hemoglobin (g/dl)	15.0±0.47	14.9±0.60	15.1±0.46	14.7±0.58
Hematocrit (%)	45.0±1.71	44.6±1.87	45.2±1.56	44.4±1.75
MCV (fl)	60.6±1.20	61.0±1.38	60.2±0.95	60.0±1.97
MCH (pg)	20.2±0.41	20.5±0.51	20.0±0.28	20.0±0.75
MCHC (g/dl)	33.2±0.42	33.5±0.49	33.3±0.50	33.2±0.49
Platelets ($\times10^9$/L)	1130±110	1190±140	1190±88.0	1250±69.1
Prothrombin time (sec)	14.6±0.47	14.2±0.39	14.0±0.38**	13.9±0.42**
Reticulocytes (‰)	19.6±4.09	18.9±7.58	18.4±5.15	15.5±6.19
Leukocytes ($\times10^9$/L)	10.5±2.26	10.9±2.35	10.5±0.976	11.3±1.86
Neutrophils ($\times10^9$/L)	1.21±0.292	1.53±0.905	1.29±0.899	1.37±0.737
Eosinophils ($\times10^9$/L)	0.14±0.087	0.12±0.106	0.12±0.159	0.14±0.116
Basophils ($\times10^9$/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lymphocytes($\times10^9$/L)	9.12±2.05	9.10±1.84	8.99±0.865	9.72±2.33
Monocytes ($\times10^9$/L)	0.13±0.189	0.13±0.217	0.12±0.179	0.12±0.163
Female				
Erythrocytes ($\times10^{12}$/L)	7.47±0.331	7.51±0.309	7.46±0.281	7.40±0.388
Hemoglobin (g/dl)	15.0±0.56	14.8±0.78	15.1±0.69	14.9±0.77
Hematocrit (%)	43.9±1.95	43.7±2.16	43.9±2.16	43.2±2.57
MCV (fl)	58.8±0.97	58.1±1.62	58.8±1.41	58.4±1.45
MCH (pg)	20.1±0.42	19.8±0.76	20.2±0.56	20.1±0.69
MCHC (g/dl)	34.1±0.69	34.0±0.69	34.3±0.79	34.4±0.75
Platelets ($\times10^9$/L)	1160±67.0	1220±134	1250±133	1210±137
Prothrombin time (sec)	15.1±0.38	15.2±0.85	14.9±0.65	14.8±0.78
Reticulocytes (‰)	18.4±5.15	15.5±6.19	16.4±7.65	21.5±7.91
Leukocytes ($\times10^9$/L)	7.73±1.93	6.73±1.54	8.16±2.33	7.94±1.97
Neutrophils ($\times10^9$/L)	1.02±0.645	0.75±0.364	1.08±1.32	0.68±0.201
Eosinophils ($\times10^9$/L)	0.04±0.046	0.02±0.047	0.05±0.085	0.07±0.082
Basophils ($\times10^9$/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lymphocytes ($\times10^9$/L)	6.63±1.66	5.91±1.51	6.98±1.40	7.16±2.04
Monocytes ($\times10^9$/L)	0.04±0.076	0.05±0.086	0.05±0.071	0.04±0.071

[a]Values are presented as means±SD. MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration
**Significant difference at the $p<0.01$ level when compared with the control group.

Hematology and Serum Biochemistry

The prothrombin time of males at 500 and 1000 mg/kg was significantly decreased. In female rats, no significant changes were observed (Table 3). In male rats, no significant changes in serum biochemistry were observed (data not shown). The total cholesterol level of females at 500 and 1000 mg/kg was significantly increased when compared with the negative control group (Table 4).

