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Mouse Single Oral Dose Toxicity Study of DHU001, a Polyherbal Formula

Seong-Soo Roh[1] and Sae-Kwang Ku[2]

[1]*Dept. of Herbology, College of Oriental Medicine,*
[2]*Development Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, Gyeongsan 712-715, Korea*

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This study was conducted to obtain acute information of the oral dose toxicity of DHU001, a polyherbal formula in male and female mice. In order to calculated 50% lethal dose (LD_{50}) and approximate lethal dose (LD), test material was once orally administered to male and female ICR mice at dose levels of 2000, 1000, 500, 250 and 0 (vehicle control) *ml*/kg (body weight). The mortality and changes on body weight, clinical signs, gross observation, organ weight and histopathology of principle organs were monitored 14 days after treatment with DHU001. We could not find any mortalities, DHU001 treatment-related clinical signs, changes on the body and organ weights, gross and histopathological findings. The results obtained in this study suggest that LD_{50} and approximate LD in mice after single oral dose of DHU001 were considered over 2000 mg/kg in both female and male mice.

Key words: DHU001, Polyherbal formula, Single oral dose toxicity, Mice, Histopathology

INTRODUCTION

DHU001 is a mixed herbal formula consisted of 7 types of aqueous extracts; Ficis fructus, Liriopis tuber, Platycodi radix, Schisandrae fructus, Glycyrrhizae radix, Zingiberis rhizome and Menthae herba, and developed for respiratory disorders. Among 7 types of herbal components of DHU001, Liriopis tuber (Park and Geon, 2003), Platycodi radix (Kim *et al*., 2004), Schisandrae fructus (Narimanian *et al*., 2005; Rhyu *et al*., 2006), Glycyrrhizae radix (Sun and Pan, 2006), Zingiberis rhizome (Aimbire *et al*., 2007; Ghayur *et al*., 2008) and Menthae herba (Shin, 2003) have been used to treat various respiratory symptoms. In addition, anti-inflammatory effects on the acute inflammation of DHU001 itself, were also already reported (Back *et al*., 2008). As increase of the concern in the functional food and well being in life, the demands and consumption of functional food originated from natural sources are increased (Lee *et al*., 2003). However, the toxicological aspects about these natural origin-functional foods has been neglected because of the reasons that they have been used as various purpose for long times. Therefore, it is considered that more detail and systemic toxicological studies should be tested to control the abuse and potential toxicities even if they have been used as traditional folk medicine.

The objective of the present study was to obtain the primary safety information about DHU001, a polyherbal formula being developed for respiratory disorders, and further clarifies their safety for clinical use. In order to observe the 50% lethal dose (LD_{50}), approximate lethal dosage (ALD), test articles were once orally administered to female and male ICR mice at dose levels of 2000, 1000, 500, 250 and 0 (control) mg/kg (body weight) according to the recommendation of KFDA Guidelines (2005). The mortality, changes on body weight, clinical signs and gross observation were monitored during 14 days after oral administration of DHU001. Organ weights and histopathology of 12 types of principle organs were also monitored.

MATERIALS AND METHODS

Experimental animals. Each of twenty-five female and male ICR mice (6-wk old upon receipt, SLC, Japan) was used after acclimatization for 8 days. Five animals were allocated per a polycarbonate cage in a temperature (20~25°C) and humidity (40~45%) controlled room. Light : dark cycle was 12 h : 12 h and feed (Samyang, Korea) and water were supplied free to access. All animals were overnight fasted (about 18 h) before dosing and terminal necropsy.

Correspondence to: Sae-Kwang Ku, Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, 290, Yugok-dong, Gyeongsan-si, Gyeongsangbuk-do 712-715, Korea
E-mail: gucci200@hanmail.net

Table 1. Herbal composition of DHU001 used in this study

Herbs	Scientific name	Amounts (g)
Ficis fructus	*Ficus carica* Linn.	140
Liriopis tuber	*Liriope spicata* Lour.	45
Platycodi radix	*Platycodon grandiflorum* Jacq.	60
Schisandrae fructus	*Schisandra chinensis* Baill	22.5
Glycyrrhizae radix	*Glycyrrhiza uralensis* Fisch	15
Zingiberis rhizoma recens	*Zingiber officinale* Roscoe	15
Menthae Herba	*Mentha arvensis* Linne var piperascens	20
Total	7 types	317.5

All herbs were purchase from from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea) and yield 11% aqueous extracts were acquired.

