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Original article

A Pilot Study on Single-dose Toxicity Testing of Scolopendrid Pharmacopuncture in Sprague-Dawley Rats

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Key Words
acupuncture, pharmacopuncture, Scolopendrid Pharmacopuncture, toxicity test

Abstract

Objectives: This study was performed to analyze single dose toxicity and the lethal dose of Scolopendrid Pharmacopuncture in rats.

Methods: All experiments were conducted at the Korea Testing & Research Institute (KTR), an institution authorized to perform non-clinical studies, under the regulations of Good Laboratory Practice (GLP). Sprague-Dawley rats were chosen for the pilot study. Doses of Scolopendrid pharmacopuncture, 0.1, 0.5, and 1.0 mL, were administered to the experimental group, and 1.0 mL doses of normal saline solution were administered to the control group. This study was conducted under the approval of the Institutional Animal Ethic Committee.

Results: No deaths or abnormalities occurred in any of the groups. No significant changes in the weight, hematological parameters or clinical chemistry were noted between the control group and the experimental group. To check for abnormalities in organs and tissues, we used microscopy to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs or tissues.

Conclusion: The above findings suggest Scolopendrid Pharmacopuncture is a relatively safe to use for treatment. Further studies on the subject should be conducted to yield more concrete evidence.

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1. Introduction

The Scolopendrid Subspinipes Mutilans are dried centipedes which belong to the family scolopendrida [1]. The centipedes are so named because the shape of the scolopendrid resembles the human vertebra. Scolopendrid Subspinipes Mutilans, ground and mixed with alcohol, is taken as a home remedy to reduce the pain of joint diseases [2]. The scolopendrid was first

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mentioned in Sinnongbonchogyeong, a text in the Compendium of Material Medica that was used to treat infantile convulsion, erysipelas, kerion, scrofulosis and biting injuries [3, 4].

Pharmacopuncture treatment is a therapy that combines acupuncture with pharmacotherapy. As a single procedure, it can produce both the effects of acupuncture and herbal medicine. Moreover, a synergistic effect would be expected from this treatment because pharmacopuncture treatment does not pass through the digestive system; rather, it works fast and can be used with orally-administered medications. It is also an effective treatment for unconscious patients who are unable take medicine [5].

Scolopendrid pharmacopuncture is a complex therapy based on chemical stimulation using the biochemical pharmacological action of the scolopendrid and the physical stimulus at a meridian point. It has been used effectively for the treatment of joint pain and nerve entrapment syndrome [6, 7].

Although Scolopendrid pharmacopuncture is widely used nowadays, objective single-dose toxicity testing of it has not been conducted yet. The current research trend for single-dose toxicity testing of extracts is to study acute and subacute toxicity through the procedure of Good Laboratory Practice (GLP). All the experiments for this research were conducted at the Korea Testing & Research Institute (KTR), an institution authorized to perform non-clinical studies, under the GLP.

This study was performed to analyze the single-dose toxicity and the approximate lethal dose of the scolopendrid pharmacopuncture in rats.

