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Safety Evaluation of Kyungokgo-gamibang Administration Based on Hematological, Biochemical, Protein, and Lipid Profiles in Dogs

Doo-won Song*†, Ga-won Lee*†, Woong-bin Ro*, Heyong-seok Kim*, Hyun-min Kang*, Jong-won Kim*, Soo-bin Park*, Yang-seon Moon, Chang-su Na*** and Hee-myung Park*[1]**

**Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea*
***Scholl of Korean Medicine, Dongshin University, Naju 58245, Korea*
****Nawoori (Ltd), Naju 58245, Korea*

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Abstract : Kyungokgo-gamibang, Kyungokgo with Iksuyongjingo and *Sparassis crispa*, is a traditional Korean medicine used for restorative effects. This study aimed to evaluate the safety of Kyungokgo-gamibang in healthy beagle dogs. In the single-dose oral toxicity study, three beagle dogs were orally administered 2,000, 1,000, and 500 mg/kg of Kyungokgo-gamibang and were observed for 14 days. In the repeated-dose oral toxicity study, nine healthy dogs were orally administered 0.2 g/kg of Kyungokgo-gamibang (n = 3, low-dose group), 1 g/kg of Kyungokgo-gamibang (n = 3, high-dose group), or normal saline (n = 3, control group) twice a day for 8 weeks. The hematological, serum biochemical, urine, protein, and lipid profiles were evaluated to investigate the adverse effects of the Kyungokgo-gamibang. During the study period, the dogs demonstrated no clinical signs and the hematological, serum biochemical, urine, protein, and lipid analyses revealed unremarkable findings. The study results suggest that Kyungokgo-gamibang can be safely administered to dogs without any adverse effects.

Key words **:** Kyungokgo-gamibang, traditional Korean medicine, safety, adverse effect, dogs.

Introduction

Oriental medicines have a long history of preventing and treating diseases in both humans and companion animals, and their use is effective with few adverse effects (15,22,33). However, there are some concerns regarding their scientific efficacy and safety. In the last two decades, efforts have been made to scientifically evaluate and validate the efficacy and safety of oriental medicine (6).

The use of oriental medicines in veterinary medicine has increased, and several studies on veterinary herbal medicines have been reported (1,25,27,30). In a previous study, Hai Zao Hu Tang, a Chinese herbal formula, improved clinical signs in a cat with hypothyroidism (30). Mammosol, another Chinese herbal formula, extends the survival in dogs with mammary gland tumors, while Yunnan Baiyao improves clotting and platelet functions, which control bleeding, in dogs with hemangiosarcoma (1,25,31). Another Chinese herbal formula, HipGuard, is used as an alternative to surgery in dogs with patellar luxation (27).

Kyungokgo is a traditional Korean herbal medicine, comprising four main ingredients— Ginseng Radix, Rehmanniae Radix, Hoelen, and honey (13). Kyungokgo has been widely used in oriental medicine for its restorative effects, including anti-inflammatory and antioxidative effects (2). This suggests that Kyungokgo might improve gastric ulcer, anti-fatigue activity, and exercise capacity (10,28).

Kyungokgo-gamibang comprises Kyungokgo with Iksuyongjingo and *Sparassis crispa*. Kyungokgo-gamibang is a more effective antioxidant and immunity booster than the original Kyungokgo (18). However, until recently, Kyungokgo-gamibang had not been used or evaluated in veterinary medicine. Hence, this study aimed to investigate the safety of Kyungokgo-gamibang in dogs.

Materials and Methods

Test compound

Kyungokgo-gamibang extract, used as the test substance in this study, was provided by Nauri after setting the material and mixing ratio.

Study design

Single-dose oral toxicity study

The study included three Beagle dogs. The highest dose of the test substance was based on the maximum dose range used in the single-dose toxicity test of healthy functional foods. Therefore, Kyungokgo-gamibang extract was divided into three concentrations (2,000, 1,000, and 500 mg/kg), and each dog was orally administered each concentration of Kyungokgo-gamibang extract once. Adverse effects, including clinical signs and mortality, were observed for 14 days after the first oral administration. The toxicity of the test substance was evaluated at 0, 1, 3, 7, and 14 days after the first administration through physical and laboratory examination, serum protein electrophoresis (SPE), and lipoprotein electro-

†Doo-won Song and Ga-won Lee contributed equally to this work.
[1]Corresponding author.
E-mail : parkhee@konkuk.ac.kr

phoresis (LPE).