Animals were marked by picric acid.

Preparation of DHU001. The herbal compositions of DHU001 were listed in Table 1. Each herbal component was purchased from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea) after confirmation of the morphology under microscopy. Approximated amounts of each herbal component was mixed (317.5 g) and boiled in 2 *l* of distilled water for 2 hours and then filtrated. The filtrate was decompressed using a rotary vacuum evaporator (Lab. Camp, Daejeon, Korea) and lyophilized in a programmable freeze-dryer (IlShin Lab, Daejeon, Korea). Total acquired lyophilized extracts were 34.93 g (yield 11%). Powders of extracts were stored in a desiccator to protect against light and moisture. It was well dissolved up to 100 mg/*ml* concentration levels and appeared to be a deep brown solution. The test article was single orally administered at a dosage volume of 20 *ml*/kg using distilled water as vehicle.

Grouping and dosing. The animals were distributed into 8 groups of 5 mice per group upon receipt. All 7 herbal components of DHU001 have been used as folk medicine and ingredients of medicinal food for long times without revealed toxicological data. The highest dosage level was selected as 2000 mg/kg according to the recommended by KFDA (2005-60, 2005) and Organization for Economic Co-Operation and Development (OECD) (2001) guidelines, and the limited dosages, 1000, 500 and 250 mg/kg, were selected using common ratio 2. In addition, a vehicle control group was added. Animal was once orally dosed using a sonde attached to a syringe of 1ml after overnight fasting (about 18 hr, water was not restricted). Feed and water were restricted further for about 3 h.

Observation of clinical signs. All abnormal clinical signs were recorded before and after dosing at least twice a day based on the functional observational battery test (Irwin, 1968; Dourish, 1987).

Body weight changes. Body weights were measured at the day of dosing (Day 0) immediately before treatment, 1, 2, 7, 13 and 14 days after dosing. In addition, to reduce the individual body weight differences of animals at initial dosing, body weigh gains during Day 0~Day 7, Day 7~Day 13 and Day 0~Day 13 were also calculated based on measured body weight at each day.

Necropsy. All unscheduled dead animals were grossly observed immediately after finding and all survived animals were subjected to terminal necropsy. Animals were asphyxiated by carbon dioxide and gross necropsy was performed in all animals at Day 14 after overnight fasting (about 18 h, water was not restricted).

Specific organs grossly observed: Lung, heart, kidney, spleen, testis, liver, pancreas, epididymis, popliteal lymph node, ovary, brain, and uterus.

Organ weight measurement. The absolute organ weight was measured and then relative organ weight (% of body weight) was calculated for the following organs of all experimental animals when sacrificed.

Measured organs: Lung, heart, kidney (left), spleen, testis (left), liver, pancreas (splenic lobes), epididymis (left), popliteal lymph node (left), ovary (left), brain, and uterus.

Histopathology. Principle organs listed below were sampled at terminal necropsy, and fixed in 10% NBF (neutral buffered formalin). After 18 h of fixation, paraffin embedding was conducted and 3~4 μm sections were prepared by routine histological methods. Representative sections of each specified organs were stained with Hematoxylin & Eosin for light microscopical examination.

Specific organs sampled: Lung, heart, kidney (left), spleen, testis (left), liver, pancreas (splenic lobes), epididymis (left), popliteal lymph node (left), ovary (left), brain, and uterus.

Statistical analyses. Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the obtained data were analyzed by one way ANOVA test followed by Tukey HSD test to determine which pairs of

group comparison were significantly different. In case of significant deviations from variance homogeneity were observed at Levene test, a non-parametric comparison test, the Mann-Whitney U-Wilcoxon Rank Sum W test was conducted to determine the specific pairs of group comparison, which are significantly different. LD_{50} and 95% confidence limits were calculated by Probit method. Statistical analyses were conducted using SPSS for Windows (Release 14.0K, SPSS Inc., USA) and a p-value of less than 0.05 was considered to be a significant difference. In addition, degree of clinical signs, gross and histopathological findings were subdivided into 3 degrees: 3+ Severe, 2+ moderate, 1+ slight.

