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Original article

A Pilot Study on Single-dose Toxicity Testing of *Hominis placenta* Pharmacopuncture in Sprague-Dawley Rats

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Key Words

aqua acupuncture, herbal acupuncture, *Hominis placenta*, pharmacopuncture, sprague-dawley rats, toxicity test

Abstract

Objectives: This study was performed to analyze the toxicity and to find the lethal dose of the test substance *Hominis placenta* pharmacopuncture when used as a single-dose in 6 week old, male and female Sprague-Dawley (SD) rats.

Methods: All experiments were conducted at Biotoxtech (Chungwon, Korea), an institution authorized to perform non clinical studies, under the regulations of Good Laboratory Practice (GLP). SD rats were chosen for the pilot study. Doses of *Hominis placenta* pharmacopuncture extracts, 0.125, 0.25 and 0.5 mL, were administered to the experimental group, and 0.5 mL doses of normal saline solution were administered to the control group. This study was conducted under the approval of the Institutional Animal Ethics Committee.

Results: No deaths or abnormalities occurred in any of the groups. Also, no significant changes in body weights were observed among the groups, and no significant differences in hematology/biochemistry, necropsy, and histopathology results were noted. Hematologically, some changes in the male rats in two experimental groups were observed, but those changes had no clinical or toxicological meaning because they were not dose dependent. Histopathological tests on the injected parts showed cell infiltration in the male rats in one of the experimental groups; however, that result was due to spontaneous generation and had no toxicological meaning. Therefore, this study showed that *Hominis placenta* pharmacopuncture had no effect on the injected parts in terms of clinical signs, body weight, hematology, clinical chemistry, and necropsy.

Conclusion: As a result of single-dose tests of the test substance *Hominis placenta* pharmacopuncture in 4 groups of rats, the lethal dose for both males and females exceeded 0.5 mL/animal. Therefore, the above findings suggest that treatment with *Hominis placenta* pharmacopuncture is relatively safe. Further studies on this subject are needed.

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1. Introduction

Pharmacopuncture is a new form of acupuncture treatment combining acupuncture and herbal medicine. While the existing acupuncture treatment incorporates physical stimulation of associated meridians and acupoints, pharmacopuncture adds chemical stimulation to the existing acupuncture treatment [1].

Hominis placenta is isolated from pregnant women after delivery. Fetal nutrition breathing and defecation all take place through the placenta [2]. *Hominis placenta* is a rich resource of various bioactive substances, such as polydeoxyribonucleotides, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), peptides, amino acids, enzymes and trace elements [3].

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Hominis placenta pharmacopuncture is generally used these days, but toxicity tests have never been done. All experiments in this research were conducted at Biotoxtech (Chungwon, Korea), an authorized institution for non clinical studies, under the regulations of Good Laboratory Practice (GLP).

2. Materials and Methods

The *Hominis placenta* pharmacopuncture extract was prepared by adhering to Korea-Good Manufacturing Practice (K-GMP) in a clean room in a laboratory at the Korean Pharmacopuncture Institute. The pH was controlled to between 7.0 and 7.5. The completed extract was stored in a refrigerator (2.1 − 5.3°C).

The animals used in this study were 6 week old Sprague-Dawley (SD) rats. The reason SD rats were chosen is that they have generally been used in stability tests of medicine, so the data obtained in this study should be easily compared with many other databases. The mean weights of the rats were 185.1 − 205.9 g and 149.7 − 167.2 g for the male and the female rats, respectively. For all animals, a visual inspection was done, and all animals were weighed using a CP3202S system scale (Sartorius, Germany). After 7 days of acclimatization, the rats general symptoms and changes in weight were observed. The weights were recorded on the last day of acclimatization. No abnormalities were noted. The temperature of the lab was 21.1 − 24.1°C, and the humidity was 40.7% − 64.5%. Sufficient food (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C) and ultra violet (UV)-filtered water were provided. Group separations were done after 7 days of acclimatization. The animals were randomly distributed into 4 groups of 5 male and 5 female rats per group (Table 1): the control, low dose, mid dose and high dose groups.

