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Original Article

A Repeated-dose Oral Toxicity Study of *Orostachys japonicus* Extract in Sprague-Dawley Rats

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A 12-week repeated-dose oral toxicity study of water-soluble *Orostachys japonicus* extract (WOJ) was performed in Sprague-Dawley (SD) rats of both genders. Each group of ten rats was orally administered in doses of either 0 or 250 mg/day over a 12-week period. As a result, no WOJ-related changes were observed in terms of survival rate, clinical signs, body weight, or food intake. In addition, no difference in organ weight between the control and treated groups was detected. Furthermore, serum biochemistry parameters revealed some changes within normal ranges although significant decreases in total-bilirubin in the females. In spite of some alterations in serum biochemistry, the clinical signs, body weight changes from food intake, and autoptical remarks indicated that WOJ was not toxic. This study suggests that repeated treatment of *O. japonicus* very low toxicity and the NOAEL (no observed adverse effect dose) of WOJ exceeds 250 mg/kg in the SD rats.

Key Words: *Orostachys japonicus*, SD rat, Repeated-dose toxicity study

INTRODUCTION

Orostachys japonicus (*O. japonicus*), a perennial herbaceous plant belonging to the family Crassulaceae, is traditionally used as a folk medication. It can be collected in summer through autumn for use as a medicinal ingredient, after removing the root and drying the plant in the sun. Previous studies on *O. japonicus* have revealed the presence of friedelin, epi-friedlanol, grutinone, glutinol, triterpenid, β-sitosterol, campesterol, fatty acid ester, kaempferol, quercetin, flavonoid, and aromatic acid (Park et al., 1991; Yoon et al., 2000; Yoon et al., 2005; Ma et al., 2009). *O. japonicus* has long been used as a cure for cancer in herbal medicine, and there has been a considerable amount of research on other possible uses of the plant as an anti-inflammation, anti-cancer, and anti-oxidant (Shin et al., 2008; Ryu et al., 2010; Jeong et al., 2011). However, more scientific research on *O. japonicus* is required due to the lack of fundamental data regarding its physiological activity. For many years, natural plants have been used in various folk medications and proven to be effective, in addition to recently drawing attention as an alternative to modern medical science. Herbal medicines, made from natural plants, have been widely used in collaboration with Western medicine as disease treatments or invigorants, and they have also recently been used as ingredients for health supplements and folk medications. However, little research has been conducted on the toxicities of natural plants, although many questions have been raised regarding their safeties (Stein et al., 2001; Wirth et al., 2005). At the core of the controversy is the level of pesticide residue and heavy metals in natural plants, in addition to possible allergies and liver, kidney, and heart damage that could be caused by the possible natural toxins in those plants (Mei et al., 2006; Kim et al., 2010). For the controversy to be resolved, accurate information

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on the toxicities of natural plants is required. Therefore, systematic research regarding the toxicities of natural plants should be a prerequisite for their use in pharmaceutical products or health supplements. Compared to the abundance of information on pharmacological actions and clinical values of *O. japonicus* extract, there has been little study on the safety of *O. japonicus* extract intake on a mid- to long-term basis. Therefore, to investigate the safety of *O. japonicus*, we carried out a repeated-dose toxicity study in a Sprague-Dawley (SD) rat by administering *O. japonicus* extract over a 12-week period.

MATERIALS AND METHODS

Materials

O. japonicus was provided by GEOBUGIWASONG Ltd. (Miryang, Korea). The plant was dried and sliced, and then the water-soluble *O. japonicus* extract (WOJ) was extracted by boiling the plant material in water for 3 h under high pressure. The WOJ was collected via filtration through filter paper, was freeze-dried (Clean-Vac 24T, Biotron, Korea) to obtain a powder, and was stored at -4℃.