RESULTS

Mortalities. No unscheduled or DHU001-treatment related mortalities were detected in all dose levels tested in this study. At termination, all of animals (5/5; 100%) were survived in all dose levels tested including vehicle control.

Clinical signs. In this study, DHU001-treatment related abnormal clinical signs were not observed during observation periods regardless of male and female mice.

Changes on body weights and gains. No significant changes on body weight and gains were detected in all dosing groups tested compared to the vehicle control in all dose levels tested (Fig. 1 and 2, Table 2).

Changes on the organ weight. No significant changes on the absolute and relative organ weight of 12 principle organs were observed in all dosing groups tested compared to the vehicle control except for significant ($p < 0.05$) increase of absolute and relative weights of thymus in DHU001 1000 mg/kg-treated male group as compared with equal gender of vehicle control (Table 3 and 4).

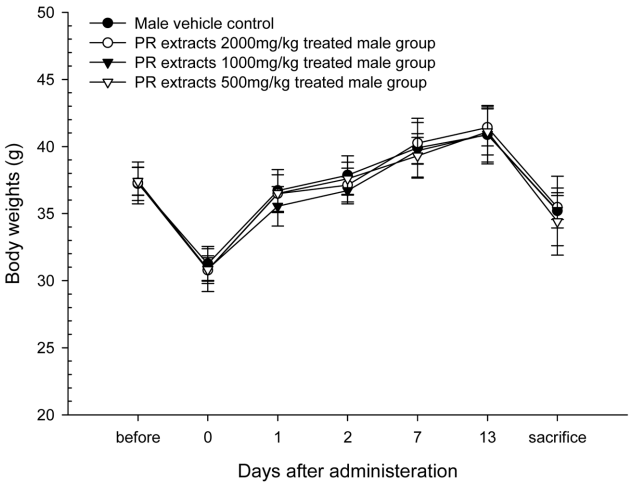

Fig. 1. Body weight changes in male mice after once orally dose of DHU001. No significant changes were detected in all DHU001 treated groups as compared with vehicle control. Before means 1 day before administration; Day 0 means at administration; All animals at sacrifice and Day 0 overnight fasted; Values are expressed as mean ± S.D. of five mice.

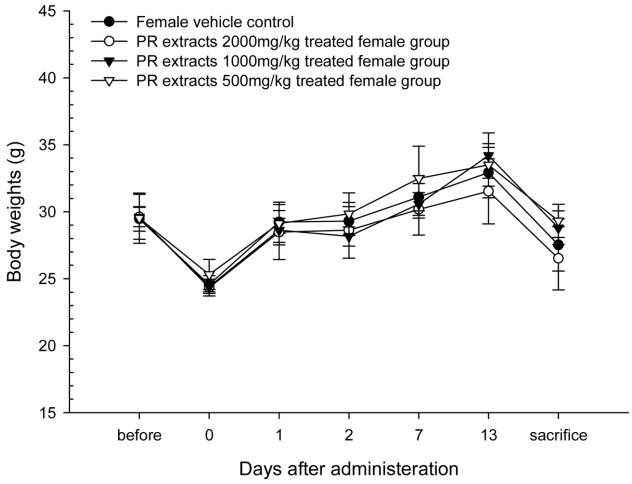

Fig. 2. Body weight changes in female mice after once orally dose of DHU001. No significant changes were detected in all DHU001 treated groups as compared with vehicle control. Before means 1 day before administration; Day 0 means at administration; All animals at sacrifice and Day 0 overnight fasted; Values are expressed as mean ± S.D. of five mice.

