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Original article

Study of Intravenous Single-Dose Toxicity Test of *Bufonis venonum* Pharmacopuncture in Sprague-Dawley Rats

Ki-Rok Kwon[1], Jun-Sang Yu[2], Seung-Ho Sun[3], Kwang-Ho Lee[4]*

[1]Research Center of the Korean Pharmacopuncture Institute, Seoul, Korea
[2]Department of Sasang Constitutional Medicine, Sangji University College of Korean Medicine, Wonju, Gangwon, Korea
[3]Department of Internal Medicine, Sangji University College of Korean Medicine, Wonju, Gangwon, Korea
[4]Department of Acupuncture & Moxibustion Medicine, Sangji University College of Korean Medicine, Wonju, Gangwon, Korea

Key Words

Bufonis venonum, Chan-Su, pharmacopuncture, toad venom, toxicity test

Abstract

Objectives: *Bufonis venonum* (BV) is toad venom and is the dried, white secretions of the auricular and the skin glands of toads. This study was performed to evaluate the toxicity of intravenous injection of *Bufonis venonum* pharmacopuncture (BVP) through a single-dose test with sprague-dawley (SD) rats.

Methods: Twenty male and 20 female 6-week-old SD rats were injected intravenously in the caudal vein with BVP or normal saline. The animals were divided into four groups with five female and five male rats per group: the control group injected with normal saline, the low-dosage group injected with 0.1 mL/animal of BVP, the medium-dosage group injected with 0.5 mL/animal of BVP and the high-dosage group injected with 1.0 mL/animal of BVP. We performed clinical observations every day and body weight measurements on days 3, 7 and 14 after the injection. We also conducted hematology, serum biochemistry, and histological observations immediately after the observation period.

Results: No mortalities were observed in any experimental group. Paleness occurred in the medium- and the high-dosage groups, and congestion on tails was observed in females in the medium- and the high-dosage groups. No significant changes in weight, hematology, serum biochemistry, and histological observations that could be attributed to the intravenous injection of BVP were observed in any experimental group.

Conclusion: The lethal dose of intravenously-administered BVP in SD rats is over 1.0 mL/animal.

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1. Introduction

Bufonis venonum (BV), "Chan-Su" in Chinese and "Somso or Sumsu" in Korean, is a well-known traditional Korean medicine obtained from the skin venom gland of a toad, such as *Bufo gargarizans Cantor* or *Bufo melanostictus Schneider*. It is mainly produced in China's Hebei, Shandong, Jiangsu and Zhejiang provinces. Formulations of toad venom have been widely applied in China, Japan, Korea and other Oriental countries for a long time [1, 2].

BV has been used in the treatment of various diseases, including cancer, arrhythmia, and various heart diseases. Recent studies have shown that an extract of a species of Chinese toad has several functions, including the abilities to kill several kinds of tumor cells and increase immunity as well as antitumor and leu-

***Corresponding Author**
Kwang-Ho Lee. Department of Acupuncture & Moxibustion, Sangji University College of Oriental Medicine, 283, Woosan-dong, Wonju, Gangwon 220-955, Korea.
Tel: +82-33-741-9257 Fax: +82-33-732-2124
E-mail: redphilips@hanmail.net

kopoietic effects [3]. It is one of the major components of "Liu-Shen-Wan" and "Kyushin", both of which are traditional Chinese medicines. "Liu-Shen-Wan" has been used for the treatment of tonsillitis, sore throat, and furuncle because of its local anesthetic and antibiotic actions. "Kyushin" is used for the treatment of palpitation and anhelation and is reported to have a cardiotonic effect, an excitatory action on respiration, as well as a local anesthetic action. The cardiotonic effect of Kyushin has been suggested to be due to the action of bufadienolides such as bufalin, cinobufagin, and resibufogenin [4]. Especially, BV is extremely cardiotoxic, even in small dose, and acts rapidly to alter intracellular calcium stores in cardiomyocytes and possibly acts at sites other than the $Na^{2+}K^{+}$ ATPase either directly or indirectly *via* changes in calcium concentrations [5].

However, most toxic compounds of toad venom are steroids similar to digoxin. These induce not only gastrointestinal symptoms, such as nausea, vomiting, and abdominal discomfort, but also digitalis toxicity-like cardiac effects, including bradycardia, atrioventricular conduction block, ventricular tachycardia, ventricular fibrillation, and sudden death [6]. Thus, BV has been used carefully by clinicians [3].

Bufonis venonum pharmacopuncture (BVP) is a pharmacopuncture that is produced by using various substances extracted from the toad venom. We identified the safety of intramuscular injection of BVP by conducting a single-dose toxicity test [7]. This time, we conducted an intravenous single-dose toxicity test of BVP in sprague-dawley (SD) rats to determine its safety.