The expected dose of *Hominis placenta* pharmacopuncture for clinical applications is 1.0 mL. In a pilot study (Biotoxtech Study No.: B13473P), 0.5 mL/animal of *Hominis placenta* pharmacopuncture was injected into each male and female rat; no deaths were observed. From this result, the doses for *Hominis placenta* pharmacopuncture were set as follows: animals in group 1 (G1, the control group) were injected with 0 mL/animal of pharmacopuncture and 0.5 mL/animal of normal saline solution (Choongwae Pharma Corp., Korea), animals in group 2 (G2, the low dose group) were injected with 0.125 mL/animal of pharmacopuncture, animals in group 3 (G3, the mid dose group) were injected with 0.25 mL/animal of pharmacopuncture, and animals in group 4 (G4, the high dose group) were injected with 0.5 mL/animal of pharmacopuncture. Using a disposable syringe (1 mL, 26 G), all rats were injected with a single dose in the left thigh muscle. This study was conducted under the approval of the Institutional Animal Ethics Committee of Biotoxtech.

The general symptoms (types of toxic symptoms, revealing times, recovery times, etc.) and the mortality were examined 30 minutes, and 1, 2, 4 and 6 hours after the injection on the day of dosing (day 0). From the 1st day to the 14th day of treatment, the general symptoms were examined once a day. The body weights were measured immediately before treatment and at 3, 7 and 14 days after injections.

All animals were fasted for more than 18 hours before the necropsy. The rats were anesthetized by using isoflurane, and blood samples were taken from the abdominal aorta (15 days after injection). An automatic hematology analyzer (ADVIA 2120i, SIEMENS, Germany) was used to analyze blood for the hematological examinations (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). For the biochemical tests, the blood remaining after carrying out the hematological tests was centrifuged at 3,000 rpm for 10 minutes, and the serum was collected. Biochemical test results were measured by using an automatic analyzer (7180, HITACHI, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany) (Table 4).

After the observations, organs and tissues from the entire bodies of all surviving animals were visually inspected. Tissue samples of all the animals were fixed in 10% neutral buffered formalin. Routine histological methods, like trimming, dehydration, and paraffin embedding, were conducted on the fixed organs and tissues. Fixed samples were sliced and stained with hematoxylin & eosin (H&E).

All the results from the experiments were analyzed by using statistical analysis system (SAS) software (version 9.3, SAS Institute Inc., U.S.A.). A Bartlett test was conducted to evaluate the homogeneity of the variance and the significance [4]. The one-way analysis of variation (ANOVA) test was conducted when homogeneity of the variance was recognized, and the Dunnett's *t*-test was conducted post-hoc [5]. The Kruskal-Wallis test was conducted when heterogeneity of the variance was recognized, and the steel test was conducted post-hoc.

Table 1 Groups of animals

Group	Dose (mL/animal)	Injection (mL/animal)	Number of animals (serial numbers)	
			Male	Female
G1: Control group	0	0.5	5 (1101 − 1105)	5 (2101 − 2105)
G2: Low-dose group	0.125	0.125	5 (1201 − 1205)	5 (2201 − 2205)
G3: Mid-dose group	0.25	0.25	5 (1301 − 1305)	5 (2301 − 2305)
G4: High-dose group	0.5	0.5	5 (1401 − 1405)	5 (2401 − 2405)

Table 2 Hematologic examination

Measurement	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, Peroxidase stain
WBC differential counting		
Neutrophils (NEU)	%	
Lymphocytes (LYM)		
Monocytes (MONO)		
Eosinophils (EOS)		
Basophils (BASO)		
Reticulocytes (Reti)	%	Flow cytometry, RNA stain

Table 3 Coagulation test

Measurement	Unit	Measuring method
Prothrombin test (PT)	Second	Coagulation time method
Activated partial thromboplastin time (APTT)	Second	

Table 4 Blood chemical test

Measurement	Unit	Measuring method
Prothrombin test (PT)	U/L	JSCC (UV Kinetic)
Activated partial thromboplastin time (APTT)	U/L	JSCC (UV Kinetic)
Alkaline phosphatase (ALP)	U/L	4-nitrophenyl-phosphate, 2Na (JSCC Transferable)
Gamma glutamyltranspeptidase (GGT)	U/L	International federation of clinical chemistr (IFCC)
Blood urea nitrogen (BUN)	mg/dL	Urease Glutamate dehydrogenase (GLDH)
Creatinine (Crea)	mg/dL	Jaffe
Total bilirubin (T-Bili)	mg/dL	Vanadate oxidation
Total protein (TP)	g/dL	Biuret
Albumin (Alb)	g/dL	Brom cresol green (BCG)
Albumin/globulin ratio (A/G ratio)	–	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	Orthocresolphthalein complexone (OCPC)
Chloride (Cl)	mmol/L	Ion selective electrode
Sodium (Na)	mmol/L	
Potassium (K)	mmol/L	

HMMPS, N-(3-sulfopropyl)-3-methoxy-5methylaniline; G6PDH, Glucose-6-phosphate dehydrogenase.