Table 2. Body weight gains after oral treatment of DHU001

Group	Intervals		
	Day 0[a]~Day 7	Day 7~Day 13	Day 0~Day 13
Male			
Vehicle control	7.06 ± 0.36	0.96 ± 1.01	8.02 ± 0.75
2000 mg/kg	6.22 ± 0.96	1.68 ± 0.90	7.90 ± 1.82
1000 mg/kg	6.44 ± 1.21	2.50 ± 1.63	8.94 ± 0.83
500 mg/kg	7.08 ± 1.08	1.68 ± 0.47	8.76 ± 1.17
250 mg/kg	7.10 ± 1.52	1.42 ± 0.82	8.52 ± 2.17
Male			
Vehicle control	4.68 ± 0.31	1.90 ± 0.57	6.58 ± 0.84
2000 mg/kg	4.98 ± 0.78	2.64 ± 1.65	7.62 ± 1.92
1000 mg/kg	4.66 ± 0.81	1.82 ± 2.55	6.48 ± 2.72
500 mg/kg	4.54 ± 1.26	2.34 ± 1.54	6.88 ± 0.35
250 mg/kg	3.80 ± 1.12	2.44 ± 0.36	6.24 ± 1.25

Values are expressed as mean ± S.D. of five mice, g; [a] Day of treatment.

Necropsy findings. No significant changes on the gross findings of 12 principle organs were observed in all dosing groups tested compared to that of vehicle control except for some accidental findings such as congestion spots of lung, atrophy of thymus, spleen atrophy or hypertrophy, atypical white foci of liver, hypertrophy of popliteal lymph nod and

Table 3. Changes on the absolute organ weights after oral treatment of DHU001

Group	Organs: Male											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis L	Liver	Pancreas S	Brain	Epididy-mis L	Lymph node L[a]
Vehicle control	0.204 ± 0.011	0.180 ± 0.007	0.051 ± 0.011	0.349 ± 0.024	0.008 ± 0.002	0.124 ± 0.040	0.129 ± 0.009	1.861 ± 0.089	0.240 ± 0.021	0.491 ± 0.025	0.050 ± 0.003	0.008 ± 0.003
2000 mg/kg	0.204 ± 0.013	0.188 ± 0.008	0.052 ± 0.010	0.342 ± 0.038	0.008 ± 0.002	0.119 ± 0.018	0.123 ± 0.015	1.902 ± 0.213	0.233 ± 0.027	0.496 ± 0.012	0.046 ± 0.007	0.010 ± 0.003
1000 mg/kg	0.204 ± 0.011	0.186 ± 0.009	0.076 ± 0.009*	0.347 ± 0.030	0.012 ± 0.006	0.139 ± 0.051	0.113 ± 0.011	1.975 ± 0.231	0.232 ± 0.023	0.502 ± 0.015	0.047 ± 0.006	0.011 ± 0.002
500 mg/kg	0.205 ± 0.016	0.189 ± 0.014	0.061 ± 0.003	0.330 ± 0.063	0.008 ± 0.003	0.115 ± 0.020	0.127 ± 0.015	1.753 ± 0.304	0.228 ± 0.020	0.503 ± 0.039	0.046 ± 0.005	0.011 ± 0.003
250 mg/kg	0.207 ± 0.016	0.181 ± 0.012	0.049 ± 0.015	0.358 ± 0.042	0.010 ± 0.003	0.144 ± 0.037	0.140 ± 0.019	1.845 ± 0.261	0.224 ± 0.035	0.506 ± 0.029	0.048 ± 0.010	0.028 ± 0.040
	Organs: Female											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	Lymph node L
Vehicle control	0.175 ± 0.010	0.140 ± 0.005	0.074 ± 0.022	0.196 ± 0.020	0.008 ± 0.001	0.131 ± 0.022	0.035 ± 0.012	1.345 ± 0.112	0.174 ± 0.014	0.475 ± 0.016	0.141 ± 0.054	0.008 ± 0.004
2000 mg/kg	0.178 ± 0.009	0.146 ± 0.009	0.084 ± 0.020	0.200 ± 0.020	0.008 ± 0.001	0.138 ± 0.034	0.034 ± 0.010	1.464 ± 0.181	0.189 ± 0.018	0.482 ± 0.026	0.095 ± 0.012	0.010 ± 0.003
1000 mg/kg	0.180 ± 0.014	0.141 ± 0.009	0.064 ± 0.028	0.196 ± 0.038	0.008 ± 0.003	0.131 ± 0.032	0.024 ± 0.006	1.374 ± 0.278	0.205 ± 0.026	0.494 ± 0.018	0.117 ± 0.042	0.013 ± 0.003
500 mg/kg	0.178 ± 0.014	0.136 ± 0.006	0.058 ± 0.013	0.196 ± 0.020	0.008 ± 0.002	0.115 ± 0.021	0.030 ± 0.008	1.331 ± 0.093	0.181 ± 0.013	0.468 ± 0.022	0.152 ± 0.058	0.011 ± 0.003
250 mg/kg	0.176 ± 0.011	0.151 ± 0.018	0.073 ± 0.008	0.207 ± 0.027	0.010 ± 0.001	0.154 ± 0.029	0.033 ± 0.013	1.421 ± 0.196	0.195 ± 0.024	0.491 ± 0.027	0.191 ± 0.055	0.010 ± 0.002

