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

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**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-
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 t nonsili stis, 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⁺−K⁺ ATPase either directly or indirectly via changes in calcium concentrations [5].

However, most toxic compounds of toad venom are steroid 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 113.3 to 122.7 g. The animals were housed in a room maintained at 20.0 – 23.0°C 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 Biotech 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 149.1 – 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 10 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 thrombin time (APTT) were measured by using the Coagpresta 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). Pale
ness 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-depend-
ent, 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, cinobufotinin, cinobufotalin and cinobuf-
fotalidin [8]. However, components of BV could vary by us-
ing 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 con-
centrations 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. Ap-
plication of serotonin to the skin can also cause itching in
humans. In several conditions of chronic itching, includ-
ing allergic contact dermatitis and atopic dermatitis, the
patient’s skin exhibits increased levels of serotonin. Sero-
tonin can also elicit pain in humans [13].

For the above reasons, we conducted an intravenous sin-
gle-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 | Gain Day 0 — 14 |
|         |                            | 0    | 3    | 7  | 14            |
| Female  | G1 (0)                     | Mean 158.4 | 6.4 | 5 | 171.8 | 210.1 | 51.7 |
|         | N                           | S.D. 4.9 | 7.5 | 5 | 186.4 | 12.9 | 9.9 |
|         |                             | N      | 5 | 5 | 201.2 | 5 |  | 5 |
| Female  | G2 (0.1)                    | Mean 159.5 | 10.3 | 9.2 | 172.7 | 215.2 | 55.7 |
|         | N                           | S.D. 9.2 | 9.2 | 5 | 187.4 | 13.9 | 8.0 |
|         |                             | N      | 5 | 5 | 213.2 | 5 |  | 5 |
| Female  | G3 (0.5)                    | Mean 160.9 | 5.1 | 6.8 | 172.6 | 215.5 | 54.6 |
|         | N                           | S.D. 6.8 | 8.8 | 5 | 189.0 | 15.0 | 10.8 |
|         |                             | N      | 5 | 5 | 210.8 | 5 |  | 5 |
| Female  | G4 (1.0)                    | Mean 160.4 | 7.1 | 5.2 | 170.9 | 214.3 | 53.9 |
|         | N                           | S.D. 5.2 | 6.4 | 5 | 186.3 | 9.4 | 5.3 |
|         |                             | N      | 5 | 5 | 209.7 | 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  | 4   | 4   | 5 | 5 | 5 | 5 |
|       |                         |                | Congestion*   | 1   | 1   |   |   |   |   |
|       | G4 (1.0)               | 5              | NOA Paleness  | 3   | 4   | 5 | 5 | 5 | 5 |
|       |                         |                | Congestion*   | 2   | 1   |   |   |   |   |

Table 3  Mean hematology parameters

(Sex: Male)

| Group/Dose (mL/animal) | Mean S.D. N | RBC (×10⁶ cells/μL) | HGB (g/dL) | HCT (%) | RBC Indices | RBC Indices | RBC Indices | RBC Indices | RETI (%) |
|------------------------|-------------|---------------------|------------|---------|--------------|--------------|--------------|--------------|----------|
|                        |             |                     |            |         | MCV (FL)     | MCH (pg)     | MCHC (g/dL)  | PLT (×10³ cells/μL) |         |
| G1 (0)                 | Mean        | 6.89 5              | 14.0       | 43.0    | 62.4         | 20.3         | 32.6         | 1237         | 5.4      |
|                        | S.D.        | 0.19 5              | 0.4        | 1.2     | 1.2          | 0.3          | 0.4          | 122          | 0.8      |
|                        | N           | 5 5                | 5 5        | 5 5     | 5 5          | 5 5          | 5 5          | 5 5          |          |
| G2 (0.1)               | Mean        | 7.08 5              | 14.1       | 43.0    | 60.7         | 20.0         | 32.9         | 1332         | 4.9      |
|                        | S.D.        | 0.29 5              | 0.5        | 1.5     | 1.4          | 0.5          | 0.3          | 106          | 0.5      |
|                        | N           | 5 5                | 5 5        | 5 5     | 5 5          | 5 5          | 5 5          | 5 5          |          |
| G3 (0.5)               | Mean        | 6.48 5              | 13.5       | 41.4    | 64.0         | 20.9         | 32.7         | 1321         | 6.8      |
|                        | S.D.        | 0.57 5              | 1.3        | 3.4     | 2.1          | 0.8          | 0.7          | 85           | 2.6      |
|                        | N           | 5 5                | 5 5        | 5 5     | 5 5          | 5 5          | 5 5          | 5 5          |          |
| G4 (1.0)               | Mean        | 7.02 5              | 14.3       | 43.8    | 62.4         | 20.3         | 32.6         | 1355         | 5.5      |
|                        | S.D.        | 0.18 5              | 0.5        | 1.4     | 2.1          | 0.7          | 0.1          | 217          | 1.2      |
|                        | N           | 5 5                | 5 5        | 5 5     | 5 5          | 5 5          | 5 5          | 5 5          |          |

(Continued)
(Sex: Female)

| Group/Dose (mL/animal) | Mean | S.D. | RBC (× 10^6 cells/μL) | HGB (g/dL) | HCT (%) | MCV (fl) | MCH (pg) | MCHC (g/dL) | PLT (× 10^3 cells/μL) | Reti (%) |
|------------------------|------|------|------------------------|------------|---------|---------|---------|------------|---------------------|----------|
|                        | Mean | S.D. | N                      |            |         |          |          |            |                     |          |
| G1 (0)                 | 7.40 | 0.35 | 5                      | 14.7       | 42.8    | 57.9    | 19.9    | 34.3       | 1283                | 2.7      |
| G2 (0.1)               | 6.86 | 0.19 | 5                      | 14.2       | 41.7    | 60.8    | 20.7    | 34.1       | 1275                | 3.2      |
| G3 (0.5)               | 7.02 | 0.38 | 5                      | 14.3       | 41.8    | 59.6    | 20.4    | 34.1       | 1277                | 2.8      |
| G4 (1.0)               | 7.04 | 0.32 | 5                      | 14.5       | 42.5    | 60.3    | 20.6    | 34.2       | 1200                | 3.0      |

| Group/Dose (mL/animal) | Mean | S.D. | WBC (× 10^6 cells/μL) | WBC Differential Counting (%) | PT (sec) | APTT (sec) |
|------------------------|------|------|-----------------------|-------------------------------|----------|------------|
|                        | Mean | S.D. | N                      | NEU LYM MONO EOS BASO         |          |            |
| G1 (0)                 | 4.82 | 0.91 | 5                      | 20.0 75.7 2.2 0.9 0.1        | 18.5     | 13.2       |
| G2 (0.1)               | 3.40 | 1.12 | 5                      | 16.0 80.6 1.6 0.9 0.1        | 18.4     | 13.9       |
| G3 (0.5)               | 3.86 | 1.09 | 5                      | 12.7 83.4 1.7 1.0 0.1        | 18.5     | 14.9       |
| G4 (1.0)               | 4.03 | 1.21 | 5                      | 16.9 79.6 1.6 0.8 0.1        | 18.3     | 14.8       |

*S* significantly different from control by Dunnnett’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         | 5         | 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)
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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