2. Materials and Methods

Twenty-four 5-week-old SD rats of each gender (48 total rats) were obtained from Orientbio Inc. (Gyeong-gi, Korea) and were used after a week of quarantine and acclimatization. The male rats weighed from 114.8 to 127.1 g, and female rats weighed from 111.3 to 122.7 g. The animals were housed in a room maintained at 20.0 — 23.0℃ under a relative humidity of 42.8% — 68.9%. The room was illuminated with artificial lighting from 07:00 to 19:00 hour and had 10 — 15 air changes per hour. The animals were housed in suspended stainless-steel wire-mesh cages with three animals per cage and were allowed access to sterilized tap water and commercial rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Harlan Laboratories, Inc., USA). This study's protocol was approved by the Institutional Animal Care Board of Biotoxtech Co. (Oh Chang, Korea).

The BVP (Lot No. N-001) was manufactured in a pathogen-free facility (Korean Pharmacopuncture Institute, Seoul, Korea). BV was purchased from Shandong, China, and was extracted as a hot water extract. The pharmacopuncture at a concentration of 0.1 mg/mL was filtered using 0.1-μm filtering paper. Finally, the BVP was sterilized before being used for this experiment.

After an adaptation period of 1 week, 20 healthy male SD rats and 20 healthy female SD rats were selected and assigned to 1 of 4 groups according to their average weights: control (normal saline, 1 mL/animal), low-dosage (0.1 mL BVP/animal), medium-dosage (0.5 mL BVP/animal) and high-dosage (1.0 mL BVP/animal) groups. The weights of the male rats were 183.6 — 197.6 g, and those of the female rats were 149.1 — 172.3 g. BVP or normal saline (Lot No. 12115, Choongwae Pharma Corp., Korea) was administered to the rats by intravenous injection in the caudal vein.

All animals were observed for clinical signs at 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours from the treatment and once a day starting on day 3 and ending on day 14 days. The body weight of each rat was measured before and after the injection and on the 3rd day, 7th day and 14th day after the injection. 15 days after treatment, the animals were fasted for 18 hours prior to necropsy and blood collection. Blood samples were drawn from the abdominal aorta by using a syringe needle under isoflurane anesthesia. Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and were analyzed to determine the red blood cell count (RBC), hemoglobin concentration (Hb), hematocrits (Ht), mean corpuscular cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular cell hemoglobin concentration (MCHC), platelet count, white blood cell count (WBC), differential WBC count, neutrophils (NEU), lymphocytes (LYM), monocytes MONO), eosinophils (EOS), basophils (BASO), and reticulocyte count (Reti) by using Hematology Systems (ADVIA 120, SIEMENS, Munich, Germany). The prothrombin time (PT) and the active partial prothrombin time (APTT) were measured by using the Coapresta 2000 instrument (SELISUI, Japan).

For the serum biochemistry analyses, blood samples were centrifuged at 3,000 rpm for 10 minutes and analyzed using an auto-analyzer (7180, HITACHI, Tokyo, Japan) and an electrolyte analyzer (AVL9181, Roche, Germany). Serum biochemistry parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine (Crea), total bilirubin (T-Bili), total protein (TP), albumin, albumin/globulin ratio (A/G ratio), total cholesterol (T-Chol), triglycerides (TG), phosphorus (P), glucose, calcium (Ca), chloride (Cl), sodium (Na), and potassium (K), were examined. The tissues from the injection sites on the rats were routinely processed, embedded in paraffin and sectioned into 3- to 5-μm pieces. The sections were stained with hematoxylin and eosin (H&E) for microscopic examination. Tissues were taken from all animals, and all tissues were examined microscopically.

Data on animal weights, blood chemistry and hematological results were tested by using SAS software (version 9.3, SAS Institute, Inc., Cary, NC, USA). The variance in the numerical data was checked using the Bartlett test. If the variance was homogeneous, the data were subjected to a one-way analysis of variance (ANOVA). If either of the tests showed a significant difference among the groups, the data were analyzed using the multiple comparison procedure of the Dunnett's test. If not, they were analyzed using the Kruskal-Wallis test $P < 0.05$ was considered significant.

3. Results

No treatment-related mortalities or weight changes were observed during the observation period (Table 1). Paleness occurred 10 minutes after treatment in the medium- and the high-dosage groups, and congestion was observed 30 minutes (3 cases) and 1 hour (2 cases) after treatment in female rats in the medium- and the high-dosage groups. The changes in the clinical signs in the animals seem to have been caused by the administration of BVP (Table 2). Blood chemistry and hematology showed minor changes in the animals, but those changes were not dose-dependent, which means that BVP has no toxicity (Tables 4, 5). In the histopathological examinations, no changes due to the administration of BVP were observed (Tables 5, 6).