Repeated-dose oral toxicity study

For examining the safety of Kyungokgo-gamibang extract, repeated-dose oral administration studies were conducted in nine Beagle dogs. Nine dogs were randomly assigned into two experimental groups—low-dose group (n = 3, administration of 0.2 g/kg of Kyungokgo-gamibang extract), high-dose group (n = 3; administration of 1 g/kg of extract)—and a control group (n = 3, administration of normal saline in the same amount as that administered in the low-dose group). Kyungokgo-gamibang extract was administered orally twice daily for 8 weeks in the experimental groups. Dogs in the control group were administered normal saline orally twice daily for 8 weeks. The toxicity of the test substance was evaluated at 0, 2, 4, 6, and 8 weeks after the first administration through physical and laboratory examination, SPE, and LPE.

Animals

Twelve clinically healthy, intact male beagle dogs aged approximately 2.3 years were used to investigate the toxicity and safety of Kyungokgo-gamibang extract. Among them, three dogs were used to investigate single-dose oral toxicity and nine dogs were used to evaluate the safety of Kyungokgo-gamibang extract through repeated-dose oral administration. All dogs were assessed to be normal based on physical examination, complete blood cell counts (CBCs), and serum chemistry panels. All procedures were approved by the Institutional Animal Care and Use Committee (approval number: ORIENT-IACUC-20024). All dogs were reared in the same stainless-steel dog cage (800 W × 930 L × 800 H) in a medium-sized beagle breeding room maintained at a constant temperature (23°C ± 3°C), a humidity of 50% ± 20%, and 100% prefiltered air. All dogs were caged separately, and lights were turned on for 12 h from 08:00 to 20:00. Before purchasing the animal feed, we reviewed the composition of general ingredients and the presence of contaminants in the laboratory animal diet for beagle dogs (Lab Diet® 5007, PMI Nutrition International Inc., USA) based on its analysis report and confirmed that the feed did not affect the study results. Approximately 300 g of the feed was provided once daily, and fresh groundwater treated with reverse osmosis was provided through an automatic water supply system.

Physical and laboratory examination

Bodyweight (BW), heart rate (HR), body temperature (BT), and respiratory rate (RR) of all dogs were recorded. Clinical signs, including vomiting, diarrhea, jaundice, hair loss, and stool abnormalities, were evaluated.

Hematological examinations were performed, including CBC tests (Vetscan® HM2 Hematology system; Abaxis, Inc., Union City, CA, USA), serum chemistry panels (Cobas C III instrument; Roche Diagnostics, South San Francisco, CA, USA), and electrolyte tests (9180 Electrolyte Analyzer; Roche Diagnostic, South San Francisco, CA, USA). The CBC test included tests to evaluate the white blood cell count, red blood cell count, hemoglobin level, hematocrit level, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and platelet count. Serum chemistry profiles and electrolyte tests included tests to evaluate the levels of blood urea nitrogen levels, creatinine, glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, lactate dehydrogenase, total cholesterol, triglyceride, amylase, lipase, total protein, albumin, creatine kinase, uric acid, calcium, phosphate, sodium, and potassium. Urinalysis included analysis of urine pH, glucose levels, bilirubin levels, erythrocyte levels, protein levels, and urine specific gravity.

Serum protein electrophoresis

Serum samples were collected and transported to a reference laboratory (Neodin Veterinary Science Institute, Seoul, South Korea) for SPE analysis. SPE was performed using a HYDRASYS® agarose gel electrophoresis system (Sebia, Norcross, GA, USA) according to the manufacturer's instructions. The gels were imaged and analyzed using a GELSCAN densitometer (Sebia) and PHORESISTM software (Sebia).

Lipoprotein electrophoresis

Blood samples were collected from the jugular vein of dogs after a fast of > 12 h in serum separator tubes and transported to a reference laboratory (Neodin Veterinary Science Institute, Seoul, South Korea) for LPE analysis. LPE of the serum was performed using a HYDRASYS® agarose gel electrophoresis system (Sebia) according to the manufacturer's instructions. Gels were designed and analyzed using a GELSCAN densitometer (Sebia) and PHORESISTM software. The serum was evaluated using paper electrophoresis in an HYDRASYS® agarose gel electrophoresis system (Sebia) according to the manufacturer's instructions. Gel images were read using GELSCAN, and densitometry was performed using PHORESISTM software. LPE results were expressed based on lipid fractions such as alpha, beta, percentage of previous beta structures, chylomicrons, triglycerides, and cholesterol.