2. Materials and Methods

The scolopendrid pharmacopuncture was prepared in a clean room (K-GMP) in a lab at the Korean Pharmacopuncture Institute. After the mixing process with pure water, the pH was controlled to between 7.0 – 7.5. NaCl was added to make a 0.9% isotonic solution. The completed extract was stored in a refrigerator (2.1 – 6.3℃). The animals used in this study were 6-week-old Sprague-Dawley rats. The reason Sprague-Dawley rats were chosen is that they have been widely used in stability tests of medicine, so the data obtained in this study should be easily compared with many other databases. The weights of the rats were 173.4 – 208.8 g for the males and 143.0 – 161.3 g for the females at the time of injection. All animals were visually inspected and weighed using the CP3202S system (Sartorius, Germany). During 6 days of acclimatization, the rats' general symptoms and changes in those symptoms were observed once a day. The weights were recorded on the last day of acclimatization. No abnormalities were found. The temperature of the lab was 21.0 – 23.1℃, and the humidity was 45.7% – 61.5%. Sufficient food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and UV- filtered water were provided. Groupings were done after the 6 days of acclimatization. The animals were selected by the criteria of their being close to the mean weight. In total, 20 male rats and 20 female rats were selected. The animals were distributed into 4 groups (5 mice per group, Table 1). The expected dose of scolopendrid pharmacopuncture for clinical applications is 1.0 mL. In a pilot study, 1.0 mL/animal of scolopendrid pharmacopuncture was injected into each male and female rat; no deaths were observed. Thus, 1.0 mL/animal was set as a high dose, and 0.5 mL/animal and 0.1 mL/animal were set as a mid-dose and as a low dose, respectively. In the control group, the same doses of normal saline solution were administered. The low- and mid-dose groups of rats were injected with a single dose in the left thigh muscle, and the control group and high-dose rats group were injected with 0.5 mL/site on both thigh muscles by using disposable syringes. This study was conducted under the approval of the Institutional Animal Ethics Committee. On the day of dosing (day 0), the general symptoms (the type of toxic symptoms, revealing time, recovering time, etc.) and the mortality were examined after 30 minutes, 1 hour, and 2, 4, and 6 hours. From the 1st day to the 14th day of treat-

Table 1 Groups of animals

Group	Injection (mL/animal)	Number of the animals(serial number)	
		Male	Female
G1 control group	Saline 1.0	5 (1101 – 1105)	5 (2101 – 2105)
G2 low-dose group	0.1	5 (1201 – 1205)	5 (2201 – 2205)
G3 mid-dose group	0.5	5 (1301 – 1305)	5 (2301 – 2305)
G4 high-dose group	1.0	5 (1401 – 1405)	5 (2401 – 2405)

ment, the general symptoms were examined once a day. The weight was measured immediately before treatment, and at 3, 7 and 14 days after treatment. After the rats have fasted for more than 18 hours before the necropsy, under anesthesia, blood samples were taken from the abdominal aorta (15 days after injection). Hematologic examination results were obtained by using an automatic hematology analyzer (ADVIA 120, SIEMEMS, Germany) to analyze the blood samples (Table 2). Two-mL blood samples were centrifuged for the blood coagulation test (3,000 rpm, 10 minutes). Coagulation test results were measured by using an automated coagulation analyzer (Coapresta 2000, SEKISUI, Japan) (Table 3). In addition to blood hematology, blood taken from the abdominal aorta was used in blood chemistry tests. Blood chemical test results were obtained using an automatic analyzer (7180, HITACHI, Japan) and electrolyte analyzer (AVL9181, Roche, Germany) (Table 4). After the termination of observation, all surviving animal organs and tissues were visually inspected and microscopically examined. The weight, hematologic examination and blood chemistry analysis results from the experiment were analyzed by SAS software (version 9.2, 9.3, SAS Institute Inc., USA). The Bartlett test was conducted to evaluate the homogeneity of variance and the significance. The one-way ANOVA test was conducted when homogeneity of variance was recognized, and the Kruskal-Wallis test was conducted post-hoc.

3. Results

In this study, no deaths or abnormal symptoms occurred. This was the same in all groups. Also, no changes in weight were observed in any of the groups. Evaluations were carried out every day, and on the 3rd, 7th and 14th days after administration (Table 5 , Fig. 1 and 2). The results of hematologic examinations and blood chemistry analyses

Table 2 Hematologic examination

Measuring	Unit	Measuring method
Erythrocyte count, RBC	$\times 10^6$ cells/μL	Flow cytometry
Hemoglobin, HGB	g/mL	Flow cytometry, Cyanmethemoglobin
Hematocrit, HCT	%	Calculated
Mean corpuscular volume, MCV	fl	Flow cytometry
Mean corpuscular hemoglobin, MCH	pg	Calculated
Mean corpuscular hemoglobin concentration, MCHC	g/dL	
Platelet, PLT	$\times 10^3$ cells/μL	Flow cytometry
Leucocyte count, WBC	$\times 10^3$ cells/μL	Flow cytometry,
WBC differential counting		
Neutrophils, NEU	%	Peroxidase stain
Lymphocytes, LYM		
Monocytes, MONO		
Eosinophils, EOS		
Basophils, BASO		
Reticulocytes, Reti	%	Flow cytometry, RNA stain