Animal care and treatment

Experiments were performed in four-week-old SD rats (Hyochang Science Co. Ltd., Daegu, Korea). Animals were maintained under constant temperature (25℃) and a 12 h light-dark cycle, with food and water provided *ad libitum*. Animal experiments were performed in accordance with the Inje University Laboratory Animal Ethics Commission guidelines for the care and use of laboratory animals. This study was approved by the Inje University Animal Care and Use Committee. The animals were divided into four groups (n=10), with each group having an equal gender ratio and animals of the same weight. The average weight range at the initial dose level was 143.1~146.0 g for males and 119.6~124.2 g for females. Considering the fact that the testing material was a natural substance, the dosage was set to 250 mg/kg/day, based on the results of a two-week preliminary test. The control group received only sterilized water.

General symptoms and mortality rate

During the experimental period, the mortality rate and the type/degree of symptoms, if any, were recorded for each animal.

Changes in weight and food intake

The weight was recorded on the day of initial administration, then once a week, and finally during the autopsy. Each weight recording was followed by fasting. The amount of food intake was recorded on the day of initial administration and then once per week during the experiment. The difference in intake was computed by measuring the remaining amounts of feed in each breeding box on the day after the quantitative feed input, thus deriving an average intake amount (g/rat/day). The feed efficiency ratio (FER) of the rats was evaluated using the following equation.

Feed efficiency ratio = Gain in body weight (g) / Food intake of each rat (g) × 100

Autopsy and measurement of organ weight

After the experimental period, the animals were fasted overnight prior to being sacrificed via cervical dislocation under ether anesthesia. Lesions observed with the naked eye, as well as the weights of the heart, lung, liver, spleen, kidney, and ovaries (left and right) or testicles (left and right) were immediately recorded.

Hematological and biochemical analysis

Before the experimental period, blood was drawn from the tail vein blood. At the completion of the experimental period, blood samples were collected in the anticoagulant 0.5% ethylene diamine tetraacetic acid (EDTA), and were analyzed for white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts. Hematological values were determined using a Coulter Cell Counter System (XE-2100, Sysmex Co., Hyogo, Japan). Blood was also collected without anticoagulant, and the separated serum was used to assay aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin, glucose, total cholesterol

(TC), triglyceride (TG), blood urea nitrogen (BUN), and creatinine levels. All serum samples were stored at -80℃ until analysis, and biochemical values were determined using an automated analyzer (ABVIA-2400, SIENEMX Co., New York, NY, USA).

Statistical analysis

Data was expressed as the mean ± standard deviation. Significant differences ($P < 0.05$) were determined using a Student's *t*-test.

Table 1. Change of body weights in male and female SD rats treated with WOJ for 12 weeks

		Initial weight (g)	Final weight (g)	Body weight gain rate[a)]
Male	Control	143.1 ± 3.2	468.0 ± 10.3	225.7 ± 6.7
	WOJ	146.0 ± 3.2	449.4 ± 5.1	208.4 ± 7.2
Female	Control	124.2 ± 4.4	269.2 ± 4.7	117.9 ± 7.6
	WOJ	119.6 ± 4.3	262.8 ± 5.7	120.7 ± 6.9

Values are mean ± SD (n=10).
[a)] Body weight gain rate (%) = (Final body weight - Initial body weight) / Initial body weight × 100

Table 2. Food intake and food efficiency in male and female SD rats treated with WOJ for 12 weeks

		Total food intake	Daily food intake	Food efficiency rate[b)]
Male	Control	2186.0 ± 42.4	26.0 ± 0.5	12.48 ± 0.2
	WOJ	2158.6 ± 27.5	25.7 ± 0.3	11.80 ± 0.1
Female	Control	1567.0 ± 31.8	18.7 ± 0.4	7.76 ± 0.2
	WOJ	1546.8 ± 36.8	18.4 ± 0.4	7.78 ± 0.3

Values are mean ± SD (n=10).
[b)] Food efficiency rate = Body weight gain (g) / Daily food intake (g).