Table 5 Body weights (g)

Group	Dose (mL/animal)	Sex	Mean S.D. N	Days after administration			
				0	3	7	14
G1	0	Male	Mean	198.4	228.5	267.7	334.9
			S.D.	5.6	8.9	11.7	17.3
			N	5	5	5	5
		Female	Mean	158.8	172.1	188.8	213.3
			S.D.	6.0	7.8	7.5	8.7
			N	5	5	5	5
G2	0.125	Male	Mean	195.5	224.8	262.7	326.0
			S.D.	6.5	9.1	13.3	15.0
			N	5	5	5	5
		Female	Mean	161.0	171.6	187.2	206.7
			S.D.	5.0	6.4	10.8	13.0
			N	5	5	5	5
G3	0.25	Male	Mean	196.7	227.5	267.3	332.2
			S.D.	6.8	7.9	10.7	18.0
			N	5	5	5	5
		Female	Mean	159.6	173.1	192.2	215.4
			S.D.	3.5	6.4	8.5	12.0
			N	5	5	5	5
G4	0.5	Male	Mean	197.9	225.3	263.7	327.0
			S.D.	8.4	7.5	9.0	14.7
			N	5	5	5	5
		Female	Mean	160.1	171.0	187.4	209.8
			S.D.	6.3	7.0	8.4	12.2
			N	5	5	5	5

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

3. Results

During the observation, no deaths or abnormal symptoms occurred in any of the rats in the experimental (0.125, 0.25, 0.5 mL/animal) and the control groups.

No significant changes in body weight were observed in any of the groups (Table 5, Figs 1, 2). No significant changes in the hematological test results were observed in any of the groups, except that the blood urea nitrogen (BUN) of G2 males and the total cholesterol (T-Chol) of G3 males showed statistically significant changes; however, those results had no toxicological meaning because they were not dose dependent (Table 6). No significant changes in the biochemical test results were observed in any of the groups (Table 7), and no abnormalities were noted when visual inspections of all the animals were conducted. No significant changes were noted in the local tolerance test on the injection sites, except for G3 males; however, that result was due to spontaneous generation and had no toxicological meaning because it was not dose dependent.

4. Discussion

Pharmacopuncture is the combination of pharmacology and acupuncture. It is useful for the treatment or prevention of several diseases [6]. Pharmacopuncture and herbal acupuncture were introduced to optimize the benefits of acupuncture and herbal medicine by injecting minute quantities of herbs, medicines, blood, oxygen, and allergens into acupoints [7].

Hominis placenta pharmacopuncture is used generally in clinics, and many studies on the effects of *Hominis placenta* pharmacopuncture have been done. It has been reported to have effects on sleep pattern disturbance [8], Bell's palsy [9, 10], and dysmenorrheal [11, 12]. In addition, it has been reported to have effects on kidneys and livers in rats intoxicated by using $HgCl_2$ [13]. Although it is used in clinics, safety studies on *Hominis placenta* pharmacopuncture are insufficient, so more safety studies are needed.

SD rats have been widely used in safety tests of drugs because they have consistent reactions to drugs [14]. There-

Table 6 Mean hematology parameters

Group	Dose (mL/animal)	Sex	Mean S.D. N	RBC ($\times 10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices MCV (fL)	RBC Indices MCH (Pg)	RBC Indices MCHC (g/dL)	PLT ($\times 10^3$ cells/μL)	Reti (%)
G1	0	Male	Mean	6.81	14.3	40.7	59.8	21.1	35.3	1064	4.8
			S.D.	0.54	1.1	3.6	3.5	1.0	1.1	241	1.2
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.94	15.4	42.5	53.6	19.4	36.2	1067	2.2
			S.D.	0.25	0.3	1.1	1.0	0.4	0.4	144	0.3
			N	5	5	5	5	5	5	5	5
G2	0.125	Male	Mean	7.18	14.9	42.7	59.5	20.8	34.8	1147	4.0
			S.D.	0.19	0.3	0.9	2.4	0.9	0.3	77	0.3
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.89	15.1	42.0	53.2	19.2	36.0	1073	1.8
			S.D.	0.35	0.7	1.7	1.7	0.8	0.6	141	0.4
			N	5	5	5	5	5	5	5	5
G3	0.25	Male	Mean	6.99	14.4	41.3	59.1	20.6	34.9	1194	4.2
			S.D.	0.23	0.3	0.8	1.3	0.2	0.4	186	0.7
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.77	15.2	42.4	54.6	19.5	35.8	1142	2.1
			S.D.	0.51	0.7	2.1	1.2	0.6	0.6	97	0.3
			N	5	5	5	5	5	5	5	5
G4	0.5	Male	Mean	7.15	14.7	42.0	58.8	20.6	35.1	1242	4.3
			S.D.	0.22	0.5	1.4	3.0	1.1	0.2	100	0.8
			N	5	5	5	5	5	5	5	5
		Female	Mean	7.67	15.4	42.3	55.2	20.2	36.5	1086	2.3
			S.D.	0.24	0.3	0.9	1.4	0.6	0.3	59	0.7
			N	5	5	5	5	5	5	5	5