Statistical analysis

All data are presented as mean ± standard deviation, except for single-dose oral toxicity. For the single-dose oral toxicity study, there was 1 individual by the concentration of Kyungokgo-gamibang, and they were expressed as individual values. Statistical analysis was performed using a commercial software, SPSS version 20 (IBM, Chicago, IL, USA). For the repeated-dose oral toxicity study, the Kruskal-Wallis test was used for comparisons of values among the three groups, followed by the Mann-Whitney test and post hoc test. The Wilcoxon rank test was used to compare the results before and after the Kyungokgo-gamibang administration in each group. A p value of < 0.05 was considered statistically significant.

Results

Single-dose oral toxicity

Two weeks after a single-dose oral administration, BW, BT, HR, and RR were within the normal range in all dogs (Table 1). No dogs died or demonstrated any clinical signs. There was no observable significant change in the results of laboratory examination, including CBC tests, blood smear

Table 1. Body weight and physical examination of 3 beagle dogs following single-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14
BW (kg)	500	10.2	10.2	10.6	11.6	11.5
	1000	10.1	10.1	10.4	11.3	11.4
	2000	10.3	10.2	10.3	10.8	10.6
BT (°C)	500	38.4	38.2	38.2	38.4	38.3
	1000	38.6	38.8	38.1	38.5	38.4
	2000	38.4	39.1	38.4	38.7	138.6
HR (/min)	500	132	126	126	138	128
	1000	126	132	126	126	132
	2000	138	126	132	126	128
RR (/min)	500	30	30	30	30	30
	1000	36	30	30	30	30
	2000	30	30	36	36	36

BW, body weights; BT, body temperature; D, days after a single-dose oral administration; HR, heart rate; RR, respiratory rate.

Table 2. Complete blood counts of 3 beagle dogs following single-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14	Reference range
WBC (K/μL)	500	6.3	6.01	7.3	9.7	11.3	5.2-13.9
	1000	9.5	8.8	8.4	11.8	10.8	
	2000	8.1	8.6	8.6	10.2	6.6	
HCT (%)	500	60.8	64.7	50.9	56.8	63.2	37.1-65.0
	1000	62.6	64.6	49.5	49.4	63.1	
	2000	55.7	63.4	55.9	53.3	45.5	
PLT (K/μL)	500	144	185	221	163	217	143.3-400
	1000	276	289	298	334	266	
	2000	273	226	226	222	372	

D, days after a single-dose oral administration; WBC, white blood cell; HCT, hematocrit; PLT, platelet.

Table 3. Serum chemistry and electrolytes of 3 beagle dogs following single-dose oral administration of Kyungokgo-gamibang extract.

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14	Reference range
TP (g/dL)	500	6.3	6.4	5.7	5.8	5.6	5.4-7.4
	1000	6.9	6.6	5.8	6	6.1	
	2000	5.4	5.4	5.6	5.8	5.6	
Alb (g/dL)	500	2.7	2.7	2.5	2.6	2.5	2.9-4.2
	1000	3	3	2.7	2.8	2.7	
	2000	2.7	2.7	2.8	2.9	2.7	
Glu (mg/dL)	500	92	95	99	84	89	70-118
	1000	92	99	106	79	93	
	2000	95	97	98	88	93	
BUN (mg/dL)	500	11.2	14.4	9.1	6.5	8.8	8.0-26.0
	1000	16.4	18.2	10.3	10.3	11.7	
	2000	13.6	11.5	11.3	10.5	12.2	
Cre (mg/dL)	500	0.6	0.6	0.6	0.7	0.6	0.5-1.3
	1000	0.7	0.7	0.6	0.7	0.6	
	2000	0.6	0.6	0.6	0.7	0.6	
AST (U/L)	500	25	21	20	26	21	15-43
	1000	20	26	18	21	18	
	2000	120	36	27	29	27	

Table 3. Continued

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14	Reference range
ALT (U/L)	500	52	55	49	45	205	19-70
	1000	94	92	64	46	43	
	2000	226	184	134	87	78	
ALP (U/L)	500	32	32	32	31	29	15-127
	1000	52	46	47	45	40	
	2000	40	38	44	44	41	
Na (mmol/L)	500	149.4	156.6	146.9	148.6	164.8	144-154
	1000	153.7	151.4	144	148.7	151.9	
	2000	145.8	146.2	146.2	148.8	151.2	
K (mmol/L)	500	4.3	4.4	4.4	4.5	5.3	4.1-5.3
	1000	4.1	3.69	3.99	4.01	4.8	
	2000	4.5	4.5	4.6	4.6	4.7	
Cl (mmol/L)	500	106.1	112.6	106.1	107.6	109.5	105-116
	1000	109.2	107.5	107.3	108.1	107.5	
	2000	107.2	107.4	105.3	108	107.5	

D, days after a single-dose oral administration; TP, total protein; Alb, albumin; Glu, glucose; BUN, blood urea nitrogen; Cre, creatinine; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Na, sodium; K, potassium; Cl, chloride.

