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Acute Dermal Toxicity Study of Bee Venom (*Apis mellifera* L.) in Rats

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Bee venom (*Apis mellifera* L. BV) has been used as a cosmetic ingredient for anti-ageing, anti-inflammatory and antibacterial functions. The aim of this study was to evaluate the acute toxicity after a single dermal administration of BV, BV was administered to 2 groups of Sprague-Dawley (SD) male and female rats (5 animals/group) at doses of 0 and 1,500 mg/kg body weight (BW). Mortality, clinical signs, body weight changes and gross findings were continually monitored for 15 days following the single dose. There were no unscheduled deaths in any groups during the study period. No BV related clinical signs and body weight changes were observed in any groups during the study period. There were no abnormal gross findings at necropsy on day 15 after the treatment. On the basis of the above results, it was concluded that there were no treatment-related effect on mortality, clinical signs, body weight changes and gross findings in SD rats treated with a single dermal dose of BV at dose of 1,500 mg/kg BW. Therefore, the approximate lethal dose of BV was considered to be over 1,500 mg/kg/day for both sexes of rats. BV may provide a developmental basis for a cosmetic ingredient or external application for topical uses.

***Key words*:** Bee venom, *Apis mellifera*, Dermal toxicity, Rat

INTRODUCTION

Bee venom (BV) from the honeybee (*Apis mellifera* L.) possesses a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating peptide (Son *et al*., 2007). In addition, it contains biologically active amines (histamine, epinephrine) and a few non-peptide components including lipids, carbohydrates and free amino acids (Lariviere and Melzack, 1996). BV has been used as a complementary medicine to treat such conditions as rheumatoid arthritis (Park *et al*., 2004; Son *et al*., 2007) and cancerous tumors (Jang *et al*., 2003; Ip *et al*., 2008; Wang *et al*., 2009; Soman *et al*., 2009; Park *et al*., 2011). Recently BV also has been used as a cosmetic ingredient for antiageing, anti-inflammatory and antibacterial functions. Pure BV is generally obtained by collecting a large amount of BV by electric stunning using a BV collector without harming the honey bees, removing impurities from the collected BV, and lyophilizing the resultant. We previously reported skin photoprotective action of BV through reduction of protein levels of matrix metalloproteinases which are main contributors to photoaging processes was found in our another study (Han *et al*., 2007a). The previous works have demonstrated that BV has identified antimicrobial and anti-inflammatory effects of BV against acne-inducing bacteria (Han *et al*., 2010). Also, BV augmented wound healing with concomitant inhibition of cytokines associated with fibrosis, which resulted in decreased wound size and increasing epithelial proliferation in a mouse full-thickness excision wound model (Han *et al*., 2011).

For the purpose of accessing BV further as a cosmetic ingredient and a potential external application for topical uses, we performed studies for the dermal toxicity. Assessment of a single dermal dose toxicity is an important part of any toxicology program for new consumer products to safe guard human beings against the possible adverse effects (Vinardell and Mitjans, 2008).

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MATERIALS AND METHODS

Bee venom. Colonies of natural honeybees (*Apis mellifera* L.) used in this study were maintained at the National Academy of Agricultural Science (NAAS), Suwon, Korea. BV was collected by a bee venom collecting device (Chunglin, Korea) in a sterile manner under strict laboratory conditions. In brief, the bee venom collector was placed on the

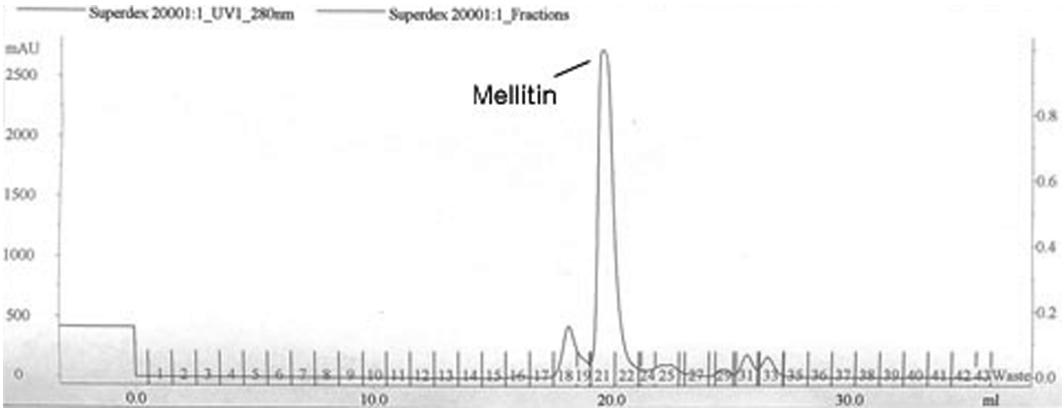

Fig. 1. Gel filtration of 12 mg PBV on Superdex Peptide. Elution with 0.02 M phosphate buffer with 0.25 M NaCl, pH 7.2. Mellitin is main component of PBV.