Group	Dose (mL/animal)	Sex	Mean S.D. N	WBC ($\times 10^3$ cells/μL)	WBC Differential Counting (%) NEU	LYM	MONO	EOS	BASO	PT (sec)	APTT (sec)
G1	0	Male	Mean	9.38	15.0	82.3	1.4	0.5	0.2	16.9	13.7
			S.D.	1.28	2.0	2.4	0.4	0.2	0.1	0.8	2.2
			N	5	5	5	5	5	5	5	5
		Female	Mean	3.10	19.0	77.9	1.4	1.2	0.1	18.2	14.6
			S.D.	0.84	8.5	8.8	0.5	0.4	0.0	0.6	1.6
			N	5	5	5	5	5	5	5	5
G2	0.125	Male	Mean	8.24	16.0	81.1	1.5	0.5	0.2	16.9	15.7
			S.D.	1.29	5.5	5.5	0.2	0.2	0.0	0.8	2.8
			N	5	5	5	5	5	5	5	5
		Female	Mean	5.19	16.6	80.4	1.4	1.0	0.2	18.4	16.4
			S.D.	3.38	5.6	6.1	0.5	0.6	0.1	0.4	1.2
			N	5	5	5	5	5	5	5	5

(continued)

G3	0.25	Male	Mean	8.58	12.5	84.2	1.6	0.5	0.2	17.6	14.9
			S.D.	2.83	2.7	3.3	0.6	0.2	0.1	0.8	1.7
			N	5	5	5	5	5	5	5	5
		Female	Mean	5.38	17.5	79.1	1.3	1.3	0.2	17.9	15.4
			S.D.	2.02	5.6	5.2	0.4	0.5	0.1	0.2	1.1
			N	5	5	5	5	5	5	5	5
G4	0.5	Male	Mean	8.38	13.3	83.8	1.4	0.5	0.1	16.8	15.6
			S.D.	0.70	3.7	4.1	0.5	0.1	0.1	0.7	2.0
			N	5	5	5	5	5	5	5	5
		Female	Mean	5.67	16.2	80.9	1.1	1.1	0.2	17.7	15.7
			S.D.	1.92	3.0	3.1	0.3	0.3	0.1	0.6	1.0
			N	5	5	5	5	5	5	5	5

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

Table 7 Mean clinical chemistry

Group	Dose (mL/animal)	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	29.2	94.2	719.5	0.36	111	9.7	0.39	0.02	82
			S.D.	3.4	15.5	148.0	0.11	3	0.9	0.02	0.03	12
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	24.2	90.7	440.8	0.78	112	13.4	0.44	0.02	63
			S.D.	4.0	17.0	86.4	0.24	6	1.6	0.04	0.01	25
			N	5	5	5	5	5	5	5	5	5
G2	0.125	Male	Mean	32.2	90.6	914.4	0.37	122	13.1*	0.40	0.01	68
			S.D.	3.6	15.7	184.6	0.15	10	3.0	0.03	0.01	5
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	25.7	91.9	417.1	0.52	105	12.2	0.41	0.01	67
			S.D.	7.4	17.6	107.0	0.14	8	1.9	0.01	0.01	12
			N	5	5	5	5	5	5	5	5	5
G3	0.25	Male	Mean	27.1	87.1	892.5	0.31	115	10.6	0.37	0.02	59†
			S.D.	1.1	14.2	80.0	0.06	7	1.0	0.03	0.01	5
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	26.4	99.6	463.0	0.56	111	12.4	0.45	0.01	68
			S.D.	4.7	23.7	106.4	0.20	4	1.5	0.03	0.01	8
			N	5	5	5	5	5	5	5	5	5
G4	0.5	Male	Mean	30.6	82.8	840.4	0.34	120	12.3	0.40	0.02	68
			S.D.	6.1	9.6	168.7	0.09	15	1.9	0.03	0.01	13
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	25.1	103.4	490.1	0.67	107	14.3	0.45	0.02	75
			S.D.	1.4	21.6	140.8	0.10	7	0.8	0.03	0.00	14
			N	5	5	5	5	5	5	5	5	5