Table 4. Protein electrophoresis results of 3 beagle dogs following single-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14	Reference range
Alpha-1 (g/dL)	500	0.25	0.26	0.25	0.26	0.34	0.25-0.60
	1000	0.26	0.25	0.26	0.25	0.27	
	2000	0.26	0.27	0.25	0.26	0.26	
Alpha-2 (g/dL)	500	1.03	1.02	0.98	0.88	0.82	0.72-1.40
	1000	0.92	0.88	0.78	0.79	0.92	
	2000	0.72	0.73	0.83	0.76	0.78	
Beta (g/dL)	500	0.67	0.67	0.69	0.65	0.63	0.63-0.89
	1000	0.67	0.64	0.66	0.66	0.63	
	2000	0.69	0.63	0.69	0.69	0.64	
Gamma (g/dL)	500	0.83	0.82	0.79	0.79	0.82	0.50-0.83
	1000	0.83	0.77	0.83	0.75	0.83	
	2000	0.82	0.89	0.81	0.83	0.78	

D, days after a single-dose oral administration; Alpha-1, alpha-1 globulin; Alpha-2, alpha-2 globulin; Beta, beta globulin; Gamma, gamma globulin.

Table 5. Lipoprotein electrophoresis results of 3 beagle dogs following single-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (mg/kg)	D0	D1	D3	D7	D14	Reference range
Alpha (mg/dL)	500	133.70	137.93	137.75	138.78	141.95	60-140
	1000	131.09	110.68	128.52	134.48	137.73	
	2000	121.03	133.20	131.36	134.30	131.67	
Beta (mg/dL)	500	21.23	21.07	19.25	24.22	23.05	< 25
	1000	5.91	18.32	20.8	23.52	19.27	
	2000	11.97	14.80	24.64	23.70	19.33	
Cholesterol (mg/dL)	500	180	192	176	157	166	135-345
	1000	174	176	156	149	151	
	2000	141	139	147	143	140	
Triglyceride (mg/dL)	500	19	47	21	26	39	19-133
	1000	23	53	48	19	36	
	2000	22	19	29	25	31	

D, days after a single-dose oral administration.

examination, serum chemistry analysis, and electrolyte tests. All CBC parameters were within the normal range (Table 2, not showing all data). All serum chemistry and electrolyte parameters showed no remarkable changes before and after single-dose oral administration in all dogs (Table 3, not showing all data). Urinalysis demonstrated no remarkable change during the 14-day study period. SPE analysis revealed that all parameters, including alpha-1, alpha-2, beta, and gamma globulin, were within the normal range in all dogs (Table 4). None of the LPE parameters showed any observable changes before and after single-dose oral administration in all dogs (Table 5).

Repeated-dose oral toxicity study

Kyungokgo-gamibang extract, orally administered to dogs weighing up to 5 g/kg for 8 weeks, did not cause any remarkable clinical signs in the experimental groups. BW and physical examination results were unremarkable (Table 6). CBC parameters were within the normal reference range in all groups (Table 7, not showing all data). There was almost no remarkable change in the results of serum chemistry analysis in all groups (Table 8, not showing all data). Urinalysis revealed no specific difference between before and after the administration of Kyungokgo-gamibang extract during the 56-day study period. There was no remarkable tendency of