Values are expressed as mean ± S.D. of five mice, g; L, left sides; S, splenic lobes; [a]Popliteal lymph node; * $p < 0.05$ as compared with equal genders of vehicle control.

Table 4. Changes on the relative organ weights after oral treatment of DHU001

Group	Organs: Male											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis L	Liver	Pancreas S	Brain	Epididy-mis L	Lymph node L[a]
Vehicle control	0.518 ± 0.038	0.457 ± 0.011	0.129 ± 0.028	0.886 ± 0.053	0.020 ± 0.006	0.316 ± 0.111	0.328 ± 0.024	4.721 ± 0.144	0.609 ± 0.063	1.245 ± 0.042	0.127 ± 0.006	0.020 ± 0.008
2000 mg/kg	0.508± 0.038	0.468± 0.052	0.129± 0.020	0.849± 0.085	0.019± 0.005	0.293± 0.027	0.305± 0.037	4.710± 0.315	0.578± 0.059	1.234± 0.066	0.114± 0.011	0.024± 0.009
1000 mg/kg	0.489 ± 0.037	0.447 ± 0.029	0.183 ± 0.029*	0.829 ± 0.026	0.029 ± 0.014	0.330 ± 0.108	0.272 ± 0.035	4.719 ± 0.335	0.556 ± 0.055	1.205 ± 0.086	0.111 ± 0.011	0.025 ± 0.005
500 mg/kg	0.509 ± 0.007	0.469 ± 0.038	0.151 ± 0.017	0.815 ± 0.122	0.019 ± 0.004	0.284 ± 0.025	0.316 ± 0.028	4.321 ± 0.398	0.565 ± 0.022	1.247 ± 0.069	0.115 ± 0.010	0.026 ± 0.006
250 mg/kg	0.517 ± 0.022	0.452 ± 0.024	0.121 ± 0.027	0.897 ± 0.096	0.024 ± 0.005	0.358 ± 0.076	0.348 ± 0.026	4.592 ± 0.330	0.557 ± 0.042	1.267 ± 0.063	0.118 ± 0.017	0.025 ± 0.005
	Organs: Female											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	Lymph node L
Vehicle control	0.575 ± 0.063	0.460 ± 0.028	0.2434 ± 0.079	0.645 ± 0.097	0.026 ± 0.003	0.433 ± 0.096	0.112 ± 0.031	4.397 ± 0.223	0.570 ± 0.055	1.561 ± 0.141	0.454 ± 0.151	0.025 ± 0.014
2000 mg/kg	0.546 ± 0.042	0.448 ± 0.033	0.255 ± 0.058	0.614 ± 0.073	0.026 ± 0.003	0.419 ± 0.093	0.104 ± 0.0134	4.470 ± 0.356	0.580 ± 0.074	1.478 ± 0.095	0.292 ± 0.045	0.031 ± 0.009
1000 mg/kg	0.586 ± 0.040	0.460 ± 0.042	0.203 ± 0.067	0.636 ± 0.077	0.027 ± 0.007	0.426 ± 0.044	0.080 ± 0.021	4.428 ± 0.328	0.668 ± 0.075	1.625 ± 0.227	0.387 ± 0.159	0.043 ± 0.011
500 mg/kg	0.576 ± 0.055	0.449 ± 0.040	0.188 ± 0.035	0.636 ± 0.090	0.026 ± 0.010	0.369 ± 0.056	0.097 ± 0.024	4.299 ± 0.320	0.586 ± 0.050	1.513 ± 0.121	0.489 ± 0.182	0.036 ± 0.011
250 mg/kg	0.549 ± 0.051	0.473 ± 0.068	0.228 ± 0.026	0.645 ± 0.092	0.030 ± 0.003	0.482 ± 0.107	0.102 ± 0.038	4.411 ± 0.436	0.609 ± 0.086	1.531 ± 0.123	0.595 ± 0.176	0.036 ± 0.019