4. Discussion

The main components of BV include bufalin, cinobufagin, resibufogenin, cinobufotoxin, cinobufotalin and cinobufotalidin [8]. However, components of BV could vary by using various extraction methods and solvents. So the choice of solvent is very important for the extraction of targeting component. The water extract of toad venom contained the greatest amount of serotonin but very small amounts of bufodienolides. In contrast, the use of MeOH or EtOH extraction solutions resulted in 5 — 26 times higher concentrations of bufodienolides, with only trace amounts of serotonin [9].

Because the BVP in this test was made through hot water extraction, the components contained mainly serotonin. Serotonin (5-hydroxytryptamine) is also one of the main ingredients in BV [10]. It is involved in various psychiatric disorders such as depression, anxiety, obsessive symptoms and impulsivity, as well as in the regulation of the feeling of satiety [11]. Therefore, BVP has therapeutic potential for treating patients with neuropsychiatric disorders such as anxiety or depression disorder [10]. Serotonin is one of the most effective pruritogens in the cheek model in rats. Application of serotonin to the skin can also cause itching in humans. In several conditions of chronic itching, including allergic contact dermatitis and atopic dermatitis, the patient's skin exhibits increased levels of serotonin. Serotonin can also elicit pain in humans [13].

For the above reasons, we conducted an intravenous single-dose toxicity test of BVP in SD rats. The results showed no treatment-related abnormalities for any of the used doses of BVP. The dose used for the high-dosage group was 1.0 mL/animal, and no dangerous signs were observed. Thus, we may conclude that the approximate lethal dose of BVP is over 1.0 mL/animal in both male and female SD rats.

Table 1 Mean body weights in grams

Sex	Group/ Dose (mL/animal)	Mean S.D. N	Days after dosing 0	3	7	14	Gain Day 0 — 14
Male	G1 (0)	Mean	192.6	219.0	260.3	324.4	131.8
		S.D.	5.1	5.3	8.2	13.1	10.4
		N	5	5	5	5	5
	G2 (0.1)	Mean	192.1	217.4	253.6	311.3	119.2
		S.D.	4.9	4.4	8.5	14.0	13.0
		N	5	5	5	5	5
	G3 (0.5)	Mean	191.9	216.0	254.8	313.3	121.5
		S.D.	5.1	5.7	7.4	10.2	11.7
		N	5	5	5	5	5
	G4 (1.0)	Mean	192.8	217.6	258.3	319.8	127.1
		S.D.	4.0	3.9	4.5	11.1	12.7
		N	5	5	5	5	5
Female	G1 (0)	Mean	158.4	171.8	188.4	210.1	51.7
		S.D.	6.4	4.9	7.5	12.9	9.9
		N	5	5	5	5	5
	G2 (0.1)	Mean	159.5	172.7	187.4	215.2	55.7
		S.D.	10.3	9.2	9.2	13.9	8.0
		N	5	5	5	5	5
	G3 (0.5)	Mean	160.9	172.6	189.0	215.5	54.6
		S.D.	5.1	6.8	8.8	15.0	10.8
		N	5	5	5	5	5
	G4 (1.0)	Mean	160.4	170.9	186.3	214.3	53.9
		S.D.	7.1	5.2	6.4	9.4	5.3
		N	5	5	5	5	5

Table 2 Summary of clinical signs

Sex	Group/ Dose (mL/animal)	No. of animals	Clinical sign	Hours (Day 0) after dosing					
				0.2	0.5	1	2	4	6
Male	G1 (0)	5	NOA	5	5	5	5	5	5
	G2 (0.1)	5	NOA	5	5	5	5	5	5
	G3 (0.5)	5	NOA Paleness	5	5	5	5	5	5
	G4 (1.0)	5	NOA Paleness	5	5	5	5	5	5
Female	G1 (0)	5	NOA	5	5	5	5	5	5
	G2 (0.1)	5	NOA	5	5	5	5	5	5
	G3 (0.5)	5	NOA Paleness Congestion*	5	4 1	4 1	5	5	5
	G4 (1.0)	5	NOA Paleness Congestion*	5	3 2	4 1	5	5	5

Table 3 Mean hematology parameters

(Sex: Male)