RESULTS

General symptoms and mortality rate

Throughout the entire experiment, the general symptoms and mortality rate were examined, with no animal deaths noted. No differences were found in comparison to the control group for otherwise observed general symptoms.

Changes in weight and feed intake

The changes in weight of the animals during the experimental period are shown in Table 1. The repeated doses of

Table 4. Abbreviation, unit and analytical methods of hematological and serum biochemical items

Items	Unit
Hematology	
WBC (White blood cell)	× 10^3/mm^3
RBC (Red blood cell)	× 10^6/mm^3
PLT (Platelet count)	× 10^3/mm^3
Serum biochemistry	
AST (Aspartate aminotransferase)	IU/l
ALT (Alanine aminotransferase)	IU/l
ALP (Alkaline phosphatase)	IU/l
TB (Total bilirubin)	mg/dl
TP (Total protein)	mg/dl
Albumin	g/dl
Glucose	mg/dl
TC (Total cholesterol)	mg/dl
TG (Triglyceride)	mg/dl
BUN (Blood urea nitrogen)	mg/dl
Creatinine	mg/dl

Table 3. Organ weights in male and female SD rats treated with WOJ for 12 weeks

	Male		Female	
	Control	WOJ	Control	WOJ
Heart	1.535 ± 0.035	1.466 ± 0.049	0.982 ± 0.028	0.984 ± 0.024
Lung	1.690 ± 0.032	1.693 ± 0.051	1.398 ± 0.028	1.311 ± 0.039
Liver	12.498 ± 0.433	11.527 ± 0.206	6.883 ± 0.319	6.792 ± 0.174
Spleen	0.800 ± 0.040	0.769 ± 0.031	0.608 ± 0.037	0.634 ± 0.032
Kidney	2.860 ± 0.075	2.858 ± 0.079	1.751 ± 0.068	1.671 ± 0.038
Ovary/Testis	3.960 ± 0.073	4.034 ± 0.047	0.117 ± 0.013	0.101 ± 0.006

Values are mean ± SD (n=10).

WOJ did not have a significant effect on the animals (male or female). The daily feed intake per individual and the FERs are shown in Table 2. Throughout the experiment, no significant differences were found between the experimental group and the control, including the WOJ in terms of the FER.

Naked-eye autopsy

No cellular or organic changes in the animals (male or female) were observed.

Table 5. Hematological values of SD rats treated with WOJ for 12 weeks

Item		Male		Female	
		Control	WOJ	Control	WOJ
RBC	Initial	8.90 ± 0.25	8.89 ± 0.23	8.25 ± 0.46	8.11 ± 0.48
	Final	8.84 ± 0.30	8.91 ± 0.19	8.36 ± 0.32	8.25 ± 0.12
WBC	Initial	6.40 ± 0.86	6.27 ± 1.06	5.27 ± 1.88	5.20 ± 1.30
	Final	6.55 ± 1.70	6.08 ± 1.10	5.34 ± 1.23	5.40 ± 1.22
PLT	Initial	694.2 ± 27.7	709.8 ± 51.6	692.9 ± 69.8	709 ± 58.8
	Final	713.2 ± 25.0	673.4 ± 24.8	747.8 ± 88.2	705.9 ± 53.8

Values are mean ± SD (n=10).

