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Skin Sensitization Study of Bee Venom (*Apis mellifera* L.) in Guinea Pigs

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Bee venom (*Apis mellifera* L., BV) has been used as a cosmetic ingredient for antiaging, anti-inflammatory and antibacterial functions. The aim of this study was to access the skin sensitization of BV, a Buehler test was conducted fifty healthy male Hartley guinea pigs with three groups; Group G1 (BV-sensitization group, 20 animals), group G2 (the positive control-sensitization group, 20 animals), and group G3 (the ethyl alcohol-sensitization group, 10 animals). The exposure on the left flank for induction was repeated three times at intervals of one week. Two weeks after the last induction, the challenge was performed on the right flank. No treatment-related clinical signs or body weight changes were observed during the study period. The average skin reaction evaluated by erythema and edema on the challenge sites and sensitization rate in the BV-sensitization group at 30 hours were 0.0 and 0%, respectively, which are substantially low compared with in positive control group (average skin reaction: 0.55, sensitization rate: 40%) and identical with in vehicle control group, representing a weak sensitizing potential. The average skin reaction and sensitization rate observed at 54 hours were 0.0 and 0% in the BV-sensitization group, respectively, and 0.25 and 20% in the positive control group, respectively. It was concluded that BV classified to Grade I, induced no sensitization when tested in guinea pigs and may provide a developmental basis for a cosmetic ingredient or external application for topical uses.

***Key words*:** Bee venom, Skin sensitization, Guinea pigs, Buehler test

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*., 2010). Recently BV also has been used as a cosmetic ingredient for antiaging, 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). For the purpose of accessing BV further as a cosmetic ingredient and a potential external application for topical uses, we performed studies for the skin sensitization. Assessment of skin sensitization potential 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 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 was stored in a refrigerator for later use.

Animals. Experiments were performed on fifty healthy, young 5 weeks old male guinea pig (Hartley Guinea pig, weight 318~369 g, Samtako Bio, Osan, Korea). Animals were visually examined at the time of receipt and housed for 11 days in the animal room. They were maintained under controlled environmental conditions (temperature 23 ± 3°C; relative humidity 55 ± 10%; 12 : 12 hours light : dark cycle; ventilation rate 10~20/hours; illumination 150~300 lux), providing *ad libitum* access to a commercial guinea pigs diet (Harlan Teklad, USA) and water. 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.

Skin sensitization test (Buehler test). Dose levels for the study were decided the result of the preliminary study (not shown). HCA (Hexylcinnamaldehyde, CAS no. 101-86-0, Sigma) solution of 10% was chosen for positive control. Table 1 is showed group assignment of animals. The induction and challenge BV was dissolved in vehicle at 10 mg/m*l* for the administration. The positive control was dissolved in 80% ethyl alcohol for the induction and in acetone for the challenge, respectively. Induction was performed on the left flank and challenge on the right flank. To use topical (patch) application, application site was cleared of hair (closely clipped). A cotton pad (2×2 cm^2) was fully loaded with test or control item solution and held on the application site by an occlusive patch for 6 hours exposure, the patch was removed and the application site was washed with tap water. The induction period was once a week total three times. The challenge period was 2 weeks after the last induction.

Individual weight of guinea pigs was determined and they were observed for any clinical signs. Mortality was recorded during the observation period. The challenge sites were cleared of hair 21 hours after removing the patch. Approximately 3 hours later and 24 hours later, skin responses were recorded according to the grading scale as Table 2 and 3. After the observation of skin response, all animals were euthanized with CO_2 overdose.

Table 1. Experiment group assignment in guinea pigs

Group	No of animal	Administration	
		Induction	Challenge
G1[a)]	20	BV	BV
G2[b)]	20	HCA[d)]	HCA
G3[c)]	10	Ethyl alcohol	HCA

[a)]Group G1: sensitized with BV and challenged with BV.
[b)]Group G2: sensitized with the HCA and challenged with HCA.
[c)]Group G3: sensitized with ethyl alcohol and challenged with HCA.
[d)]HCA(Hexylcinnamaldehyde) is positive control.

Table 2. Evaluation of primary skin response

Score	Skin response
0	No visible change
1	Dispersed or blotchy erythema
2	Moderate diffused erythema
3	Marked erythema and oedema

Table 3. Classification of skin sensitization level

Sensitization rate (%)	Grade	Classification
1~8	I	Weak
9~28	II	Mild
29~64	III	Moderate
65~80	IV	Strong
81~100	V	Extreme

Statistical analysis. The body weights collected during the study were analysed with F-test to examine variance homogeneity. The t-test was conducted to determine whether the BV-sensitization group was significantly different from the vehicle control-sensitization group. The level of significance was taken as $P < 0.05$ or 0.01. Statistical analysis was performed by using Path/Tox System (ver 4.4.4, Xybion medical Systems Corporation, USA) according to SOPs of KIT, KRICT.