Group/ Dose (mL/animal)	Mean S.D. N	RBC ($\times 10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT ($\times 10^3$ cells/μL)	Reti (%)
					MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1 (0)	Mean S.D. N	6.89 0.19 5	14.0 0.4 5	43.0 1.2 5	62.4 1.2 5	20.3 0.3 5	32.6 0.4 5	1237 122 5	5.4 0.8 5
G2 (0.1)	Mean S.D. N	7.08 0.29 5	14.1 0.5 5	43.0 1.5 5	60.7 1.4 5	20.0 0.5 5	32.9 0.3 5	1332 106 5	4.9 0.5 5
G3 (0.5)	Mean S.D. N	6.48 0.57 5	13.5 1.3 5	41.4 3.4 5	64.0 2.1 5	20.9 0.8 5	32.7 0.7 5	1321 85 5	6.8 2.6 5
G4 (1.0)	Mean S.D. N	7.02 0.18 5	14.3 0.5 5	43.8 1.4 5	62.4 2.1 5	20.3 0.7 5	32.6 0.1 5	1355 217 5	5.5 1.2 5

(Continued)

Group/ Dose (mL/animal)	Mean S.D. N	WBC ($\times 10^6$ cells/μL)	WBC Differential Counting (%)					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G1 (0)	Mean	7.97	16.6	78.8	2.6	0.5	0.2	17.0	14.3
	S.D.	1.13	2.5	1.3	1.0	0.1	0.1	0.9	1.5
	N	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	7.42	18.2	78.1	2.2	0.4	0.2	17.2	15.2
	S.D.	1.75	1.6	1.4	0.4	0.1	0.1	0.6	0.7
	N	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	7.46	17.6	78.1	2.2	0.5	0.1	17.3	14.6
	S.D.	2.35	5.9	6.0	0.7	0.3	0.1	0.7	0.9
	N	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	7.96	17.6	77.9	2.5	0.5	0.2	17.1	14.8
	S.D.	0.54	5.9	5.1	0.6	0.4	0.1	0.7	0.7
	N	5	5	5	5	5	5	5	5

(Sex: Female)

Group/ Dose (mL/animal)	Mean S.D. N	RBC ($\times 10^6$ cells/μL)	HGB (g/dL)	HCT (%)	RBC Indices			PLT ($\times 10^3$ cells/μL)	Reti (%)
					MCV (fL)	MCH (pg)	MCHC (g/dL)		
G1 (0)	Mean	7.40	14.7	42.8	57.9	19.9	34.3	1283	2.7
	S.D.	0.35	0.5	1.5	1.8	0.8	0.5	164	0.4
	N	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	6.86	14.2	41.7	60.8†	20.7	34.1	1275	3.2
	S.D.	0.19	0.5	1.4	0.6	0.3	0.3	141	0.3
	N	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	7.02	14.3	41.8	59.6	20.4	34.1	1277	2.8
	S.D.	0.38	0.6	1.9	1.5	0.8	0.7	160	0.4
	N	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	7.04	14.5	42.5	60.3*	20.6	34.2	1200	3.0
	S.D.	0.32	0.9	2.5	1.1	0.7	0.8	154	0.3
	N	5	5	5	5	5	5	5	5

Group/ Dose (mL/animal)	Mean S.D. N	WBC ($\times 10^6$ cells/μL)	WBC Differential Counting (%)					PT (sec)	APTT (sec)
			NEU	LYM	MONO	EOS	BASO		
G1 (0)	Mean	4.82	20.0	75.7	2.2	0.9	0.1	18.5	13.2
	S.D.	0.91	6.6	6.8	0.7	0.4	0.1	0.3	1.6
	N	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	3.40	16.0	80.6	1.6	0.9	0.1	18.4	13.9
	S.D.	1.12	4.2	4.4	0.7	0.2	0.1	0.8	1.8
	N	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	3.86	12.7	83.4	1.7	1.0	0.1	18.5	14.9
	S.D.	1.09	3.7	3.2	0.5	0.3	0.1	0.2	1.1
	N	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	4.03	16.9	79.6	1.6	0.8	0.1	18.3	14.8
	S.D.	1.21	3.9	3.5	0.6	0.3	0.0	0.7	0.8
	N	5	5	5	5	5	5	5	5

*Significantly different from control by Dunnett's *t*-test. $P < 0.05$, †$P < 0.01$.