Table 3 Coagulation test

Measuring elements	Unit	Measuring method
Prothrombin test, PT	second	Coagulation time method
Activated partial thromboplastin time, APTT	second	

are shown in Tables 6 and 7, respectively. No meaningful changes were noted on necropsy, and after histological examination of all of the groups, no significant changes related to injections in the brain, lungs, liver, kidney or spinal cord were noted.

4. Discussion

Much experimental research on and clinical trials of scolopendrid pharmacopuncture have been done. Hong reported that scolopendrid has the effects of reducing pains and alleviating fever and relaxing smooth muscles [8]. Kim reported antipyretic, analgesic and anticonvulsive effects of scolopendrid. The effects of scolopendrid have been reported to be greater when the head and the legs are removed [9]. In a safety study of scolopendrid pharmacopuncture, Lim *et al*. conducted a microbiological examination for 6 weeks. This study analyzed blood hematology, blood chemistry and urine samples obtained from 20 patients who had been treated with scolopendrid pharmacopuncture. The results showed no abnormal findings after scolopendrid pharmacopuncture treatment [10]. In a clinical trial, Koh *et al*. reported that out of 70 patients diagnosed with Herniated Nucleus Pulposus (HNP) and treated with scolopendrid pharmacopuncture for 7 days, 80% of the patients adapted to daily and social life with satisfaction after discharge [11].

This study was performed to prove more objectively the safety of scolopendrid pharmacopuncture than previously recorded. Sprague-Dawley rats were chosen for this study. Scolopendrid pharmacopuncture, 0.1-mL, 0.5-mL, and 1.0-mL doses, was administered to the experimental group, and normal saline solution, a 1.0-mL dose, was administered to the control group. No significant differences in the clinical signs, weights, and results of hematological examinations or blood chemistry analyses were observed between the control group and the experimental group. The necropsy for checking for abnormalities in organs and tissues showed no significant histological findings. To assess the toxicity of scolopendrid pharmacopuncture, further studies about the acute and the chronic harmful effects and the relations with the capacity reaction are necessary. Animal testing is the most fundamental and basic

Table 4 Blood chemical test

Measuring elements	Unit	Measuring method
Alanine aminotransferase, ALT	U/L	JSCC (UV Kinetic)
Aspartate aminotransferase, AST	U/L	JSCC (UV Kinetic)
Alkaline phosphatase, ALP	U/L	4-Nitrophenyl-phosphate, 2Na (JSCC Transferable)
Gamma glutamyltranspeptidase, GGT	U/L	IFCC
Blood urea nitrogen, BUN	mg/dL	Urease-GLDH
Creatinine, Crea	mg/dL	Jaffe
Total bilirubin, T-Bili	mg/dL	Vanadate oxidation
Total protein, TP	g/dL	Biuret
Albumin, Alb	g/dL	BCG
A/G ration	-	Calculated
Total cholesterol, T-Chol	mg/dL	Cholesterol oxidase-HMMPS
Triglycerides, TG	mg/dL	GPO-HMMPS Glycerol blanking
Phosphorus, P	mg/dL	Fiske Subbarow
Glucose, Glu	mg/dL	Hexokinase-G6PDH
Calcium, Ca	mg/dL	OCPC
Chloride, Cl	mmol/L	
Sodium, Na	mmol/L	Ion-selective electrode
Potassium,K	mmol/L	