(continued)

Group	Dose (mL/animal)	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	42	5.4	2.3	0.73	8.65	9.9	140	4.6	105
			S.D.	19	0.2	0.0	0.04	0.60	0.2	1	0.3	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	15	5.8	2.6	0.85	6.79	9.8	140	4.5	107
			S.D.	6	0.3	0.1	0.09	0.49	0.4	1	0.2	1
			N	5	5	5	5	5	5	5	5	5
G2	0.125	Male	Mean	41	5.3	2.3	0.78	8.45	9.6	141	4.5	106
			S.D.	15	0.2	0.1	0.06	0.57	0.2	2	0.3	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	13	5.8	2.6	0.83	7.46	10.0	141	4.5	106
			S.D.	6	0.2	0.2	0.04	0.92	0.4	1	0.4	2
			N	5	5	5	5	5	5	5	5	5
G3	0.25	Male	Mean	49	5.2	2.3	0.77	8.51	9.7	140	4.6	105
			S.D.	6	0.1	0.1	0.05	0.25	0.2	1	0.2	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	20	5.8	2.6	0.84	7.01	9.9	140	4.5	105
			S.D.	14	0.2	0.1	0.02	0.46	0.2	1	0.3	1
			N	5	5	5	5	5	5	5	5	5
G4	0.5	Male	Mean	30	5.3	2.3	0.75	8.15	9.8	140	4.4	104
			S.D.	18	0.1	0.1	0.03	0.42	0.3	0	0.1	1
			N	5	5	5	5	5	5	5	5	5
		Female	Mean	17	5.8	2.6	0.80	7.34	10.0	139	4.5	105
			S.D.	10	0.3	0.1	0.05	0.45	0.3	1	0.2	1
			N	5	5	5	5	5	5	5	5	5

Significantly different from control by Dunnett's *t*-test: $^{*}P < 0.05$, $^{\dagger}P < 0.01$. 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; A/G ratio, albumin/globulin ratio; P, phosphorus; Ca, calcium; Na, sodium; K, potassium; Cl, chloride.

fore, they are considered to be appropriate for use as test animals.

This study was performed to provide objective safety data for *Hominis placenta* pharmacopuncture. During the observation, no deaths or abnormal clinical symptoms occurred in any of the groups. No significant changes in body weight were observed in any of the groups. Hematologically, some changes in the G2 and the G3 male group were observed, but those changes had no clinical or toxicological meaning. No significant change in the biochemical test results were observed in any of the groups. Visual inspection after necropsy showed no visual abnormalities in the male and the female animals in any groups. Histopathological tests on the injected parts showed a cell infiltration in the G3 male group; however, the result was due to spontaneous generation and had no toxicological meaning.

Further studies should be conducted to evaluate the toxicity more precisely for clinical applications. We need to study the acute and the chronic side effects of *Hominis placenta* pharmacopuncture and its relations to the capacity reaction more. Also, the toxicity and the safety of *Hominis placenta* pharmacopuncture for use on humans must be investigated.

5. Conclusions

The objective of this study was to analyze the single-dose toxicity of *Hominis placenta* pharmacopuncture extracts. All experiments were conducted under the regulations of GLP at Biotoxtech, an institution authorized to perform non clinical studies. The results showed that administration of 0.5 mL/animal of *Hominis placenta* pharmacopuncture extracts did not cause any changes in weight or any mortalities in SD rats. This study indicates that *Hominis placenta* pharmacopuncture can be used as a safe treatment.