Table 6. Biochemical values of SD rats treated with WOJ for 12 weeks

Items		Male		Female	
		Control	WOJ	Control	WOJ
AST	Initial	114.17 ± 8.09	129.67 ± 6.35	114.33 ± 8.97	120.33 ± 5.61
	Final	140.17 ± 12.58	118.67 ± 14.34	136.33 ± 13.11	129.00 ± 10.01
ALT	Initial	39.17 ± 2.91	36.33 ± 1.48	32.50 ± 1.02	33.00 ± 1.53
	Final	38.17 ± 3.50	43.83 ± 11.43	23.33 ± 2.39	28.33 ± 4.13
ALP	Initial	591.17 ± 27.66	444.33 ± 10.01	474.33 ± 31.51	387.17 ± 22.86
	Final	183.33 ± 9.71	160.17 ± 12.37	146.33 ± 16.37	158.83 ± 8.55
TB	Initial	0.10 ± 0.0	0.10 ± 0.0	0.10 ± 0.0	0.10 ± 0.0
	Final	0.10 ± 0.0	0.08 ± 0.02	0.18 ± 0.02	0.12 ± 0.02*
TP	Initial	5.38 ± 0.29	5.17 ± 0.05	5.27 ± 0.19	5.25 ± 0.10
	Final	6.04 ± 0.13	5.88 ± 0.24	6.19 ± 0.16	6.16 ± 0.23
Albumin	Initial	3.89 ± 0.15	3.74 ± 0.04	3.96 ± 0.12	3.95 ± 0.07
	Final	3.84 ± 0.09	3.76 ± 0.10	4.19 ± 0.09	4.12 ± 0.16
Glucose	Initial	118.38 ± 5.52	107.05 ± 5.81	99.83 ± 4.42	122.68 ± 6.41
	Final	238.70 ± 12.54	221.45 ± 15.40	144.85 ± 19.18	152.43 ± 16.81
TC	Initial	113.98 ± 8.50	124.83 ± 1.71	106.07 ± 7.59	106.90 ± 3.42
	Final	96.73 ± 6.46	100.05 ± 7.64	85.73 ± 3.16	88.92 ± 6.57
TG	Initial	38.38 ± 1.36	41.82 ± 7.09	34.30 ± 1.89	31.82 ± 5.18
	Final	48.45 ± 7.22	48.45 ± 3.41	35.32 ± 2.70	36.13 ± 1.72
BUN	Initial	10.13 ± 0.38	16.10 ± 0.68	14.20 ± 1.57	17.88 ± 1.01
	Final	20.67 ± 0.63	20.32 ± 0.48	21.13 ± 1.21	20.35 ± 0.91
Creatinine	Initial	0.38 ± 0.03	0.33 ± 0.02	0.32 ± 0.03	0.40 ± 0.03
	Final	0.57 ± 0.02	0.48 ± 0.05	0.62 ± 0.03	0.67 ± 0.03

Values are mean ± SD (n=10).
*Significantly different from control at $P<0.05$.

Organ weight

The weights of the internal organs of the controls and each experimental group are shown in Table 3. A slight, but not significant, difference was observed between the experimental and control groups.

Hematological and biochemical analysis

The analyses of hematological and biochemical parameters provide an index of the functional changes in the hematopoietic system and the whole body, helping to determine the presence of infection or disease (Ryu et al., 2009). The hematological parameters of SD rats following WOJ treatment are shown in Table 5. Some hematological parameters of the WOJ-treated rats differed slightly from those of the control group, although some parameters were in the normal range. Table 6 shows the biochemical profiles of the treated and control groups. In the serum biochemical analysis, a significant reduction of TB was observed in the females, but no significant change was found in the males.

DISCUSSION

During the experiment, neither significant clinical symptoms nor animal death were observed. After the initial dose of WOJ, a gradual increase in weight was observed in both the experimental and control groups. However, no significant change in weight resulting from a continued dosage of WOJ was observed. No statistical significance in the daily feed intake per individual or FER was found between the two groups. No significant conclusion was reached based on the naked-eye observations of the animal organs at the end of the experiment. No significant differences in the weights of the internal organs were observed between the experimental and control groups. In hematological and biochemical analysis, a significant reduction in TB was observed in the females, but no significant change was found in the males. The AST value reflects liver disease along with hepatic cell damage and necrosis, while the ALT value reflects hypertrophy and other conditions of the liver (Ryu et al., 2009; Lagarto et al., 2011). The differences in the AST and ALT values between the control and experimental groups were not significant, and the mean AST and ALT values in both groups were within normal ranges. These results suggest that animals treated with WOJ exhibited no signs of hepatotoxicity. Since no male-female correlation was found, the TB difference between the genders may have been a spontaneous phenomenon without any relation to the treatment of the WOJ. We observed subtle changes in the dietary efficiency ratio, organ weight, and the factors in the hematological and biochemical analysis between the treated and control groups. However, no toxicity was detected according to the general symptoms, weight changes, feed intakes, and autopsy diagnoses. Moreover, there was no diagnostic reason to conclude a significant toxicological change in the experimental group due to repeated doses with WOJ. Therefore, we concluded that the NOAEL (no observed adverse effect level) of *O. japonicus* exceeds 250 mg/kg and suggest that *O. japonicus* extract does not induce oral toxicity in SD rats. This study provides a useful foundation for studying and developing new functional substances based on *O. japonicus*.