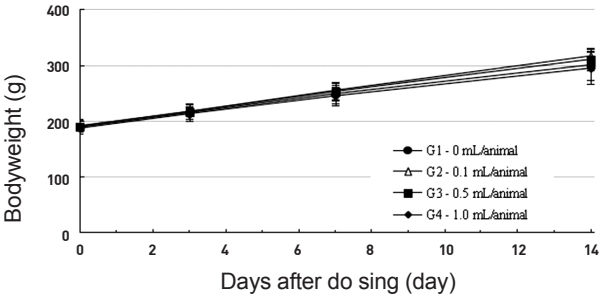

Figure 1 Body weights in male SD rats

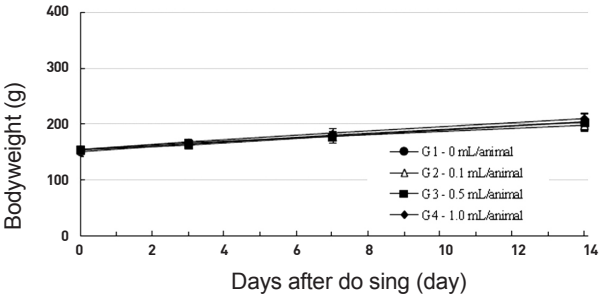

Figure 2 Body weights in female SD rats

Table 5 Body weights (g)

Group	Dose of SP	Sex	Mean S. D. N	Days after administration			
				0	3	7	14
G1	0	Male	Mean	189.5	215.7	249.4	300.7
			S. D.	8.4	11.1	16.2	25.9
			N	5	5	5	5
		Female	Mean	150.9	164.0	180.6	203.4
			S. D.	6.9	7.1	6.2	7.6
			N	5	5	5	5
G2	0.1	Male	Mean	191.6	218.9	255.1	316.6
			S. D.	12.8	13.7	16.4	15.6
			N	5	5	5	5
		Female	Mean	153.7	163.1	177.8	198.1
			S. D.	4.9	6.5	10.0	10.4
			N	5	5	5	5
G3	0.5	Male	Mean	191.1	217.1	253.0	311.4
			S. D.	11.5	13.0	15.5	19.7
			N	5	5	5	5
		Female	Mean	154.1	165.5	178.0	204.1
			S. D.	6.3	5.3	7.8	14.7
			N	5	5	5	5
G4	1.0	Male	Mean	188.6	213.1	246.2	295.8
			S. D.	9.2	12.3	17.4	28.9
			N	5	5	5	5
		Female	Mean	154.0	167.2	183.1	210.6
			S. D.	3.8	5.0	9.0	9.9
			N	5	5	5	5

S.D., standard deviation; N, number of animals.

Table 6 Mean Hematology Parameters

Group	Dose of SP	Sex	Mean / S. D. / N	RBC (×10³ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT (×10³ cells/μL)	Reti(%)
							MCV(fl)	MCH (pg)	MCHC (g/dL)		
G1	0	Male	Mean	7.15	14.6	44.9	63.0	20.4	32.4	1315	4.3
			S. D.	0.43	0.4	1.3	2.0	0.8	0.5	95	0.5
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.14	14.3	42.5	59.6	20.0	33.6	1277	2.7
			S. D.	0.25	0.6	1.9	2.4	0.8	0.3	68	0.3
			N	5	5	5	5	5	5	5	5
G2	0.1	Male	Mean	6.99	14.4	44.6	63.7	20.7	32.4	1367	5.0
			S. D.	0.42	0.5	1.9	1.9	0.7	0.2	111	0.9
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.13	14.4	42.8	60.0	20.3	33.7	1296	2.5
			S. D.	0.37	0.5	1.7	1.2	0.5	0.4	175	0.5
			N	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	6.94	14.2	43.5	62.7	20.4	32.6	1281	4.9
			S. D.	0.22	0.3	0.7	1.8	0.7	0.3	52	0.4
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.31	14.6	43.7	59.7	19.9	33.3	1284	2.9
			S. D.	0.18	0.5	1.4	1.5	0.5	0.3	108	0.8
			N	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	7.01	14.1	43.4	61.9	20.1	32.5	1330	4.3
			S. D.	0.29	0.4	1.5	2.0	0.6	0.3	220	0.9
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.20	14.4	43.3	60.2	19.9	33.2	1447	3.0
			S. D.	0.30	0.3	0.9	1.5	0.5	0.3	51	0.6
			N	5	5	5	5	5	5	5	5