RESULTS

Mortality, clinical signs and body weight. The BV application to the guinea pig skin revealed no appreciable clinical signs throughout the observation period of 31 days and there was no mortality seen (Table 4). Also there was no significant change in body weight of the guinea pigs from BV application during the observation period (Fig. 1). In the skin sensitization test, no fur, erythema, scab or any other reactions were observed in BV (Table 5).

Observation of application sites. Approximately 30 and 54 hours from the start of the challenge application (24 and 48 hours from the removing patch), the skin reaction was observed. The average skin reaction scores in the groups BV, positive control, and ethyl alcohol at 30 hours were 0.0, 0.55, and 0.0, respectively (Table 6). The sensitization rates at the same time point were 0, 40, and 0%, respectively. The average skin reaction scores in the group BV, positive control, and negative control at 54 hours were 0.0, 0.25, and 0.0, respectively. The sensitization rates at the same time point were 0, 20, and 0%, respectively.

Table 4. Mortality of skin sensitization in guinea pigs

Group	Dosing Phase					Final mortality
	1 day	≤ 1 weeks	≤ 2 weeks	≤ 3 weeks	≤ 4 weeks	
G1[a)]	0	0	0	0	0	0/20
G2[b)]	0	0	0	0	0	0/20
G3[c)]	0	0	0	0	0	0/10

[a)]Group G1: sensitized with BV and challenged with BV.
[b)]Group G2: sensitized with the HCA and challenged with HCA.
[c)]Group G3: sensitized with ethyl alcohol and challenged with HCA.

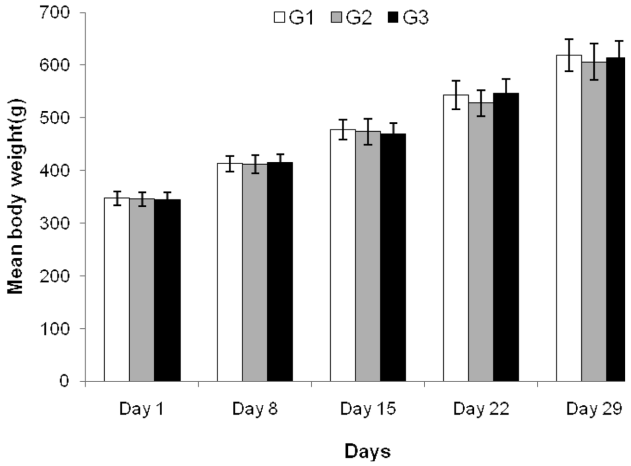

Fig. 1. Body weights of skin sensitization in guinea pigs. Group G1; sensitized with BV and challenged with BV, Group G2; sensitized with the HCA and challenged with HCA, Group G3: sensitized with ethyl alcohol and challenged with HCA.

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). This study was performed to determine the skin sensitively of BV. In Buehler test of a skin sensitization test using guinea pigs, the induction with patch application was repeated three times at intervals of one week and the challenge application was performed 2 weeks after the last induction application. The average skin reaction score and sensitization rate in the BV group at 30 hours were 0 and 0%, respectively. The skin response on the same site at 54 hours showed the average skin reaction of 0 and sensitization rate of 0%.

Table 5. Clinical signs of skin sensitization in guinea pigs

Group	G1[a)]		G2[b)]		G3[c)]	
	#[d)]	%	#	%	#	%
Fur, Hair, Coat loss of fur	0/20[e)]	0	10/20	50	0/10	0
Skin Scab	0/20	0	20/20	100	0/10	0
Erythema	0/20	0	20/20	100	0/10	0

[a)]Group G1: sensitized with BV and challenged with BV.
[b)]Group G2: sensitized with the HCA and challenged with HCA.
[c)]Group G3: sensitized with ethyl alcohol and challenged with HCA.
[d)]Number of animals.
[e)]Number of animals with sign/Total of animals observed.

Table 6. Results of skin sensitization in guinea pigs

Group	No. of animals	Average sensitization score[a)]		Sensitization rate[b)]		Maximization grade	
		30 h	54 h	30 h	54 h	Grade	Classification
G1[c)]	20	0.0	0.0	0%	0%	I	Weak
G2[d)]	20	0.55	0.25	40%	20%	III	Moderate
G3[e)]	10	0.0	0.0	0%	0%	I	Weak

[a)]Average sensitization score = (∑Sensitization score of each animal)/Number of animals observed.
[b)]Sensitization rate(%) = (Number of animals with positive response/Number of animals observed) × 100.
[c)]Group G1: sensitized with BV and challenged with BV.
[d)]Group G2: sensitized with the HCA and challenged with HCA.
[e)]Group G3: sensitized with ethyl alcohol and challenged with HCA.

In conclusion, skin sensitization level of BV was classified as Grade I. BV is not considered to induce skin sensitization since the skin sensitization ratio was 0%. Since BV 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 BV for the possible adverse effects with the intent of using BV in cosmetic and medical applications.

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