Values are expressed as mean ± S.D. of five mice, % of body weight at sacrifice; L, left sides; S, splenic lobes; [a] Popliteal lymph node; * $p < 0.05$ as compared with equal genders of vehicle control.

Table 5. Necropsy findings after oral treatment of DHU001

Group	Male					Female				
	Vehicle control	2000 mg/kg	1000 mg/kg	500 mg/kg	250 mg/kg	Vehicle control	2000 mg/kg	1000 mg/kg	500 mg/kg	250 mg/kg
Lung										
Normal	4/5	4/5	5/5	3/5	4/5	4/5	4/5	4/5	4/5	5/5
Congestion	1/5	1/5	0/5	2/5	1/5	1/5	1/5	1/5	1/5	0/5
Thymus										
Normal	4/5	4/5	5/5	5/5	4/5	4/5	5/5	4/5	3/5	5/5
Atrophy	1/5	1/5	0/5	0/5	1/5	1/5	0/5	1/5	2/5	0/5
Spleen										
Normal	3/5	5/5	4/5	3/5	4/5	3/5	3/5	4/5	3/5	4/5
Atrophy	2/5	0/5	0/5	1/5	0/5	1/5	1/5	0/5	2/5	0/5
Hypertrophy	0/5	0/5	1/5	1/5	1/5	1/5	1/5	1/5	0/5	1/5
Liver										
Normal	4/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5
Atypical Foci	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Epididymis/Uterus										
Normal	5/5	5/5	5/5	5/5	5/5	3/5	5/5	4/5	3/5	2/5
Edematous changes	0/5	0/5	0/5	0/5	0/5	2/5	0/5	1/5	2/5	3/5
Lymph node[a]										
Normal	4/5	3/5	3/5	4/5	4/5	4/5	4/5	3/5	3/5	5/5
Hypertrophy	1/5	2/5	2/5	1/5	1/5	1/5	1/5	2/5	2/5	0/5

Observed animals/total observed animals (five mice per group); [a] Bilateral popliteal lymph node.

edematous changes of uterus. They were randomly detected throughout the whole experimental groups including each gender of vehicle controls, and most of these sporadic gross findings do not show any dose-dependent frequencies encounted (Table 5).

Histopathological findings. No significant changes on the histopathological findings of 12 principle organs were observed in all dosing groups tested compared to that of vehicle control except for some sporadic accidental findings such as hypertrophy of lung alveolus wall as congestion, depletion of lymphoid cells or focal hemorrhages in the cortex of thymus, focal necrosis and inflammatory cell infiltration in liver and desquamation of the uterus mucosa. They were randomly detected throughout the whole experimental groups including each gender of vehicle controls,

Table 6. Histopathological findings after oral treatment of DHU001

Group	Male					Female				
	Vehicle control	2000 mg/kg	1000 mg/kg	500 mg/kg	250 mg/kg	Vehicle control	2000 mg/kg	1000 mg/kg	500 mg/kg	250 mg/kg
Lung										
Normal	4/5	4/5	5/5	3/5	5/5	4/5	4/5	4/5	3/5	4/5
Congestion	1/5	1/5	0/5	2/5	0/5	1/5	1/5	1/5	2/5	1/5
Thymus										
Normal	3/5	5/5	5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5
Focal hemorrhage	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
DE*	1/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Liver										
Normal	4/5	5/5	5/5	5/5	4/5	5/5	4/5	5/5	5/5	5/5
IF-FN*	1/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	0/5	0/5
Epididymis/Uterus										
Normal	5/5	5/5	5/5	5/5	5/5	3/5	5/5	4/5	3/5	2/5
DM*	0/5	0/5	0/5	0/5	0/5	2/5	0/5	1/5	2/5	3/5

Observed animals/total observed animals (five mice per group); * Abbreviations: DE, decreases of lymphoid cells; IF-FN, inflammatory cell infiltration with necrosis; DM, desquamation of mucosa.

and most of these sporadic histopathological findings do not show any dose-dependent frequencies encounted (Table 6).