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REFERENCES

Jeong JH, Ryu DS, Suk DH, Lee DS. Anti-inflammatory effects of ethanol extract from *Orostachys japonicus* on modulation of signal pathways in LPS-stimulated RAW 264.7 cells. BMB Reports. 2011. 44: 399-404.

Kim YS, Song MY, Park JD, Song KS, Ryu HR, Chung YH, Chang HK, Lee JH, Oh KH, Kelman BJ, Hwang IK, Yu IJ. Subchronic oral toxicity of silver nanoparticles. Part Fibre Toxicol. 2010. 7: 20.

Lagarto A, Bueno V, Guerra I, Valdés O, Vega Y, Torres L. Acute and subchronic oral toxicities of *Calendula officinalis* extract in Wistar rats. Exp Toxicol Pathol. 2011. 63: 387-391.

Ma CJ, Jung WJ, Lee KY, Kim YC, Sung SH. Calpain inhibitory flavonoids isolated from *Orostachys japonicus*. J Enzyme Inhib Med Chem. 2009. 24: 676-679.

Mei N, Arlt VM, Phillips DH, Heflich RH, Chen T. DNA adduct formation and mutation induction by aristolochic acid in rat kidney and liver. Mutat Res. 2006. 602: 83-91.

Park HJ, Young HS, Park KY, Rhee SH, Chung HY, Choi JS. Flavonoids from the whole plants of *Orostachys japonicus*. Arch Pharm Res. 1991. 14: 167-171.

Ryu DS, Kim SH, Lee DS. Effect of *Salicornia herbacea* polysaccharides on the activation of immune cells *in vitro* and *in vivo*. Food Sci Biotechnol. 2009. 18: 1481-1486.

Ryu DS, Baek GO, Kim EY, Kim KH, Lee DS. Effects of polysaccharides derived from *Orostachys japonicus* on induction of cell cycle arrest and apoptotic cell death in human colon cancer cells. BMB Reports. 2010. 43: 750-755.

Shin JH, Lee SJ, Cha JY, Seo JK, Cheon EW, Sung NJ. The antioxidants activities of Wa-song (*Orostachys japonicus* A. Berger) on edible oil and fat. Korean J Food Cookery Sci. 2008. 24: 748-756.

Stein U, Greyer H, Hentschel H. Nutmeg (myristicin) poisoning - report on a fetal case and a series of cases recorded by a poison information centre. Forensic Sci Int. 2001. 118: 87-90.

Wirth JH, Hudgins JC, Paice JA. Use of herbal therapies to relieve pain: a review of efficacy and adverse effects. Pain Manag Nurs. 2005. 6: 145-167.

Yoon Y, Kim KS, Hong SG, Kang BJ, Lee MY, Cho DW. Protective effects of *Orostachys japonicus* A. Berger (Crossuloceae) on H_2O_2-induced apoptosis in GTI-1 mouse hypothalamic neuronal cell line. J Ethnopharmacol. 2000. 69: 73-78.

Yoon NY, Min BS, Lee HK, Park JC, Choi JS. A potent anti-complementary acylated sterol glucoside from *Orostachys japonicus*. Arch Pharm Res. 2005. 28: 892-896.