Table 6. Body weight and physical examination of 9 beagle dogs following repeated-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56
BW (kg)	0 (Control)	10.07 ± 0.12	10.93 ± 0.21	11.27 ± 0.21	11.20 ± 0.35	11.63 ± 0.46
	0.2	9.70 ± 0.20	10.93 ± 0.21	11.43 ± 0.35	11.60 ± 0.56	12.20 ± 0.46
	1	9.70 ± 0.26	10.87 ± 0.29	11.43 ± 0.31	11.20 ± 0.56	11.87 ± 0.23
BT (°C)	0 (Control)	38.20 ± 0.10	38.30 ± 0.20	38.53 ± 0.15	38.50 ± 0.10	38.37 ± 0.21
	0.2	38.40 ± 0.26	38.30 ± 0.26	38.63 ± 0.12	38.50 ± 0.10	38.27 ± 0.15
	1	38.30 ± 0.26	38.40 ± 0.10	38.40 ± 0.20	38.37 ± 0.15	38.50 ± 0.20
HR (/min)	0 (Control)	134.00 ± 3.46	130.00 ± 3.46	128.00 ± 3.46	128.00 ± 3.46	136.00 ± 3.46
	0.2	132.00 ± 6.00	134.00 ± 6.93	132.00 ± 6.00	132.00 ± 6.00	130.00 ± 3.46
	1	128.00 ± 3.46	128.00 ± 3.46	132.00 ± 6.00	134.00 ± 6.93	132.00 ± 6.00
RR (/min)	0 (Control)	34.00 ± 3.46	32.00 ± 3.46	32.00 ± 3.46	32.00 ± 3.46	34.00 ± 3.46
	0.2	32.00 ± 3.46	32.00 ± 3.46	34.00 ± 3.46	34.00 ± 3.46	32.00 ± 3.46
	1	34.00 ± 3.46	34.00 ± 3.46	34.00 ± 3.46	30.00 ± 0.00	34.00 ± 3.46

BW, body weights; BT, body temperature; D, days after a first oral administration; HR, heart rate; RR, respiratory rate.

Table 7. Complete blood counts of 9 beagle dogs following repeated-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56	Reference range
WBC (K/μL)	0 (Control)	8.05 ± 2.31	8.55 ± 2.06	7.00 ± 0.38	6.53 ± 0.42	7.97 ± 1.49	5.2-13.9
	0.2	5.73 ± 1.25	7.04 ± 1.80	7.19 ± 0.63	6.25 ± 0.80	8.70 ± 0.30	
	1	6.29 ± 0.79	8.31 ± 4.24	7.07 ± 1.19	5.87 ± 1.19	10.18 ± 3.92	
HCT (%)	0 (Control)	53.87 ± 8.56	48.93 ± 7.52	53.27 ± 7.44	58.77 ± 3.69	59.23 ± 5.70	37.1-65.0
	0.2	56.93 ± 4.22	55.03 ± 5.92	50.77 ± 2.75	54.33 ± 3.17	49.67 ± 3.15	
	1	60.63 ± 1.57	45.80 ± 3.67	46.10 ± 3.32	54.80 ± 6.70	46.53 ± 3.11	
PLT (K/μL)	0 (Control)	250.67 ± 58.52	273.00 ± 36.66	233.67 ± 20.40	226.67 ± 17.90	202.33 ± 35.23	143.3-400
	0.2	200.00 ± 23.39	238.00 ± 76.96	235.33 ± 7.64	257.67 ± 58.53	274.00 ± 21.66	
	1	254.33 ± 37.61	200.67 ± 150.98	287.33 ± 30.89	238.67 ± 21.13	309.33 ± 39.25	

D, days after a first oral administration; WBC, white blood cell; RBC, red blood cell; HCT, hematocrit; PLT, platelet.

Table 8. Serum chemistry and electrolytes of 9 beagle dogs following repeated-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56	Reference
TP (g/dL)	0 (Control)	6.03 ± 0.21	5.80 ± 0.26	5.93 ± 0.49	6.10 ± 0.10	6.07 ± 0.12	5.4-7.4
	0.2	6.50 ± 0.56	6.30 ± 0.36	6.50 ± 0.52	6.43 ± 0.25	6.30 ± 0.10	
	1	5.47 ± 0.38	5.63 ± 0.25	5.93 ± 0.25	6.07 ± 0.31	5.73 ± 0.40	
Alb (g/dL)	0 (Control)	2.77 ± 0.06	2.80 ± 0.17	3.03 ± 0.35	3.20 ± 0.00	3.33 ± 0.23	2.9-4.2
	0.2	2.83 ± 0.12	3.30 ± 0.44	3.20 ± 0.20	3.13 ± 0.23	3.27 ± 0.29	
	1	2.70 ± 0.00	3.40 ± 0.10	2.90 ± 0.17	3.10 ± 0.17	3.00 ± 0.20	