DISCUSSION

In the present study, we investigated the acute toxicity of single oral dose with DHU001, a polyherbal formula to female and male mice as a part of the safety test. DHU001 were once orally administered to female and male ICR mice at dose levels of 2000, 1000, 500, 250 and 0 mg/kg according to the recommendation of KFDA Guidelines (2005). The mortality, changes on body weight, clinical signs and gross observation were monitored during 14 days after treatment of DHU001, and organ weight and histopathology of 12 types of principle organs were also conducted.

As the results, we could not find any mortality, clinical signs, changes in the body weight and gross findings and some sporadic gross findings In addition, no significant changes on the organ weight and histopathology of 12 types of principle organs were detected in the present study except for dose independent sporadic accidental changes.

The body weight detected in this study was well corresponded to the body weight ranges of same aged normal mice (Plata and Murphy, 1972; Yamaguchi *et al*., 1983) in all tested groups including both male and female vehicle control, respectively. It means DHU001 did not induce any harmful changes on the body weights.

In KFDA Guidelines (2005) and OECD Guidelines (#423, 2001), the recommended highest dose of test materials were 2000 mg/kg or the maximum solubility, and they also recommended that in case of single dose toxicity in mice, the dosage volume were below 20 *ml*/kg in case of clear soluble materials but 10 *ml*/kg in suspensions. In the present study, the highest dose of DHU001 was selected as 2000 mg/kg because all 7 individual herbal components of DHU001 have been used as folk medicine and ingredients of medicinal food for long times and no revealed toxicological data was available, base on the recommendation of KFDA (2005-60, 2005) and OECD Guidelines (#423, 2001), and treated in a volume of 20 *ml*/kg using distilled water as vehicle because DHU001 were clearly dissolved upto 100 mg/*ml*, at least in the present study.

Significant ($p < 0.05$) increases of absolute and relative thymic weights in DHU001 1000 mg/kg male mice were considered unrelated to DHU001 treatment because they did not show dose-dependency with no specific changes on the histopathological profiles of thymus, and the other organ weights measured in this study well corresponded to the normal mice organ weight ranges as previously (Plata and Murphy, 1972; Yamaguchi *et al*., 1983).

Edematous changes in the uterus at gross findings and related desquamation of uterus mucosa at histopathological observation in the present study were also considered as a result of differences of physiological estrus cycles (Banks, 1986; Pineda, 1989) not DHU001 treatment relative changes. They were also detected in vehicle control. The atypical white foci detected in one mouse of male vehicle control and DHU001 250 mg/kg treated groups revealed as slight focal necrosis and inflammatory cell infiltrations at histopathological observations.

Congestion spots of lung, atrophy of thymus, spleen atrophy or hypertrophy and hypertrophy of popliteal lymph nod detected as gross findings, and hypertrophy of lung alveolus wall as congestion, depletion of lymphoid cells or focal hemorrhages in the cortex of thymus detected in histopathological findings were considered as accidental findings and they were not considered to be DHU001 treatment related abnormal gross or histopathological findings because they were restricted in some individual animals and most of them were also observed in male and female vehicle controls. In addition, most of them were rarely observed in normal mice (Lee *et al*., 2005, 2006).

Recently Notified Guidelines by KFDA (2005-60, 2005) and OECD Guidelines (#423, 2001) recommended that the highest oral dose of test materials was 2000 mg/kg. The LD_{50} and ALD in mice after single oral dose of DHU001 were detected over 2000 mg/kg in both male and female in the present study.

The results obtained in this study suggest that LD_{50} and approximate LD in mice after single oral dose of DHU001 were considered over 2000 mg/kg in both female and male mice, and is likely to be safe in humans.

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