Gross findings and organ weight

At necropsy, one case of atrophy of testis, epididymis, prostate and seminal vesicle were observed in male at 500 mg/kg,

Table IV. Serum biochemical findings in male rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	0	250	500	1000
Aspartate aminotransferase (IU/L)	123±32.5[a]	115±34.9	133±23.1	117±25.7
Alanine aminotransferase (IU/L)	40.2±11.9	40.4±10.5	48.9±11.9	43.3±8.69
Alkaline phosphatase (IU/L)	753±158	793±221	678±158	704±176
Blood urea nitrogen (mg/dl)	12.9±2.66	12.8±3.03	12.0±0.91	12.4±1.82
Creatinine (mg/dl)	0.43±0.080	0.42±0.097	0.46±0.088	0.38±0.055
Glucose (mg/dl)	124±19.2	138±30.8	123±16.0	119±13.9
Total cholesterol (mg/dl)	61.0±12.5	63.2±17.2	64.0±10.7	59.0±8.25
Total bilirubin (mg/dl)	0.08±0.011	0.08±0.022	0.09±0.013	0.08±0.007
Total protein (g/dl)	5.78±0.524	6.17±1.23	6.09±0.154	5.91±0.424
Albumin (g/dl)	4.20±0.330	4.36±0.649	4.31±0.133	4.20±0.204
Creatine phosphokinase (IU/L)	744±424	640±384	671±323	565±270
Triglyceride (mg/dl)	30.7±8.14	39.2±20.3	30.4±10.7	30.2±9.50
Calcium (mg/dl)	9.62±0.811	10.1±1.89	10.1±0.297	9.86±0.437
Inorganic phosphate (mg/dl)	9.00±1.15	9.46±1.77	9.53±0.730	8.90±0.624
Phospholipid (mg/dl)	93.0±11.9	96.2±26.8	93.7±8.69	87.5±9.37
Albumin/Globulin (ratio)	2.68±0.316	2.89±1.84	2.44±0.181	2.49±0.269
Sodium (nmol/L)	142±1.17	142±0.94	142±1.51	142±0.92
Potassium (nmol/L)	4.76±0.538	4.61±0.269	4.98±0.677	4.67±0.238
Chloride (nmol/L)	105±0.82	104±1.03	104±1.15	105±0.99

[a]Values are presented as means±SD.

Table V. Serum biochemical findings in female rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	0	250	500	1000
Aspartate aminotransferase (IU/L)	116±17.6[a]	119±26.7	129±27.1	117±34.2
Alanine aminotransferase (IU/L)	27.2±3.47	26.7±3.07	28.3±3.85	26.2±3.32
Alkaline phosphatase (IU/L)	490±105	424±64.3	419±98.3	474±121
Blood urea nitrogen (mg/dl)	15.7±3.28	15.3±2.98	16.5±3.83	15.7±3.42
Creatinine (mg/dl)	0.42±0.073	0.45±0.096	0.44±0.077	0.47±0.114
Glucose (mg/dl)	118±21.5	114±10.1	123±20.0	126±20.5
Total cholesterol (mg/dl)	58.8±8.15	60.8±14.5	73.5±17.9*	73.5±8.91*
Total bilirubin (mg/dl)	0.08±0.012	0.09±0.010	0.08±0013	0.09±0.009
Total protein (g/dl)	5.82±0.331	5.61±0.335	5.73±0.463	5.72±0.244
Albumin (g/dl)	4.26±0.174	4.14±0.146	4.17±0.216	4.18±0.143
Creatine phosphokinase (IU/L)	898±282	917±418	968±411	833±469
Triglyceride (mg/dl)	11.5±5.55	10.4±3.37	11.9±3.96	11.4±3.60
Calcium (mg/dl)	9.67±0.489	9.54±0.494	9.75±0.500	9.66±0.371
Inorganic phosphate (mg/dl)	9.12±0.872	9.15±0.766	8.92±0.953	8.89±0.919
Phospholipid (mg/dl)	99.4±12.6	101±21.2	117±26.2	117±9.49
Albumin/Globulin (ratio)	2.76±0.264	2.86±0.375	2.73±0.320	2.73±0.159
Sodium (nmol/L)	140±1.51	139±1.95	140±2.45	139±1.48
Potassium (nmol/L)	5.25±0.511	5.10±0.313	5.20±0.440	5.05±0.670
Chloride (nmol/L)	109±3.06	108±1.70	108±1.63	109±2.20

[a]Values are presented as means±SD.
*, **Significant difference at the $p<0.05$ and $p<0.01$ levels, respectively, when compared with the control group.

but there were no treatment-related gross pathological findings in the other males and females of any treatment group (data not shown). No treatment-related changes in organ weight were observed in all rats of both sexes (data not shown).