Table 8. Continued

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56	Reference
Glu (mg/dL)	0 (Control)	82.00 ± 1.00	91.67 ± 1.53	81.33 ± 12.01	80.00 ± 4.58	89.00 ± 2.00	70-118
	0.2	92.33 ± 1.53	98.67 ± 1.53	80.00 ± 5.57	90.00 ± 5.20	92.67 ± 6.11	
	1	89.00 ± 3.00	90.33 ± 9.07	73.00 ± 5.29	91.67 ± 9.50	94.33 ± 7.64	
BUN (mg/dL)	0 (Control)	10.33 ± 0.47	13.10 ± 3.05	12.93 ± 1.36	11.17 ± 1.61	14.07 ± 2.05	8.0-26.0
	0.2	19.63 ± 7.67	14.00 ± 2.29	13.17 ± 2.27	12.93 ± 1.76	13.47 ± 2.26	
	1	20.30 ± 6.02	12.37 ± 0.57	11.13 ± 0.95	14.00 ± 2.70	11.50 ± 3.60	
Cre (mg/dL)	0 (Control)	0.70 ± 0.10	0.60 ± 0.10	0.67 ± 0.12	0.67 ± 0.06	0.67 ± 0.06	0.5-1.3
	0.2	0.70 ± 0.10	0.63 ± 0.06	0.63 ± 0.06	0.70 ± 0.10	0.63 ± 0.06	
	1	0.67 ± 0.06	0.60 ± 0.00	0.60 ± 0.00	0.70 ± 0.00	0.60 ± 0.10	
AST (U/L)	0 (Control)	25.67 ± 4.16	25.00 ± 3.61	33.33 ± 5.69	24.33 ± 2.08	38.67 ± 7.77	15-43
	0.2	41.00 ± 7.94	32.67 ± 5.03	39.00 ± 8.66	31.00 ± 7.21	38.00 ± 9.54	
	1	40.00 ± 9.54	22.33 ± 4.16	34.67 ± 7.77	26.33 ± 6.66	34.00 ± 7.21	
ALT (U/L)	0 (Control)	67.33 ± 15.89	69.33 ± 10.69	66.00 ± 12.49	68.00 ± 13.08	63.33 ± 17.79	19-70
	0.2	74.33 ± 2.08	74.00 ± 3.61	76.00 ± 1.00	73.33 ± 3.06	72.33 ± 3.79	
	1	58.00 ± 23.07	41.67 ± 29.84	35.67 ± 12.50	53.00 ± 21.17	48.67 ± 21.22	
ALP (U/L)	0 (Control)	26.67 ± 2.52	26.67 ± 4.73	31.67 ± 9.02	22.67 ± 4.51	18.00 ± 5.29	15-127
	0.2	40.33 ± 18.01	44.67 ± 9.29	44.67 ± 12.50	40.33 ± 3.51	36.00 ± 4.58	
	1	20.33 ± 7.51	27.33 ± 11.68	27.00 ± 7.94	22.67 ± 4.73	19.33 ± 5.86	
Na (mmol/L)	0 (Control)	148.37 ± 0.72	152.53 ± 3.30	150.87 ± 1.33	145.53 ± 2.05	144.23 ± 0.67	144-154
	0.2	154.13 ± 5.79	151.40 ± 0.61	151.37 ± 0.55	145.80 ± 0.98	143.63 ± 1.45	
	1	152.57 ± 3.40	150.17 ± 0.47	151.40 ± 0.17	148.93 ± 4.19	143.80 ± 0.92	
K (mmol/L)	0 (Control)	4.65 ± 0.38	4.77 ± 0.31	4.52 ± 0.48	4.38 ± 0.50	4.57 ± 0.52	4.1-5.3
	0.2	4.62 ± 0.12	4.57 ± 0.13	4.55 ± 0.13	4.51 ± 0.34	4.27 ± 0.44	
	1	4.86 ± 0.33	4.64 ± 0.18	4.24 ± 0.29	4.60 ± 0.06	4.46 ± 0.16	
Cl (mmol/L)	0 (Control)	109.53 ± 0.06	109.67 ± 1.68	107.87 ± 1.00	112.47 ± 1.21	111.07 ± 0.93	105-116
	0.2	112.60 ± 4.82	107.30 ± 0.85	108.37 ± 0.21	111.80 ± 0.61	108.97 ± 0.55	
	1	113.83 ± 2.86	109.27 ± 0.45	109.37 ± 0.29	115.67 ± 3.20	111.03 ± 1.42	

D, days after a first oral administration; TP, total protein; Alb, albumin; Glu, glucose; BUN, blood urea nitrogen; Cre, creatinine; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Na, sodium; K, potassium; Cl, chloride.