Group	Dose of SP	Sex	Mean / S. D. / N	WBC (×10³ cells/μL)	WBC Differential Counting (%)					PT (sec)	APTT (sec)
					NEU	LYM	MONO	EOS	BASO		
G1	0	Male	Mean	6.67	15.9	79.9	2.1	0.5	0.1	17.6	13.9
			S. D.	1.95	2.5	1.5	0.4	0.2	0.1	0.4	0.4
			N	5	5	5	5	5	5	5	5

(Continued)

Group	Dose of SP	Sex	Mean S. D. N	WBC (×10³ cells/μL)	WBC Differential Counting (%)					PT (sec)	APTT (sec)
					NEU	LYM	MONO	EOS	BASO		
G1	0	Female	Mean	3.06	18.5	77.0	2.1	0.8	0.2	18.3	14.3
			S. D.	0.32	5.9	6.2	0.6	0.2	0.1	0.4	1.0
			N	5	5	5	5	5	5	5	5
G2	0.1	Male	Mean	8.49	14.7	80.9	2.3	0.4	0.2	17.2	14.1
			S. D.	1.10	3.9	4.6	0.6	0.2	0.1	0.7	0.6
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.09	13.7	81.9	2.0	0.8	0.2	18.1	12.9
			S. D.	1.03	1.8	2.0	0.5	0.3	0.1	0.7	1.6
			N	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	7.89	17.5	77.3	2.5	0.7	0.2	18.1	14.3
			S. D.	1.38	6.9	7.8	0.6	0.4	0.0	0.5	0.5
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.45	13.6	81.9	2.0	0.8	0.2	18.1	12.9
			S. D.	1.03	1.8	2.0	0.5	0.3	0.1	0.7	1.6
			N	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	7.08	16.8	78.9	2.2	0.4	0.2	17.7	13.6
			S. D.	1.17	4.2	4.0	0.5	0.1	0.0	0.6	1.2
			N	5	5	5	5	5	5	5	5
		Female	Mean	4.59	14.3	81.8	1.6	0.9	0.2	18.3	14.0
			S. D.	1.94	4.4	4.7	0.4	0.3	0.1	0.9	0.6
			N	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of animals; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular cell hemoglobin concentration; WBC, white blood cell; PLT, platelet; PT, prothrombin time; APTT, active partial thromboplastin time; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, Eosinophils; BASO, basophils; Reti, reticulocytes.

Table 7 Mean clinical chemistry

Group	Dose of SP	Sex	Mean S. D. N	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glu (mg/dL)	BUN (mg/dL)	Crea (mg/dL)	T-Bili (mg/dL)	T-Chol (mg/dL)
G1	0	Male	Mean	31.4	85.6	958.2	0.43	125	11.2	0.36	0.03	64
			S. D.	4.9	14.6	214.6	0.21	7	1.3	0.02	0.01	13
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22.1	72.9	435.9	0.59	128	11.2	0.39	0.02	71
			S. D.	2.8	9.3	119.1	0.16	11	1.1	0.01	0.01	10
			N	5	5	5	5	5	5	5	5	5

(Continued)