Histopathological findings

In males, one case of chronic progressive nephropathy and cardiomyopathy, two cases of cystic tubule in kidney, four cases of microgranuloma were observed in control group. One case of seminiferous tubular atrophy, aspermia and atrophy of prostate gland were observed at 500 mg/kg and one case of chronic progressive nephropathy, cystic tubule and liver necrosis, two case of fatty change of liver were observed at 1000 mg/kg. In females, one case of chronic inflammation and mineralization of kidney, chronic inflammation of lung, three cases of chronic progressive nephropathy, granuloma and fatty change in liver were observed in control group. One case of granuloma in liver, two cases of chronic progressive nephropathy, mineralization of kidney and fatty change in liver were observed at 1000 mg/kg (Table 5).

Table VI. Histopathological findings in male and female rats treated with AS6 for 4 weeks

Dose (mg/kg/day)	Male				Female			
	0	250	500	1000	0	250	500	1000
Kidney								
Chronic progressive nephropathy	1	0	0	1	3	0	0	0
Cystic tubule	2	0	0	1	0	0	0	2
Chronic inflammation	0	0	0	0	1	0	0	2
Mineralization	0	0	0	0	1	0	0	0
Heart								
Cardiomyopathy	1	0	0	0	0	0	0	0
Liver								
Necrosis	0	0	0	1	0	0	0	0
Microgranuloma	4	0	0	0	3	0	0	1
Fatty change	0	0	0	2	3	0	0	2
Lung								
Chronic inflammation	0	0	0	0	1	0	0	0
Testis								
Seminiferous tubular Atrophy	0	0	1	0	–	–	–	–
Epididymis								
Aspermia	0	0	1	0	–	–	–	–
Prostate								
Atrophy	0	0	1	0	–	–	–	–
Seminal vesicle								
Atrophy	0	0	1	0	–	–	–	–

DISCUSSION

Triterpenoids are an interesting group of compound that exists widely in plants. AS6, a triterpenoid compound with ursane structure, is a new hepatoprotective agent effective in decreasing chemical-induced liver injury and inhibiting chronic liver cirrhosis. The present study showed that the 4-week repeated oral administration of AS6 did not cause any clinical signs up to dose of 1000 mg/kg. In the subacute toxicity study, the loss of fur in male rats at 1000 mg/kg was considered as an accidental sign because of its low frequency. Decreased food consumption and increased water consumption were also considered as an accidental finding because it was observed in only one sex and low frequently. The increased prothrombin time and increased total cholesterol level was not considered as a treatment-related toxic effect because it was within the normal physiological range of SD rats (Wolford *et al*, 1986; Kang *et al.*, 1995). Atrophy of testis, epididymis, prostate and seminal vesicle observed in male at 500 mg/kg was not considered as a treatment-related toxic effect because it occurred at low frequency and dose-independent, therefore it was judged to be an accidental finding. In addition, the histopathological findings such as seminiferous tubular atrophy, aspermia and atrophy of prostate gland, chronic progressive nephropathy, cystic tubule and liver necrosis, fatty change of liver, mineralization of kidney, chronic inflammation of lung, granuloma in liver were not considered to be treatment-related. Because these changes are common and well known in normal Sprague-Dawley rats (Boorman, 1990; Greaves, 1990; Haschek and Rousseaux, 1998) and occurred with similar frequency in the control and highest dose groups.

Based on the results, it was concluded that the 4-week repeated oral dose of AS6 did not cause any toxicity at below dose of 1000 mg/kg. In the condition of this study, target organ was not observed, and the NOAEL of AS6 was considered to be 1000 mg/kg/day for both male and female rats.

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