hive, and the bees were given enough electric shock to cause them to sting a glass plate from which dried bee venom was later scraped off. The collected venom was purified by method of Han *et al*. (2007b). Purified BV (PBV) was stored in a refrigerator for later use. Fig. 1 shows that PBV used in the experiment were confirmed with size exclusion gel chromatography (AKTA explorer, USA) by dissolving in 0.02 M phosphate buffer with 0.25 M NaCl adjusted to pH 7.2 using a Superdex Peptide column (Amersham Biosciences, USA).

Animals. Experiments were performed on twenty-four healthy, young 7 weeks old male SD rat (Sprague-Dawley rat, weight 192.5~201.6 g, Orient Bio, Seoungnam, Korea) and 9 weeks old female SD rat (Sprague-Dawley rat, weight 190.6~205.6 g, Orient Bio, Seoungnam, Korea). All animals were visually examined on acquisition. Only the animals remained in good physical condition during the 7 day acclimatization in the animal room were selected for the test.

Housing conditions. They were maintained under controlled environmental conditions (temperature 23 ± 3^{o}C; relative humidity $50 \pm 10\%$; 12 : 12 h light : dark cycle; ventilation rate 10~20/h; illumination 150~300 Lux). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) in Korea Institute of Toxicology, KRICT and conducted in the facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International (1998). Two animals for the quarantine period and one animal for the observation after the treatment period per cage were housed in a stainless wire-mesh cage (255 W × 465 L × 200 H mm) throughout the study. Cage cards showing the study number and the animal number were attached on each cage.

Diet and water. Pelleted chow for experimental animals was purchased from PMI Nutrition International (PMI Nutrition International, IN, USA). Heavy metal and insecticide contaminations were examined by the supplier prior to supply. The pellet chow was gamma-ray irradiated and given *ad libitum*. Microbial screening was conducted by the quarantine laboratory of Korea Institute of Toxicology, KRICT. No significant microbiological factors to affect the experimental results were found. Municipal tap water was given *ad libitum*, following the UV irradiation and filtration. The water was analysed at Daejeon Regional Institute of health and Environment (Daejeon, Korea). No significant contaminations to affect the experimental results were found.

Dosage and group assignment. Dose levels for the study were decided the result of the preliminary study (not shown). Dose level of 1,500 mg/kg BW was selected for the limit dose in this study. Table 1 is showed group assignment of animals. Healthy animals were grouped using the Path/Tox System (Version 4.2.2, Xybion Medical Systems Corporation, USA) according to the body weight following the quarantine and acclimatization. Each animal was identified by tail staining with ink marker, tail tattooing and the cage cards according to SOPs of KIT.

Preparation and administration of the PBV. On the treatment day, the PBV was weighed at designated amount. The PBV was dissolved and suspended in distilled water as vehicle. The concentration measurement of the prepared

Table 1. Experiment group assignment in SD rats

Group	Sex	No. of animals	Volume (ml/kg)	Dose (mg/kg)
Vehicle control	Male	5	3	0
	Female	5	3	0
PBV	Male	5	3	1,500
	Female	5	3	1,500

PBV was not conducted. The hairs on the dorsal skin surface (About 6×8 cm^2) of animals were carefully shaved 24 hours before application. The shaved skin was covered and taped with the gauze (About 4×4 cm^2) of three-fold evenly spread with either PBV of vehicle control for 24 hours. On the next day, gauze and tape were removed and the skin was washed with saline. Dermal route is the intended administered once a day (A 24 h exposure period). The administration volume of the test item was calculated with a dose volume of 3 ml/kg body weight according to the body weight of the animal on the treatment day using Path/Tox System (Version 4.2.2).

Observation and examination. Clinical signs and mortality were monitored at 1, 2, 3, 4, 5 and 6 hours after dosing and once a day until Day 15. Individual body weights were measured just before PBV administration (Day 1), and on Day 2, 5, 8 and 15 after the PBV administration. On day 15 after the treatment, all animals were euthanized by exsanguinations from abdominal aorta and cava under CO2 gas overdose, and necropsied with special attention to all vital organs.