Group	Dose of SP	Sex	Mean S. D. N	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Glu (mg/dL)	BUN (mg/dL)	Crea (mg/dL)	T-Bili (mg/dL)	T-Chol (mg/dL)
G2	0.1	Male	Mean	26.2	77.6	799.6	0.33	125	11.8	0.36	0.03	60
			S. D.	3.1	9.2	116.1	0.12	16	1.9	0.03	0.01	9
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22.3	68.6	461.5	0.66	121	11.1	0.39	0.03	76
			S. D.	4.2	7.9	119.2	0.24	11	2.0	0.01	0.01	16
			N	5	5	5	5	5	5	5	5	5
G3	0.5	Male	Mean	33.4	79.3	805.7	0.36	113	10.7	0.37	0.04	65
			S. D.	3.8	12.8	169.0	0.24	15	1.2	0.02	0.01	18
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	23.2	75.1	475.1	0.55	129	12.8	0.41	0.02	74
			S. D.	5.2	13.4	131.6	0.15	11	1.8	0.02	0.01	13
			N	5	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	29.5	71.4	844.8	0.42	127	13.3	0.36	0.03	61
			S. D.	3.9	2.7	80.7	0.14	13	1.9	0.01	0.01	15
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22.1	67.2	441.6	0.43	135	12.0	0.38	0.02	76
			S. D.	2.1	11.0	68.1	0.12	15	1.8	0.04	0.01	11
			N	5	5	5	5	5	5	5	5	5

Group	Dose of SP	Sex	Mean S. D. N	TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G1	0	Male	Mean	40	5.2	2.3	0.79	8.61	10.0	139	4.8	102
			S. D.	16	0.1	0.1	0.05	0.30	0.2	2	0.2	2
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	14	5.6	2.6	0.85	7.23	10.1	140	4.5	102
			S. D.	2	0.2	0.1	0.07	0.40	0.1	2	0.2	2
			N	5	5	5	5	5	5	5	5	5
G2	0.1	Male	Mean	54	5.3	2.3	0.77	8.32	10.2	139	4.9	103
			S. D.	27	0.1	0.1	0.03	0.50	0.3	1	0.2	3
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	15	5.5	2.5	0.83	7.44	10.0	140	4.5	102
			S. D.	6	0.1	0.1	0.06	0.27	0.2	1	0.2	1
			N	5	5	5	5	5	5	5	5	5

(Continued)

Group	Dose of SP	Sex	Mean S. D. N	TG (mg/dL)	TP (g/dL)	Alb (g/dL)	A/G ratio	P (mg/dL)	Ca (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
G3	0.5	Male	Mean	37	5.2	2.2	0.77	8.34	10.0	138	4.7	103
			S. D.	5	0.2	0.1	0.04	0.43	0.3	2	0.2	3
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	14	5.5	2.5	0.83	7.67	10.1	140	4.7	102
			S. D.	4	0.2	0.1	0.06	0.30	0.1	1	0.3	1
			N	5	5	5	5	5	5	5	5	5
G4	1.0	Male	Mean	35	5.1	2.2	0.78	8.33	10.1	139	4.8	102
			S. D.	13	0.3	0.1	0.06	0.55	0.3	3	0.2	2
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	22	5.5	2.5	0.83	6.98	10.3	140	4.7	103
			S. D.	8	0.3	0.1	0.04	0.48	0.3	1	0.1	1
			N	5	5	5	5	5	5	5	5	5

S.D., standard deviation; N, number of animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltranspeptidase; Glu, glucose; BUN, blood urea nitrogen; Crea, creatinine; T-Bili, total bilirubin; T-Chol, total cholesterol; TG, triglycerides; TP, total protein; Alb, albumin.

way to study safety assessments. For the study of toxicity, from the Korea Food & Drug Administration has a testing protocol guideline [12], and all the experiments should be conducted following GLP regulations. In this study, the LD_{50} of scolopendrid pharmacopuncture can be assumed to be above 1.0 mL in both male and female rats, indicating that this dose is safe to use and does not cause histological abnormalities.

5. Conclusion

The objective of this study was to analyze the single-dose toxicity of scolopendrid pharmacopuncture. All experiments were conducted at the KTR, an institution authorized to perform non-clinical studies, under the regulations of GLP. Results show that the administration of 1.0 mL/animal of scolopendrid pharmacopuncture does not cause any changes in weight or any mortality. This would indicate that this dosage of scolopendrid pharmacopuncture can be safely used for treatment.

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Conflict of interest

The authors declare that there are no conflicts of interest

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