Table 9. Protein electrophoresis results of 9 beagle dogs following repeated-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56	Reference range
Alpha-1 (g/dL)	0 (Control)	0.23 ± 0.01	0.31 ± 0.04	0.25 ± 0.01	0.25 ± 0.02	0.24 ± 0.03	0.25-0.60
	0.2	0.22 ± 0.04	0.28 ± 0.01	0.25 ± 0.03	0.24 ± 0.02	0.27 ± 0.01	
	1	0.25 ± 0.02	0.25 ± 0.03	0.27 ± 0.01	0.22 ± 0.02	0.25 ± 0.01	
Alpha-2 (g/dL)	0 (Control)	0.71 ± 0.12	0.82 ± 0.10	0.76 ± 0.05	0.73 ± 0.06	0.68 ± 0.07	0.72-1.40
	0.2	0.84 ± 0.11	0.86 ± 0.06	0.85 ± 0.12	0.74 ± 0.01	0.71 ± 0.05	
	1	0.79 ± 0.15	0.86 ± 0.14	0.77 ± 0.06	0.71 ± 0.12	0.66 ± 0.15	
Beta (g/dL)	0 (Control)	0.67 ± 0.21	0.71 ± 0.12	0.64 ± 0.02	0.61 ± 0.06	0.63 ± 0.07	0.63-0.89
	0.2	0.73 ± 0.15	0.67 ± 0.10	0.69 ± 0.16	0.67 ± 0.10	0.64 ± 0.06	
	1	0.60 ± 0.11	0.63 ± 0.13	0.63 ± 0.15	0.60 ± 0.17	0.59 ± 0.20	
Gamma (g/dL)	0 (Control)	1.23 ± 0.05	1.24 ± 0.06	1.18 ± 0.09	1.14 ± 0.02	1.11 ± 0.08	0.50-0.83
	0.2	1.55 ± 0.37	1.57 ± 0.30	1.55 ± 0.35	1.41 ± 0.31	1.42 ± 0.27	
	1	1.27 ± 0.23	1.20 ± 0.30	1.41 ± 0.44	1.31 ± 0.34	1.26 ± 0.35	

D, days after a first oral administration; Alpha-1, alpha-1 globulin; Alpha-2, alpha-2 globulin; Beta, beta globulin; Gamma, gamma globulin.

increase or decrease in the SPE parameters after the administration of Kyungokgo-gamibang extract or saline in both the experimental groups or the control group, respectively (Table 9). In the LPE analysis, no remarkable changes were observed before and after administration of Kyungokgo-gamibang extract (Table 10).

Table 10. Lipoprotein electrophoresis results of 9 beagle dogs following repeated-dose oral administration of Kyungokgo-gamibang extract

Parameters	Dose (g/kg)	D0	D14	D28	D42	D56	Reference range
Alpha (mg/dL)	0 (Control)	116.44 ± 24.51	127.75 ± 24.71	114.24 ± 27.09	115.03 ± 24.37	113.07 ± 21.37	60-140
	0.2	143.79 ± 40.26	129.15 ± 24.84	114.97 ± 20.95	115.52 ± 16.62	113.66 ± 21.32	
	1	127.17 ± 22.85	157.16 ± 40.58	133.21 ± 34.79	135.63 ± 48.34	112.74 ± 32.67	
Beta (mg/dL)	0 (Control)	18.65 ± 3.56	33.78 ± 6.49	27.09 ± 8.43	27.64 ± 2.55	14.26 ± 0.92	<25
	0.2	28.88 ± 13.03	36.18 ± 4.85	39.37 ± 4.27	28.48 ± 6.71	16.00 ± 3.59	
	1	29.44 ± 9.94	39.18 ± 4.87	34.25 ± 2.39	31.70 ± 0.88	20.26 ± 3.56	
Cholesterol (mg/dL)	0 (Control)	123.33 ± 24.83	138.67 ± 24.34	125.00 ± 23.07	129.00 ± 24.43	114.67 ± 21.22	135-345
	0.2	153.00 ± 38.59	143.00 ± 30.61	136.67 ± 24.58	130.33 ± 20.26	117.67 ± 22.19	
	1	137.67 ± 31.53	169.00 ± 43.21	152.67 ± 37.86	152.00 ± 47.47	122.33 ± 30.66	
Triglyceride (mg/dL)	0 (Control)	11.33 ± 1.53	22.33 ± 6.66	16.33 ± 9.02	13.67 ± 1.53	12.67 ± 1.53	19-133
	0.2	19.67 ± 15.95	22.33 ± 4.04	17.67 ± 5.13	13.67 ± 4.93	12.00 ± 5.00	
	1	18.33 ± 7.02	27.33 ± 6.81	14.33 ± 4.93	15.33 ± 1.15	10.67 ± 3.06	

D, days after a first oral administration.