Statistical analysis. The data for clinical signs and body weights were analysed using Path/Tox System (Version 4.4.4, Xybion medical Systems Corporation, USA) according to SOPs of KIT, KRICT.

RESULTS

The PBV application to the SD rats skin revealed no appreciable clinical signs throughout the observation period of 15 days and there was no mortality seen (Table 2 and 3). In the dermal toxicity test, no erythema, eschar, edema or any other reactions were observed in either intact or abraded sites of all rabbits which were treated with either PBV or distilled water (Table 2). There was no significant change in body weight of the SD rats from PBV application during the observation period (Fig. 2). Also, there were no prominent gross lesions observed in all animals (Table 4).

Table 2. Mortality of dermal toxicity in SD rats

Sex	Dose (mg/kg)	Dosing phase			Final mortality
		1 day	≤ 1 weeks	≤ 2 weeks	
Male	0	0	0	0	0/5
	1,500	0	0	0	0/5
Female	0	0	0	0	0/5
	1,500	0	0	0	0/5

Table 3. Summary of clinical signs of dermal toxicity in SD rats

Sex	Group	Dose (mg/kg)	No. of animals	Test site	
				Erythema & Escher	Edema
Male	Vehicle control	0	5	0	0
	PBV	1,500	5	0	0
Female	Vehicle control	0	5	0	0
	PBV	1,500	5	0	0

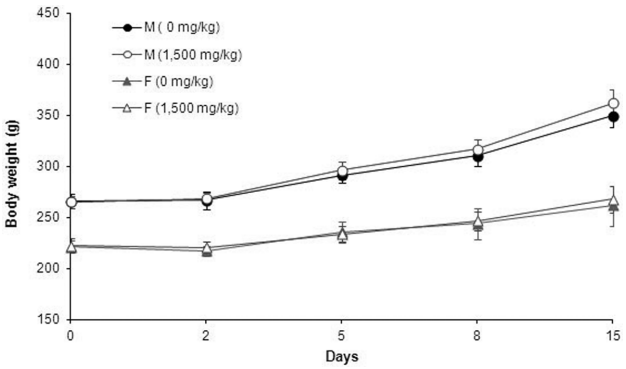

Fig. 2. Body weights of dermal toxicity in SD rats. M; Male SD rats, F; Female SD rats, 1,500 mg/kg ; treatment of 1,500 mg/kg PBV.

Table 4. Gross findings of dermal toxicity in SD rats

Sex	Group	Dose (mg/kg)	Incidence of gross findings	
			No. of observations	Observed no remarkable finding
Male	Vehicle control	0	5	5
	PBV	1,500	5	5
Female	Vehicle control	0	5	5
	PBV	1,500	5	5

DISCUSSION

BV therapy is a treatment modality that may be thousands of years old (Piek, 1986) and involves the application of live bee stings to patient skin or, in more recent years, the injection of BV into the skin with a hypodermic needle (Castro *et al*., 2005; Baek *et al*., 2006). BV also has been reported to be effective in treating allergies, scarring, burns, and skin diseases (Han *et al*., 2007a). The EU Research Project CAESAR was responsible for developing robust QSARs for five toxicological endpoints of regulatory importance, one of which was skin sensitization. A skin sensitizer is a substance that will induce an allergic response following skin contact. Substances are classed as skin sensitizers, if there is evidence in humans that the substance can induce sensitization by skin contact in a substantial number of persons, or where there are positive results from an appropriate animal test (Chaudhry *et al*., 2010). In the assessment and evaluation, dermal toxicity is important initial test. This study was performed to determine the dermal toxicity of PBV.

The results of the study, a decreased body weights were thought to be caused by the stress given by taping for the treatment, since it was happened in both control and treatment-related effects on mortality, clinical signs and gross finding at necropsy. In conclusion, a single dermal dose to PBV had no toxic effects on mortality, clinical signs, body weight changes and gross findings in both sexes of rats at dose of 1,500 mg/kg BW. Therefore, the approximate lethal dose of test item might be higher than 1,500 mg/kg BW in both sexes of rats. Since PBV has recently been reported to possess antibacterial effect against acne-inducing bacteria and effect of wound healing (Han *et al*., 2010; Han *et al*., 2011), it is timely and appropriate to endeavour toxicological approach to PBV for the possible adverse effects with the intent of using BV in cosmetic and medical applications.

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