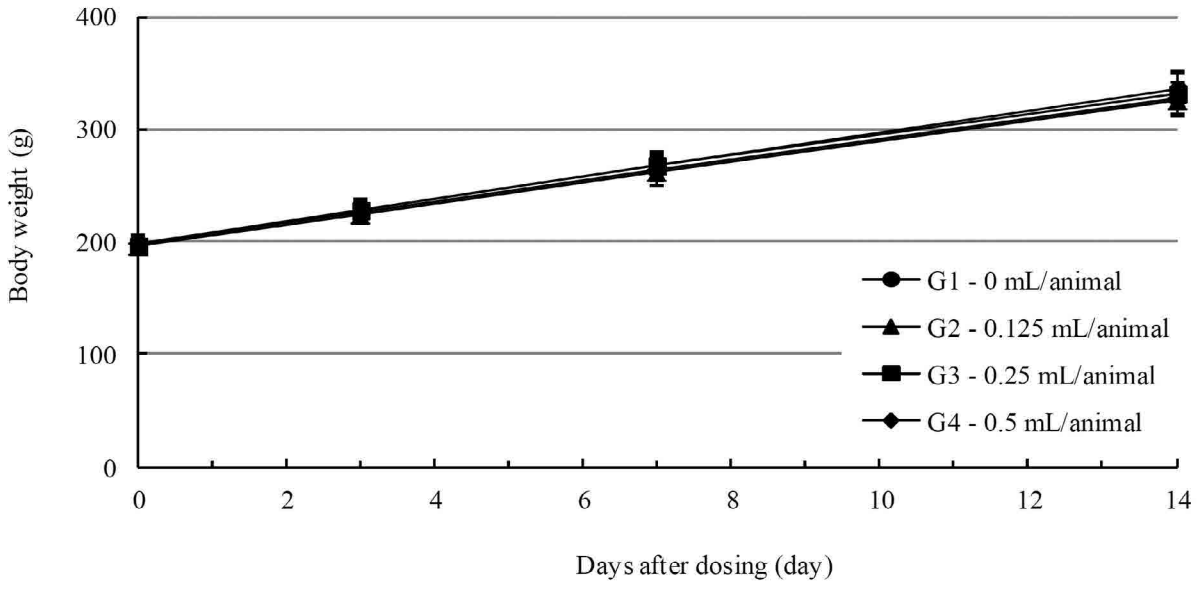

Figure 1 Body weights in male Sprague-Dawley rats.

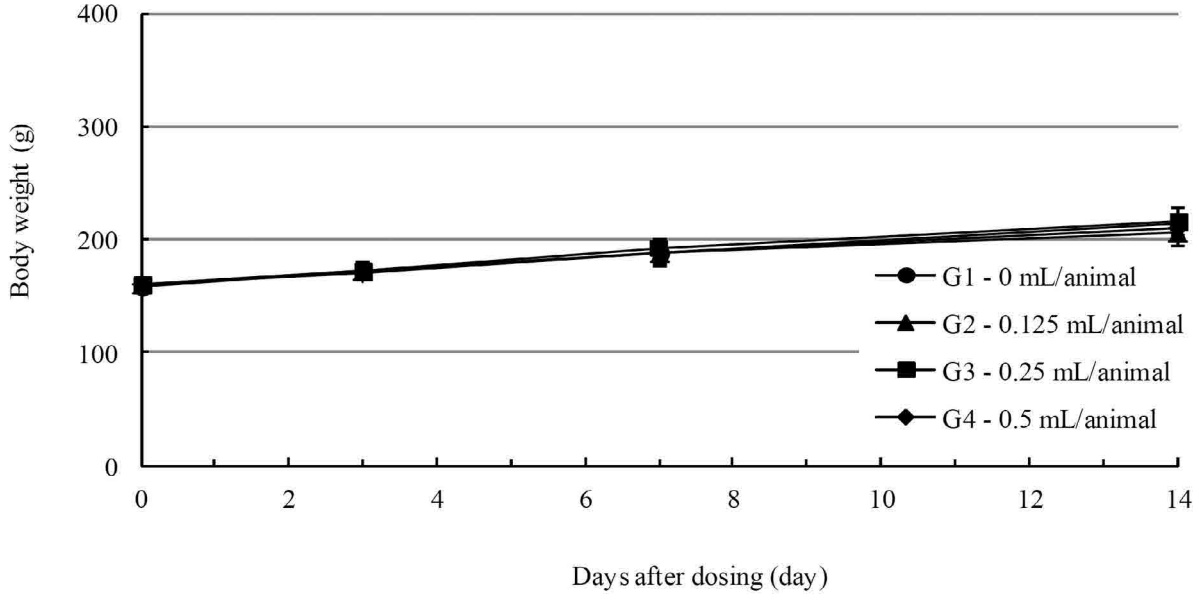

Figure 2 Body weights in female Sprague-Dawley rats.

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Conflict of interest

The authors declare that there are no conflict of interest.

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