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; 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 4 Mean clinical chemistry

(Sex: Male)

Group/ Dose (mL/animal)	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)	Mean	28.8	90.1	667.2	0.20	123	11.7	0.36	0.04	87
	S.D.	6.1	16.2	102.5	0.18	22	1.6	0.01	0.01	24
	N	5	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	29.0	75.7	831.5	0.19	129	10.8	0.34	0.03	65
	S.D.	5.0	9.5	206.6	0.08	16	0.4	0.02	0.01	8
	N	5	5	5	4	5	5	5	5	5
G3 (0.5)	Mean	29.4	78.4	731.9	0.22	127	10.9	0.34	0.03	76
	S.D.	5.8	9.7	117.3	0.14	8	1.2	0.01	0.01	17
	N	5	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	29.0	76.0	716.4	0.16	133	11.3	0.35	0.02	87
	S.D.	4.7	10.0	194.9	0.08	11	1.1	0.04	0.01	9
	N	5	5	5	5	5	5	5	5	5

Group/ Dose (mL/animal)	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)	Mean	68	5.2	2.3	0.78	8.61	10.3	138	4.8	102
	S.D.	48	0.1	0.1	0.07	0.35	0.2	1	0.2	1
	N	5	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	40	5.3	2.3	0.75	8.15	10.3	139	4.4	103
	S.D.	9	0.1	0.0	0.03	0.39	0.1	1	0.2	2
	N	5	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	48	5.2	2.2	0.75	8.60	10.2	139	4.7	104
	S.D.	25	0.2	0.1	0.02	0.34	0.2	1	0.4	2
	N	5	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	67	5.4	2.3	0.76	8.46	10.5	139	4.7	103
	S.D.	22	0.2	0.1	0.05	0.54	0.1	1	0.2	1
	N	5	5	5	5	5	5	5	5	5

(Sex: Female)

Group/ Dose (mL/animal)	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)	Mean	23.2	90.4	445.6	0.45	122	11.9	0.41	0.03	79
	S.D.	4.5	23.9	125.6	0.05	10	1.0	0.02	0.01	16
	N	5	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	25.0	107.3	578.8	0.29	130	12.4	0.41	0.03	72
	S.D.	11.0	64.3	75.1	0.08	15	1.6	0.01	0.02	13
	N	5	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	23.1	81.0	493.2	0.28*	131	11.0	0.38	0.03	75
	S.D.	5.5	17.5	120.2	0.06	10	2.1	0.02	0.01	18
	N	5	5	5	4†	5	5	5	5	5
G4 (1.0)	Mean	20.9	84.7	542.0	0.37	136	12.1	0.40	0.02	88
	S.D.	4.3	12.4	97.4	0.14	13	1.1	0.03	0.02	16
	N	5	5	5	5	5	5	5	5	5

(Continued)

Group/ Dose (mL/animal)	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)	Mean	15	5.7	2.6	0.82	7.21	10.1	139	4.7	104
	S.D.	5	0.2	0.1	0.04	0.27	0.3	1	0.4	0
	N	5	5	5	5	5	5	5	5	5
G2 (0.1)	Mean	14	5.5	2.5	0.82	7.20	9.7	140	4.8	105
	S.D.	8	0.3	0.1	0.05	0.30	0.3	1	0.3	2
	N	5	5	5	5	5	5	5	5	5
G3 (0.5)	Mean	16	5.5	2.5	0.83	7.45	10.1	138	4.7	103
	S.D.	2	0.3	0.1	0.03	0.39	0.4	1	0.3	1
	N	5	5	5	5	5	5	5	5	5
G4 (1.0)	Mean	16	5.7	2.6	0.85	7.43	10.1	139	4.6	103
	S.D.	5	0.2	0.1	0.06	0.36	0.2	1	0.3	2
	N	5	5	5	5	5	5	5	5	5

*, Significantly different from control by Dunnett's *t*-test. $P < 0.05$; †, Value below the level of detection was excluded from statistics.

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.

Table 5 Summary of necropsy findings

Sex	Male				Female			
Group	G1	G2	G3	G4	G1	G2	G3	G4
Dose (mL/animal)	0	0.1	0.5	1.0	0	0.1	0.5	1.0
No. of animals	5	5	5	5	5	5	5	5
Unremarkable findings	5	5	5	5	5	5	5	5
No. examined	5	5	5	5	5	5	5	5

External surface and all organs in body cavity were unremarkable.

Table 6 Summary of histopathological findings

Sex		Male				Female			
Organ/ Findings	Group	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mL/animal)	0	0.1	0.5	1.0	0	0.1	0.5	1.0
	No. of animals	5	5	5	5	5	5	5	5
Injection site	Remarkable findings	0	0	0	0	0	0	0	0
	No. examined	5	5	5	5	5	5	5	5

5. Conclusion

Under our experimental conditions, intravenous injection of BVP did not cause any complications. Thus, we conclude that the lethal dose of BVP is over 1.0 mL/animal in both male and female SD rats.

Conflict of interest

The authors declare that there are no conflicts of interest.

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