Discussion

Herbal medicine has been widely used in veterinary medicine, and various ingredients have been studied (3,8,9,16,26,32). Herbal medicine is relatively safer than synthetic drugs; however, it might induce more side effects than expected (17). Various kinds of herbs, including guarana, ma huang (ephedra), white willow, and Yohimbe, can cause potential toxicity in dogs (20). Suspected ephedrine and caffeine toxicosis were observed in dogs after ingestion of herbal supplements containing ma huang and guarana (19), and toxic effects such as vomiting, diarrhea, and hemoptysis were reported in a dog treated with pennyroyal oil (23). Therefore, careful monitoring and evaluation of the safety of herbal medicines are required in dogs. Since there are no published data on the administration of Kyungokgo-gamibang in dogs, scientific evaluation of its safety and efficacy is needed.

In this study, hematological, serum chemical, protein, lipid, and urinary profiles were evaluated in healthy dogs before and after oral administration of Kyungokgo-gamibang for identifying the possible toxicity. In both the single-dose and repeated-dose oral administration toxicity studies, the results of CBC tests, serum chemistry analysis, electrolyte tests, SPE, and LPE did not show significant changes and no clinical signs were observed after the administration of Kyungokgo-gamibang in all groups.

Previous studies (4,5,11,14) have reported that Kyungokgo has several effects, including prevention of osteoporosis, relief of atopic symptoms, anti-inflammatory, and anti-cancer. It has been widely used in human patients with fatigue and reduced immunity (24). One of the most representative effects of Kyungokgo is the improvement of immunity, and several previous studies have revealed its beneficial effect on the immune system (10,12,21). In a previous study using an immune suppression mouse model (21), administration of Kyungokgo significantly normalized the expression levels of several cytokines such as interferon-γ, interleukin (IL)-2, IL-12, IL-4, IL-5, and IL-13, which were altered by methotrexate toxicity. Another study in mice also reported changes in the expression levels of immune-related cytokines and alterations in CBC test results, including neutrophil counts, red blood cell counts, hemoglobin levels, and hematocrit levels, after administration of Kyungokgo-gamibang (10). In this study, the CBC test results did not change after the administration of Kyungokgo-gamibang owing to the involvement of healthy dogs without immune suppression. Similar to the results of this study, no significant change in CBC results were observed after administration of Kyungokgo in a previous human study including healthy participants.

In a mouse experiment study, significant weight loss was confirmed after administration of Kyungokgo up to 0.4 g/kg thrice daily for 3 days (29). Contrary to the results of the above study, mild weight gain was observed, without significance, after administration of Kyungokgo twice a day for 4 weeks in a human study (7). Additionally, our study results are similar to those of a human study, in which slight weight gain was observed in all groups administrated Kyungokgo-gamibang. Since BW is an index that is greatly influenced by food intake and personal physical activity, further studies are needed under equivalent conditions on a large scale.

The study results confirmed that Kyungokgo-gamibang can be safely administered without causing major side effects for a period of 8 weeks. However, there might be a few side effects that did not appear in the tests performed in this study, and the results were not assessed after administration for >8 weeks. Moreover, since this study was conducted in healthy dogs with normal clinical test values, safety in dogs with various diseases such as liver or kidney diseases should be further investigated. Therefore, for safely administering Kyungokgo-gamibang to dogs with various clinical conditions, collection of more data through various investigations in a larger population of dogs and long-term evaluations is required.

Conclusion

This study demonstrated the safety of Kyungokgo-gamibang through single-dose and repeated-dose oral administration in dogs for the first time. Single-dose oral administration

of Kyungokgo-gamibang up to 2,000 mg/kg is considered safe, while long-term oral administration does not cause any adverse effects in dogs. However, further scientific evaluation of the efficacy of Kyungokgo-gamibang is needed for applying it to veterinary medicine. The scientific data obtained from this study provide safety information about the traditionally used Korean medicine Kyungokgo-gamibang, which could be the foundation for further research in dogs.

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